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SPECIAL ISSUE

IJFMP

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Indian Journal of Forensic Medicine and Pathology

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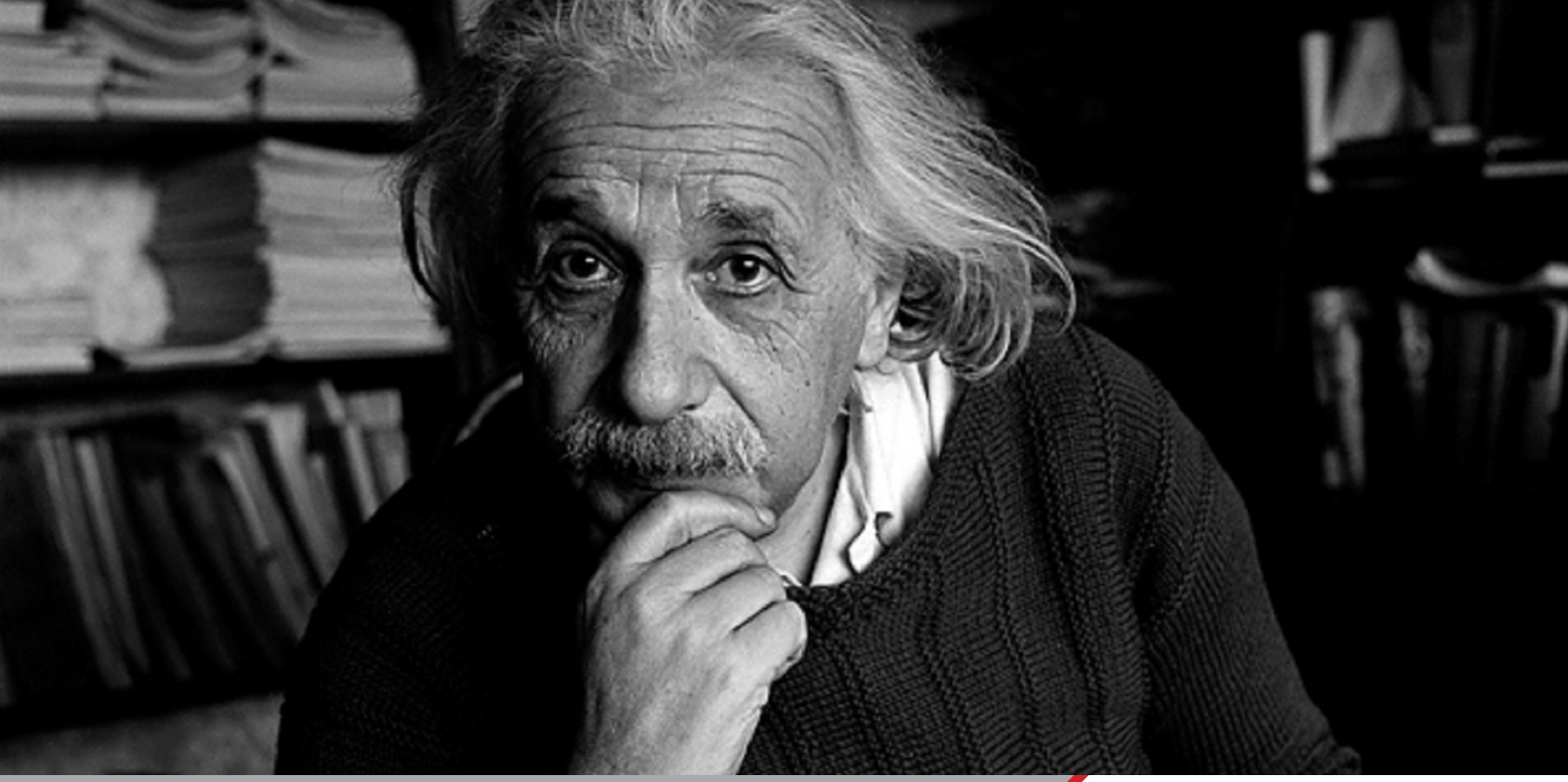

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ORIGINAL ARTICLE

Effectiveness of X-Ray Fluorescence Spectrometer Technique in Analysis of Nano-Coated Textile Material for use in Forensic Science

[1]Parshuram Singh, [2]Sapna Balayan,[3]R K Sarin,[4]Utkarsh Jain

ABSTRACT

CONTEXT: Forensic examination is conducted to detect, identify, and investigate the crime to figure out the pieces of evidence and connect it to the perpetrator of the crime. The nanoparticles play a crucial role in the forensic analysis of the evidence obtained at the crime scene. These nanoparticles can be characterized by various scanning and X-Ray techniques. The XRF technique provides an effective analysis of elemental composition of the materials.

AIMS: This study aims to perform a differential analysis of nanomaterials of coated and non-coated samples through X-Ray fluorescence spectroscopy.

MATERIALS & METHOD: Firstly, the titanium dioxide (TiO_2) nanoparticles were synthesized by using a hydrothermal method. This nanomaterial was then characterized with distinct techniques such as Dynamic light scattering (DLS), X-Ray diffraction (XRD), and Ultraviolet-visible spectroscopy (UV-VIS). Furthermore, the TiO_2 nanoparticles were coated on the surface of rexine, paint, and glass to observe the composition of elements in nano-coated and non-coated samples. Moreover, the surfaces were characterized by using SEM, and the elemental composition was determined through XRF.

RESULTS: The results exhibit a distinctive difference in the concentration of titanium obtained in glass samples. However, the analysis on rexine and paint samples shows that the difference in the quantity of titanium is less when the nanocoated and non-coated samples were analyzed.

CONCLUSIONS: It was concluded that titanium is already present during the manufacturing of rexine and paint therefore, the nanoparticle coating of TiO2 doesn't create a large difference. Besides, there was a significant difference in nanomaterials coated and non-coated glass samples.

KEY MESSAGES: The forensic investigation can be more qualitative with nanomaterial coating on the surface of glass, fabrics, and paint samples. These materials can be easily characterized with surface and X-ray techniques to provide efficient data.

KEYWORDS | x-ray fluorescence, scanning electron microscopy, dynamic light scattering, DLS

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INTRODUCTION

RECENTLY, NANOTECHNOLOGY HAS EMERGED as a harbinger of new technologies in product development due to the unique properties of the materials at their nanoscale. This study was conducted for the examination of different samples such as rexine, paint, and glass. Effective analysis was done for each sample coated with titanium dioxide (TiO_2) nanoparticles and non-coated samples by using the XRF technique for forensic applications.

METHOD & MATERIALS

Chemicals and Equipments

Titanium dioxide is widely used in consumer products including textile and paint materials. Therefore, titanium dioxide nanoparticles were selected to analyze the surface of these materials (rexine, paint, and glass) during forensic analyses. The rexine, paint, and glass slab were purchased from a local vendor. Titanium tetra isopropoxide (TTIP), ethanol, and nitric acid were procured from US-based Sigma-Aldrich. Distilled water (DW) was used for preparing the samples that were used in this research. The apparatus was autoclaved before carrying out the experiments. The samples were characterized using dynamic light scattering (DLS) (Malvern Panalytical), UV-VIS (UV-1800, Shimadzu), SEM (Zeiss EVO 18 448), and XRF (Shimadzu ED7000) techniques. The scheme at Fig. 1 below represents the steps involved in the present work.

Synthesis of TiO_2 Nanoparticles

TiO_2 nanoparticles synthesis was carried out with a laboratory-based method in which reducing agent nitric acid and precursor TTIP was used. The mixture was prepared in a ratio of 1:1:4 using TTIP, ethanol, and DW. Further, this mixture was stirred continuously for 30 minutes at room temperature. pH of the solution was maintained at neutral and kept undisturbed for 24 hours to carry out the aging process. The resultant was then autoclaved for 2 hours at 120°C. Thereafter, the resultant was cooled at room temperature and washing was done with DW to remove impurities. The final product was obtained when the resultant was autoclaved for 2 hours at 450°C and then cooled at room temperature followed by fine grinding.[1]

Sample Preparation of Rexine, Paint, and Glass

The rexine samples were prepared from a car

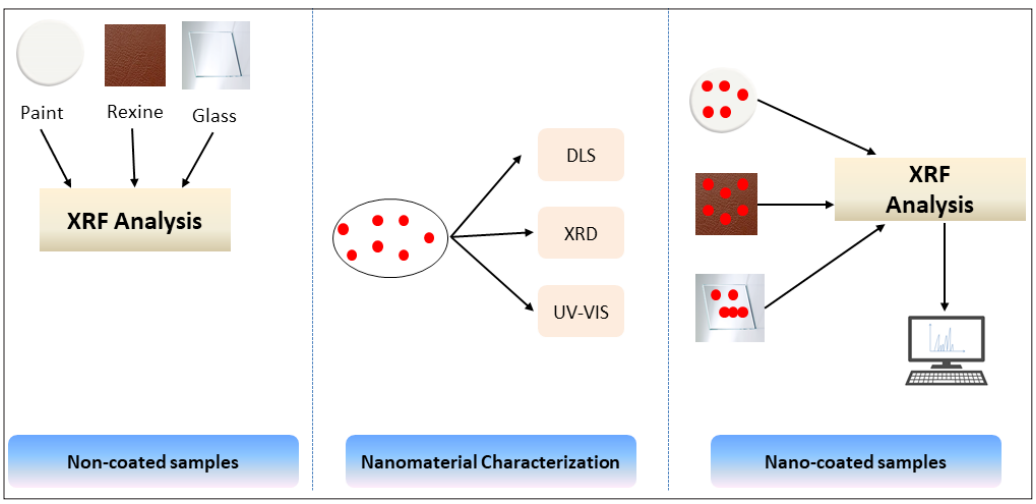


Figure 1 The Scheme represents the three techniques involved in the characterisation of samples.

cover sheet. The sample preparation of the paint was done on the surface of an aluminum sheet. The samples were divided into two parts where one part was coated using TiO_2 nanoparticles and the other was used for the control or comparison observation. Before analysis, the surface was carefully washed with DW to remove any impurities and was dried. One part of the sample was coated using TiO_2 nanoparticles.

For rexine, a solution was prepared in DW using TiO_2 nanoparticles with a concentration of 0.4 mg/mL, and the solution was then sonicated for 30 minutes. Further, it was immersed in this solution for 10 minutes. The deposition of TiO_2 nanoparticles on the surface was followed by annealing at 75°C for 60 minutes. The samples were then washed with DW in an ultrasonic bath to remove undeposited nanoparticles.

For the paint sample, the aluminum sheets were coated with paint in the presence of TiO_2 nanoparticles when the paint was dried and the flakes were then used for the analysis. Similarly, the glass samples were prepared on a glass slab while coating the TiO_2 nanoparticles on the slab surface. Figures 1(a), (b), and (c) show rexine, paint, and glass samples.

Characterization of Prepared Samples

The synthesized TiO_2 nanoparticles were characterized using different techniques. DLS was done for determining the size of the particles. To carry out the DLS analysis 1 mg/mL solution was prepared and was placed in a cuboid to determine the nanoparticle size. The XRD analysis was carried out for obtaining the crystallinity of the material (a 2mg powder sample was used for XRD analysis). The optical properties of the synthesized material were determined with UV-VIS. To perform the UV-VIS analysis a dilutes sample was prepared and was kept in the spectrophotometer. Further, the sample prepared on different surfaces like rexine, paint, and glass were characterized using SEM to study the surface of the sample and XRF analysis to determine the elemental composition of the prepared sample.

RESULTS

The size of the prepared sample was determined using DLS performed at Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida. The size of the nanoparticles was obtained within 500 nm as shown in Figure 2(a). The XRD analysis was performed for TiO_2 nanoparticles and the recorded spectrum

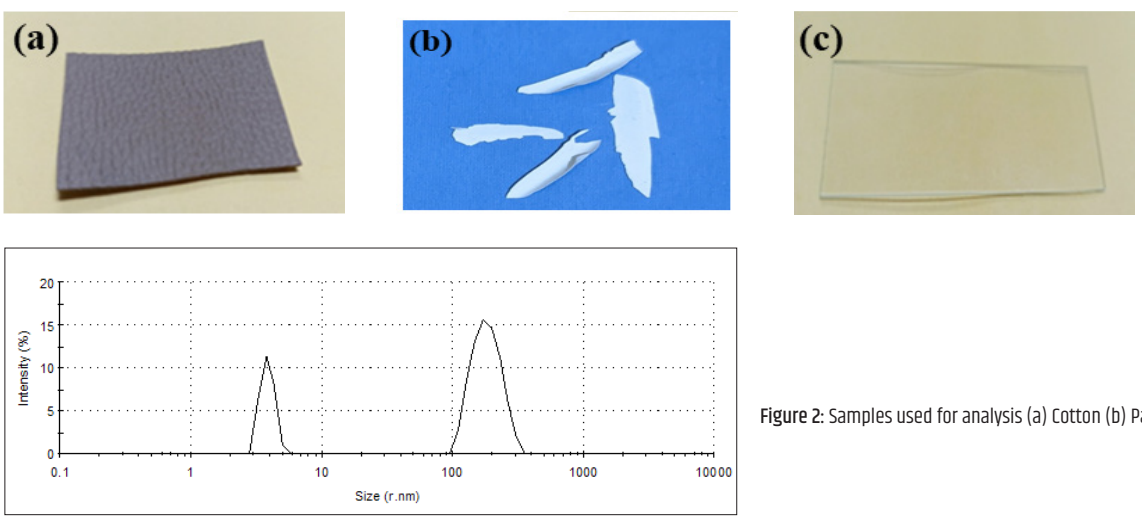


Figure 2: Samples used for analysis (a) Cotton (b) Paint (c) Glass

is illustrated in Figure 2(b). The diffraction peaks were obtained at 25.63°, 27.62°, 36.33 °, 48.39°, 54.50° confirms the synthesis of TiO2 nanomaterials[2]. Further, the UV-VIS analysis of the TiO2 nanoparticles was carried out to determine the optical properties as shown in Figure 2(c). The absorbance was obtained at room temperature and in a nano range. The sample was scanned within a wavelength ranging from 200 to 700nm. It was observed that a peak was shown at a wavelength of 312 nm showing good absorbance in the ultra visible region.[3]

Scanning Electron Microscopy

The surface of the nanoparticle-coated samples was characterized using scanning electron microscopy (EVO 18 Special Edition, Zeiss). Figure 3 (a, b) illustrates the SEM micrographs for glass and rexine samples respectively coated with TiO_2 nanoparticles. The spherical structure on the surface of the rexine, paint, and glass slab was observed in nanometer size 4-6. Hence, it was confirmed that the TiO_2 nanoparticles are present in the glass and rexine surfaces.

X-Ray Fluorescence Spectroscopy

The rexine, paint, and glass samples were examined using the XRF technique (Shimadzu ED7000). The samples were placed directly in the chamber with air atmosphere one after other. For operating the instrument a voltage of 50 KV was used and an acquisition range from 0-40 Kev. For target material rhodium (Rh) was incorporated. The sample was analyzed in the same target area in both samples.

XRF Analysis for Rexine Samples

The elemental analysis for rexine is displayed in Figure 4(a) and (b) for non-coated and coated samples respectively. The elemental profile obtained during analysis is shown in Table 1 (a) and (b) for coated and non-coated samples respectively. The obtained results show the presence of a higher percentage of chlorine (Cl) (83.83%) followed by titanium (Ti) (6.84%) and calcium (Ca) (6.67%) in both samples. The observed intensity peak exhibits no significant difference for Ti elements in both samples. This may be due to the presence of TiO_2 particles in the rexine sample having higher concentrations rather than the coated TiO_2 nanoparticles.

Determining the Composition of Paint Sample using XRF Technique

The XRF analysis carried out for the paint sample is illustrated in Figure 5. The titanium peak obtained in both the samples (non-coated and TiO_2 nanoparticle-coated) is almost similar. The XRF graph for non-coated samples is shown in Figure 5(a) whereas, the nanocoated is shown in Figure 5(b). Table 2(a) shows the elemental percentage of composition of non-coated samples and for nanocoated samples,

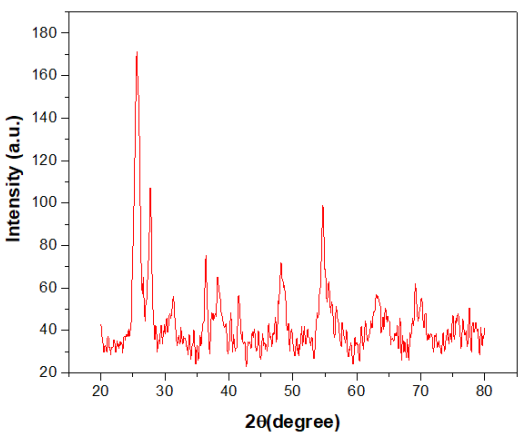


Figure 2(b)

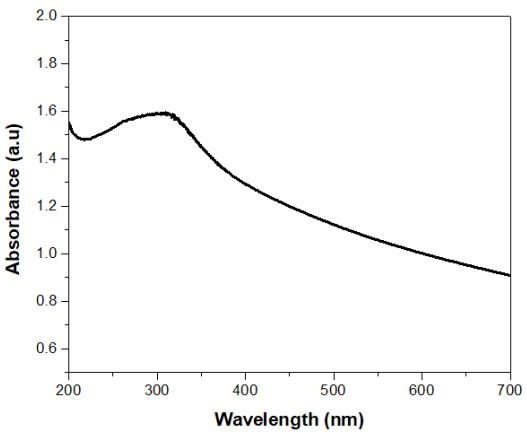


Figure 2(c)

the results are shown in Table 2(b). The sample is composed of approximately 98% for both the samples, no large difference is obtained between the titanium percentage for non and nanocoated paint samples. This was due to the presence of white color paint coated on the surface of the aluminum sheet itself containing TiO_2 particles.[7]

Analysing the Glass Sample using the XRF Technique

The surface of the glass slab was characterized under the XRF technique to study the presence of elements and their percentage on the surface. Figure 6 depicts the XRF analysis for non-coated [Figure 6(a) and nanocoated (Figure 6(b)] samples of glass slabs. A higher percentage of silicon (Si) (64.58%) was observed followed by Ca (30.7%) in both samples as shown in Table 3(a) and (b). The principal constituent of glass is silica (SiO_2) and calcium oxide (CaO) which are used to increase their durability. Whereas, the percentage of Ti was very low (0.20%) in the case of non-coated glass slab and was higher (2.02%) in the nanocoated sample. Also, a large difference was observed in peak intensity of Ti from the control sample (0.373 cps/μA) to nanoparticle coated (3.661) differentiating the two samples.

DISUCSSION

The most common piece of evidence that can be frequently collected from the crime spot for forensic analysis are fiber, fabrics, hair, paint, and glass. The evidence in cases like rape, burglary, and physical violence can be collected from the various materials at the crime scene. Presently, the evidence collected for solving the forensic analyses is mostly in the form of bloodstains and fingerprints which are then analyzed using spectroscopic techniques.[8,9] The forensic analysis of these materials is difficult due to the complex chemical composition of samples.[9]

With the new advances in technology, the textile is nowadays coated with nanomaterials. They are used for digital printing, textile coloring, and developing smart fabrics as they provide various exceptional properties such as self-cleaning, anti-bacterial, thermal retardancy, ultra-violet protection, and others.[10-15]

The imaging techniques used in surface analysis for nanomaterial characterization are Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Scanning Tunneling Microscopy (STM), and Atomic Force Microscopy (AFM).[16-18] The vibration mode of molecules is studied with spectroscopic techniques including Fourier Transform Infrared (FTIR) spectroscopy and Fourier Transform Raman (FT-RAMAN) spectroscopy.[19,20] Ultraviolet-visible spectroscopy (UV-VIS) technique is

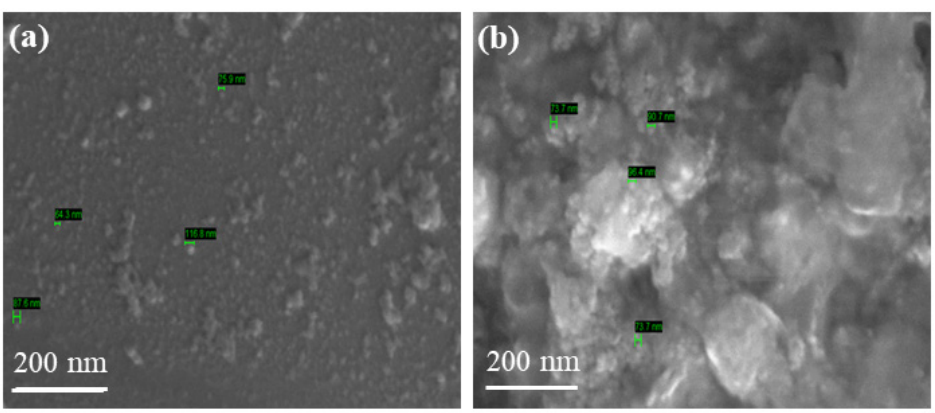


Figure 3: Scanning electron microscopy for TiO2 nanoparticle coated samples at 200 nm: (a) Cotton; (b) Glass; (c) Paint; (d) Rexine

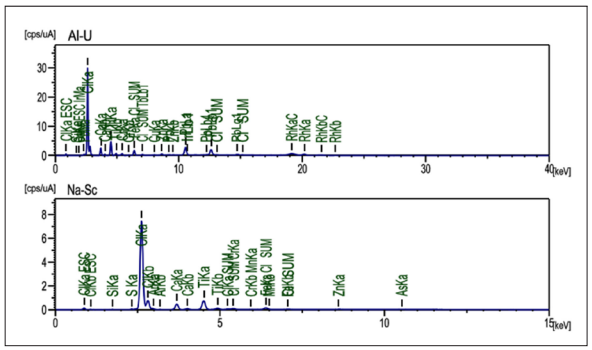
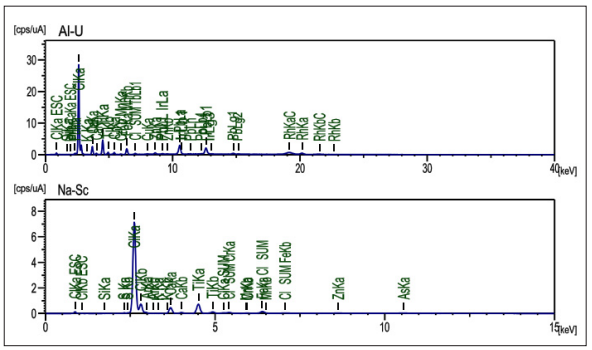


Figure 4(a):



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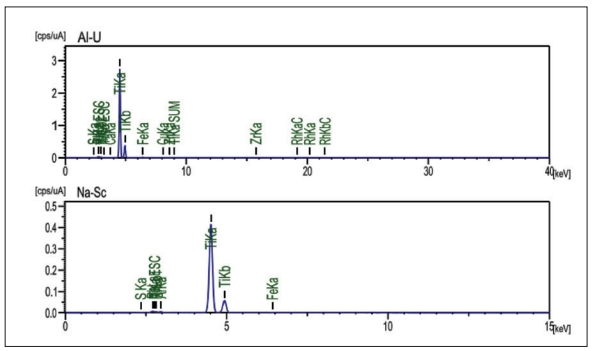
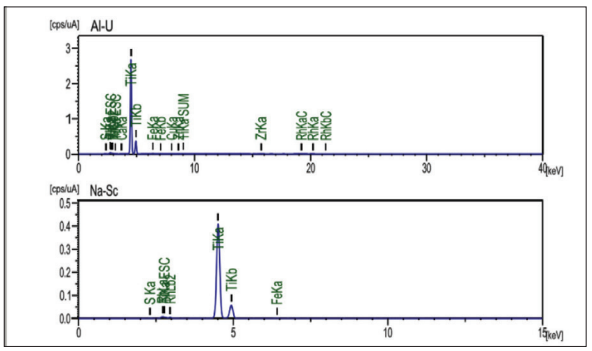


Figure 5(a):



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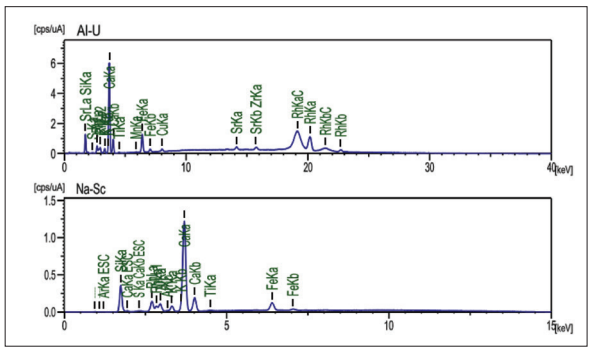

Figure 6(a):

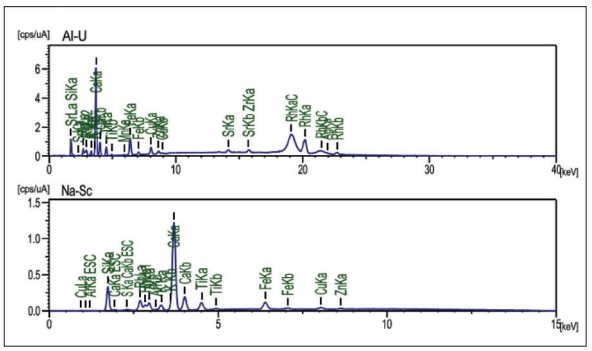

Figure 6(b):

used to study the optical properties of the nanomaterials.[21] X-ray techniques like X-Ray Diffraction (XRD), X-Ray Fluorescence (XRF) spectroscopy, and X-Ray Photoelectron Spectroscopy (XPS) are used to get information about the crystalline structure and oxidation state of the elements of nanomaterials.[22,23] Among these techniques, XRF is well known for elemental analysis in forensic investigation.[24] In an X-ray, when allowed to hit the surface of the material, electrons are ejected from the atom of an element present in the sample resulting in determining their composition. This technique facilitates in retrieving information about the elemental composition of the samples obtained from the crime scene. Samples such as paint, glass, and textile are very common in cases including murder, rape, and burglary. It can provide better analysis of these samples.[25-27] There are various advantages

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1	Cl	83.83	0.225	Quant.-FP	ClKa	75.910
2	Ti	6.84	0.059	Quant.-FP	TiKa	30.465
3	Ca	6.67	0.067	Quant.-FP	CaKa	5.139
4	Pb	0.85	0.008	Quant.-FP	PbKa	18.630
5	Si	0.59	0.058	Quant.-FP	SiKa	0.100
6	Cr	0.52	0.011	Quant.-FP	CrKa	4.311
7	Fe	0.32	0.004	Quant.-FP	FeKa	4.848
8	S	0.19	0.014	Quant.-FP	S Ka	0.255
9	Zn	0.11	0.004	Quant.-FP	ZnKa	3.683
10	Cu	0.03	0.003	Quant.-FP	CuKa	0.936

Table 1(a): X-ray Fluorescence (XRF) elemental analysis for rexine samples (a) without nanoparticle coating

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1.	Ti	98.35	0.418	Quant.-FP	TiKa	16.644
2.	Fe	0.72	0.175	Quant.-FP	FeKa	0.009
3.	S	0.57	0.029	Quant.-FP	S Ka	0.082
4.	Cu	0.092	0.023	Quant.-FP	CuKa	0.014
5.	Ca	0.091	0.019	Quant.-FP	CaKa	0.024
6.	Zn	0.091	0.005	Quant.-FP	ZnKa	0.068
7.	Zr	0.07	0.017	Quant.-FP	ZrKa	0.023

Table 2(a): XRF spectroscopy for paint samples (a) without nanoparticle coating

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1.	Si	64.58	0.643	Quant.-FP	SiKa	3.149
2.	Ca	30.71	0.144	Quant.-FP	CaKa	13.953
3.	K	2.36	0.042	Quant.-FP	K Ka	0.765
4.	Fe	1.26	0.017	Quant.-FP	FeKa	8.637
5.	S	0.61	0.061	Quant.-FP	S Ka	0.090
6.	Ti	0.20	0.024	Quant.-FP	TiKa	0.373
7.	Cu	0.11	0.007	Quant.-FP	CuKa	1.382
8.	Sr	0.05	0.003	Quant.-FP	SrKa	2.018
9.	Zr	0.046	0.003	Quant.-FP	ZrKa	1.715
10.	Mn	0.045	0.010	Quant.-FP	MnKa	0.226

Table 3(a): Elemental composition of glass samples using XRF (a) non-coated;

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1.	Cl	82.72	0.228	Quant.-FP	ClKa	72.517
2.	Ti	6.78	0.059	Quant.-FP	TiKa	29.623
3.	Ca	6.74	0.069	Quant.-FP	CaKa	5.100
4.	Pb	0.93	0.009	Quant.-FP	PbKa	19.607
5.	Fe	0.86	0.011	Quant.-FP	FeKa	12.567
6.	Si	0.82	0.066	Quant.-FP	SiKa	0.136
7.	Cr	0.52	0.011	Quant.-FP	CrKa	4.239
8.	S	0.23	0.013	Quant.-FP	S Ka	0.296
9.	K	0.12	0.025	Quant.-FP	K Ka	0.059
10.	Zn	0.11	0.004	Quant.-FP	ZnKa	3.605
11.	Cu	0.04	0.003	Quant.-FP	CuKa	1.170
12.	Ir	0.03	0.007	Quant.-FP	IrKa	0.434
13.	Mn	0.02	0.006	Quant.-FP	MnKa	0.272

Table 1 (b): X-ray Fluorescence (XRF) elemental analysis for rexine samples (b) surface coated with TiO2 nanoparticles

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1.	Ti	98.33	0.422	Quant.-FP	TiKa	16.314
2.	Fe	0.64	0.032	Quant.-FP	FeKa	0.091
3.	S	0.62	0.182	Quant.-FP	S Ka	0.008
4.	Cu	0.10	0.019	Quant.-FP	CuKa	0.027
5.	Ca	0.10	0.023	Quant.-FP	CaKa	0.016
6.	Zn	0.09	0.018	Quant.-FP	ZnKa	0.028
7.	Zr	0.08	0.004	Quant.-FP	ZrKa	0.060

Table 2(b): XRF spectroscopy for paint samples (b) with TiO_2 nanoparticle coating

S.No.	Analyte	Result (%)	Std. Dev	Calc. Proc.	Line	Intensity (cps/µA)
1.	Si	64.04	0.640	Quant.-FP	SiKa	2.925
2.	Ca	31.00	0.146	Quant.-FP	CaKa	14.052
3.	K	2.35	0.041	Quant.-FP	K Ka	0.766
4.	Ti	2.02	0.043	Quant.-FP	TiKa	3.661
5.	Fe	1.25	0.018	Quant.-FP	FeKa	8.246
6.	S	0.63	0.060	Quant.-FP	S Ka	0.094
7.	Cu	0.30	0.009	Quant.-FP	CuKa	3.688
8.	Zn	0.106	0.007	Quant.-FP	ZnKa	1.502
9.	Ag	0.104	0.013	Quant.-FP	AgKa	0.952
10.	Sr	0.06	0.004	Quant.-FP	SrKa	2.005
11.	Zr	0.05	0.004	Quant.-FP	ZrKa	1.802
12.	Mn	0.04	0.012	Quant.-FP	MnKa	0.231

Table 3(b): Elemental composition of glass samples using XRF (b) TiO2 nano-coating

associated with XRF analysis such as it is non-destructive, provides rapid results, and is easy to use. Therefore in the present work, the XRF technique is used for analyzing the elemental composition of various materials attributed to forensic investigations.

Various nanoparticles have been used in the field of forensic investigation such as gold nanoparticles which are used for storing fingerprints for a long period and possess

several advantages such as high sensitivity, selectivity, and inert nature.[28-32] The silver nanoparticles are used for enabling the visualization of fingerprints during criminal investigations.[33-35] Zinc oxide nanoparticles (ZnO-NPs) have excellent properties such as high excitation binding energy, and wide-band. Therefore, ZnO-NPs are used in the form of nano-powder to obtain latent fingerprints.[36-39] Silica Nanoparticles (SiO_2-NPs) are used for interaction with an organic compound present on the surface, besides these nanoparticles prevent photo-decomposition of the fingerprints.[40] It has been reported that titanium dioxide is used in forensic applications such as latent fingerprinting or fingermark powder because they help in decreasing the risk level during inhalation and have low toxicity for investigators.[41]

CONCLUSION

In this study, the effective analysis of distinct surfaces of rexine, paint, and glass coated with nanomaterials was performed by using XRF analysis. The study was performed with two sets of observations.

Firstly, the surface of the material was analyzed without nanoparticle coating and then the surface of the material was coated with TiO_2 nanoparticles. TiO_2 are widely used in consumer products and have several applications in forensic science due to their efficacy in fingerprinting and reducing the risk level of inhalation during investigations. Hence, TiO_2 nanomaterials were synthesized and characterized by using different techniques such as DLS, XRD, and UV-VIS. Further, the synthesized TiO_2 nanoparticles were then coated on the surface of rexine, paint, and glass. Thereafter, the surface of these samples was characterized by using SEM, and the elemental composition of these samples was obtained with XRF analysis. It was observed that a notable difference was obtained in the titanium concentration with the glass sample.

In the case of other samples (rexine and paint), a minimal difference was observed indicating the presence of higher composition of titanium which was already present during their manufacturing process. The study presented an effective approach to exhibiting the integration of nanotechnology that can play a vital role in forensic investigation to provide selective and sensitive ways for detecting and solving criminal cases with infallible evidence.

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ORIGINAL ARTICLE

Comparative Solvent Extraction Methods for Determination of Pesticide Residues in Different Food Matrices and Its Analysis by GC- FID

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ABSTRACT

CONTEXT: Pesticides are often used in agricultural sector to protect crops before and after their harvest. Pesticide residues are deposits of active components, metabolites, or breakdown products of pesticides discovered in some component of the environment. **AIM:** Pesticide residues in fruits and vegetables were determined by gas chromatography/flame ionization detector. **MATERIALS AND METHOD:** Brinjal, tomato and grapes were purchased from the local market. Extraction of the samples was carried out using ethyl acetate/methanol and acetonitrile/toluene extraction procedures to determine the percentage recovery of pesticides chlorpyrifos—an organophosphate and cypermethrin—a synthetic pyrethroids from fruits and vegetables from both the extraction procedures. The extracts were cleaned using graphitized carbon black, magnesium sulphate and primary, secondary amine (PSA).

ANALYSIS: The analysis was done using gas chromatography with flame ionization detector.

RESULTS AND CONCLUSION: The samples of brinjal, tomato and grape were spiked with known concentrations of pesticide samples. Most of the pesticides recovered 60-70% of their concentrations at 0.01-0.10mg/kg range under ideal extraction and clean-up procedures. The recovery of different pesticides was dependent on the types of extraction procedure used. For chlorpyriphos, ethyl acetate/methanol recover more of the pesticides and in case of cypermethrin, acetonitrile/toluene recover more of the pesticides. The lesser recovery of pesticides on 5 and 7 days was due to the conversion of pesticides in their metabolites that was not detected in GC-FID.

KEYWORDS | residues, GC-FID, chlorpyrifos, cypermethrin, ethyl acetate, acetonitrile

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INTRODUCTION

THE MAJORITY OF FRUITS AND VEGETABLES available in market are either artificially grown or sprayed with pesticides. Pesticides are often used to protect crops before and after their harvest. Pesticides of various types are currently used to manage pests and infections that wreak havoc on crops. Pesticidal residues are deposits of pesticides' active components, metabolites, or breakdown products discovered in some component of the environment following pesticide application, spillage, or dumping.[1] Residues exist due to overuse of a pesticide when it was applied just before harvest, even if it was a permitted pesticide; pesticides that are not authorized for illegitimate pesticide use, and inappropriate pesticide application during storage and transportation. Pesticide residue analysis is a critical step in determining the safety of specific pesticides.[1] The persistence of various pesticides left residual amounts in fruits and vegetables from a variety of areas with varying residual levels. Pesticide residue analysis is routinely performed using multi-residue methods

that include homogenization of the sample with an appropriate solvent, separation of the liquid portion of the sample from insoluble material, purification and clean up, and chromatographic determination.[2]

The Gas Chromatography (GC) analytical technique is commonly used to detect the presence of these compounds in fruits and vegetables.[2] Pesticide residues in different food matrices have been assessed using a range of analytical techniques. Most of them used gas chromatography with electron capture detection (ECD), nitrogen-phosphorus detection (NPD), or flame photometric detection (FPD). Pesticide residues in fruits and vegetables are evaluated in two steps: 1) extraction and clean-up of the target analytes from the matrices, and 2) determination of the target analytes. The first step involves the use of different techniques such as liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME), and QuEChERS (quick, easy, cheap, effective, rugged, and safe) extraction.[3]

Liquid-liquid extraction (LLE), also known as partitioning, is a separation process consisting of the transfer of a solute from one solvent to another, the two solvents being immiscible or partially miscible with each other. Organic solvents such as acetonitrile, ethyl acetate, chloroform, hexane, 1, 2-dichloromethane, etc., are usually used in Liquid-liquid extraction methods for the determination of pesticide residues in food and the environment, due to their good solubility in several immiscible liquids, such as in water and organic solvents.[4]

Ethyl acetate is shown to be an almost universal solvent, having the ability to extract many different classes of pesticides from various commodities, resulting in thousands of analyses over the years. The loss of basic pesticides in acidic crops is one of the method's drawbacks. Extraction with ethyl acetate yielded higher recoveries for polar pesticides and was somewhat faster, easier, and less expensive to perform.[1] Other method is extraction with acetonitrile and purification with dispersive solid-phase extraction (d-SPE). Acetonitrile method consumes few amounts of sample and toxic solvents. There is no need for blending, filtration, large volume quantitative transfers, evaporation/condensation steps, or solvent exchanges. This is critical because each additional step complicates the procedure and therefore causes systematic and random errors. When using acetonitrile method of extraction to determine pesticides in fruits and vegetables, matrix effects are eliminated, allowing for high recoveries of target analytes.[5]

This study used a gas chromatography-flame ionization detector to assess two extraction techniques for evaluating and determining pesticides in different food matrices. One extraction method is ethyl acetate/methanol and the other is acetonitrile/toluene. The comparison of extraction methods was done on brinjal, tomato and grapes spiked with known concentrations of pesticides. The purpose of this study was to determine the percentage recovery of pesticides from fruits and vegetables and to find out which extraction method extracts more of pesticides, is recovery will be similar to both extraction methods for both of pesticides or will be specific to pesticides. Recovery have been studied on known concentration level in three selected matrixes.

METHOD AND MATERIALS

Chemicals: The chemical reagents and stock solutions that were employed (acetic acid glacial, acetonitrile, ethyl acetate, toluene, magnesium sulphate, methanol, sodium sulphate, and sodium chloride) were obtainedfrom codon biotech. Pvt. Ltd. The pesticides studied were - Chlorpyrifos and Cypermethrin. Codon Biotech Pvt. Ltd. provided the pesticides' standards, which were verified to be >90 percent genuine. Individual stock standard solutions were prepared in hexane at a concentration of 2g/L and kept hidden in the dark. The functional solutions were made with a 20mg/L concentration.

Specimen

The fruits and vegetables used in this study (brinjal, tomatoes, and grapes) were purchased from the local markets.The samples were stored and packaged in a fresh brown bag. To protect

the bag from humidity, a little packet of silica gel was placed inside. A known volume (100ul) from working solution of pesticides were spiked into the individual samples and the samples were collected on 1,3,5 and 7 days after spiking of pesticides.

Gas Chromatography/Flame Ionization Detector

Samples were analyzed using a NUCON Model 5890 Series GC with a capillary column and a Flame ionizing detector (FID). Samples were analyzed by direct injection of 1µL into an injection port maintained at 250°C. The FID was maintained at 230°C.

The GC oven temperature was initially held at 50°C for 2 minutes and then maintained at 250°-50°C where it was maintained for the remainder of the run with a column having head pressure of 20 psi. Nitrogen gas was used as the carrier gas. Calibration curves were created for each pesticide using standard dilutions of the spiking solution.

Sample Preparation and Ethyl Acetate Extraction

Fruits and vegetable samples were cut into small bits and blended. In a mortar and pestle, a 5g amount of homogenate material was measured and grinded with 1gm of sodium sulfate and 1gm of sodium chloride to produce a fine paste after being injected with 100uL of test solution. The macerated sample was mixed and homogenized with 10ml of ethyl acetate on mechanical shaker for 1hr. After that, the mixture was then centrifuged for 30 minutes at 5000rpm. By adding a pinch of activated charcoal to the supernatant and letting it overnight, the complete organic phase was cleaned out. Filter the solution and then evaporated at 50°C in oven. The residue was then redissolved in 1mL of methanol or acetonitrile. The solution was then introduced into the GC/FID apparatus in a volume of 1uL.

Sample Preparation and Acetonitrile Extraction

Fruit and vegetable samples were cut into small bits and blended. A 7.5 gm of homogenized sample of fruits and vegetables injected with 100µL of test solution was weighed and macerated with mortar and pestle into fine paste. The macerated sample was mixed with 7.5ml of acetonitrile containing 0.75µL acetic acid in a centrifuge tube. After that, 3g of $MgSO_4$ and 0.75gm of sodium acetate were also added. The tube was shaken forcefully for 4 mins and then centrifuged at 5000 rpm for 5 mins. Clean up procedure was done by adding 300mg of $MgSO_4$ and 50mg primary-secondary amine into the supernatant liquid extract. The extract was shaken for 20sec and centrifuged at 3000rpm for 5mins. The solution was then filter and evaporated at 40°C in oven. After that, the residue was redissolved in 1mL of toluene. The solution was then introduced into the GC/FID apparatus in a volume of 1uL. Extraction and analysis were done on the same day in fruits and vegetables samples to determine the percentage recovery for each sort of matrices under investigation at 0.01mg/kg concentration level.

The pesticide recoveries were evaluated by dividing the area under the peak of analyte from the spike samples to that of the standard solutions. Figure 1 shows chromatogram of a standard solution of chlorpyrifos pesticides (1µL), showing the retention time and area under peak of standard solutions. Recoveries were determined for spiked brinjal, tomato and grapes samples (100µL) obtained after extraction by ethyl acetate and acetonitrile methods. Recoveries (Table 1, Table 2 and Table 3) were above 50% for chlorpyrifos using ethyl acetate extraction method and above 30% in acetonitrile extraction method.

DISCUSSION

Extraction Technique Comparison

When pesticide-containing foods are extracted, the percentage transfer of residues into the solvent is determined by the polarity and solubility of pesticide compounds. A single clean solvent solution cannot yield satisfactory recoveries due to the enormous varieties in polarity and solubility presented in the chemicals examined. The best extraction method was chosen based on three criteria: recovery, extract purity, and the number of

pesticides extracted. Extracting solvents with greater polarities, such as acetonitrile, acetone, ethyl acetate, and others, are often used for extraction of pesticide residues with significant polarity variations from agricultural products of fruits and vegetables, according to a review of multiresidue pesticide methods.[5] Therefore, a comparative study was conducted to determine the recovery rate and extraction efficiency of pesticides from these solvents. Ethyl acetate was chosen because it is both polar and miscible with water, allowing for good penetration into plant cells. Furthermore, unlike halogenated solvents, ethyl acetate is anti- hazardous and has lower disposal costs. Ethyl acetate has proven to be a nearly universal solvent, with thousands of analyses demonstrating its capacity to remove many distinct kinds of pesticides from numerous commodities. Pesticides are extracted from fruit and vegetable samples using the acetonitrile extraction method, involves shaking with acetic acid–acetonitrile and salting out with sodium acetate and magnesium sulphate.[5] Because magnesium sulphate hydrate is extremely soluble in water, not only it binds water but also promotes the partitioning of pesticides into the organic phase. The approach involves combining the acetonitrile extract with PSA SPE sorbent and magnesium sulphate in a dispersive SPE cleanup.[6]

Ethyl acetate has the benefit of being partly immiscible with water, which eliminates the need for the addition of other nonpolar solvents to separate water from the extract. Sodium sulphate (Na2SO4) is commonly used in multi-residue method processes to improve polar component recovery. Chlorpyrifos was removed during the extraction and cleaning steps, yielding overall results of 50, 40, and 48 percent in brinjal, tomato, and grapes, respectively by ethyl acetate method rather than of acetonitrile method. The lesser recovery of chlorpyrifos in matrices was due to loss of pesticides in clean up stage of acetonitirile method8. So, it is concluded that the average recovery of chlorpyrifos is above 50% by ethyl acetate extraction method among the different matrics.

Acetonitrile isolates less lipophilic co-extractives than acetone and ethyl acetate, the acetonitrile method yielded cleaner extracts. Product coextractants, like photosynthetic pigments, was discovered to be the least abundant in the acetonitrile extract.[9] The recovery efficiency for non-polar compound (cypermethrin) extracted using two different methods and analysed using GC-FID was as follows: acetonitrile are superior to ethyl acetate.[10] The average recovery percentage of cypermethrin in spiked samples of brinjal, tomato and grapes were 66, 55 and 51 respectively with acetonitrile extraction method rather than of ethyl acetate method. The average recovery of cypermethrin is between 60-50% in different matrics by acetonitrile extraction method. Between the extraction methodsethyl acetate extraction was found time consuming and least suitable for isolation ofmulticlass pesticide residues from samples whereas acetonitrile offers advantages inextraction selectivity and compatibility with more diverse analytical techniques. Different components with a larger molecular size such as triglycerides and pigments are often found and must be removed to enable for a more precise measurement of specific threshold residues and to avoid adverseeffects on detecting equipment, irrespective of the extraction method employed. Polarity-based extraction separation is used in many clean-up processes, such as liquid-liquid partitioning and column chromatography. PSA columns, according to this study, provided the most effective clean-up, removing the greatest number of sample matrices interferences.[10]

The lesser extraction of pesticides into their main compounds was because their residues also contains metabolites or their degradration products having different physicochemical properties. Besides of this, the climatic conditions, nature of chemicals, and the application methods also influence the degradation behavior of pesticides. Variations in recovery of pesticides residues were also shown in different matrics. Among all the matrics, brinjal and tomato have the higher recovery rate than that of grapes samples. The overall lesser recovcery of pesticides by both the exraction methods was due to the lower concentartion of spiked pesticides solution. This research underlined the need of further

CHLOROPYRIFOS

	Recovery of Residue [%] Brinjal samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	78	34.7
3 days	46	26.2
5 days	37	21.6
7 days	34	12.9

CYPERMETHRIN

	Recovery of Residue [%] Brinjal samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	41.5	90
3 days	27	78
5 days	12.5	56
7 days	12.0	40

CHLOROPYRIFOS

	Recovery of Residue [%] Tomato samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	50.6	45.6
3 days	46.2	22.9
5 days	11	20.3
7 days	3	13.3

CYPERMETHRIN

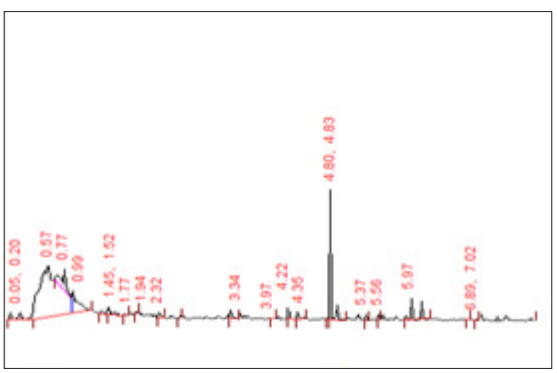
	Recovery of Residue [%] Tomato samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	37.9	86.8
3 days	18.1	78.1
5 days	16.3	24.4
7 days	10.4	24.0

CHLOROPYRIFOS

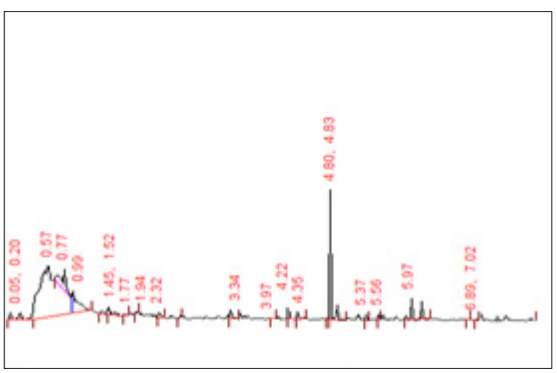
	Recovery of Residue [%] Grape samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	72	20.4
3 days	50	11.4
5 days	33	11.0
7 days	30	7.11

CYPERMETHRIN

	Recovery of Residue [%] Grape samples	
Days After Treatment	Extracted Sample with Ethyl Acetate Residue	Extracted Sample with Acetonitrile Residue
1 day	12.8	83.3
3 days	10.4	70.4
5 days	7.66	38.1
7 days	0.9	13.6



A



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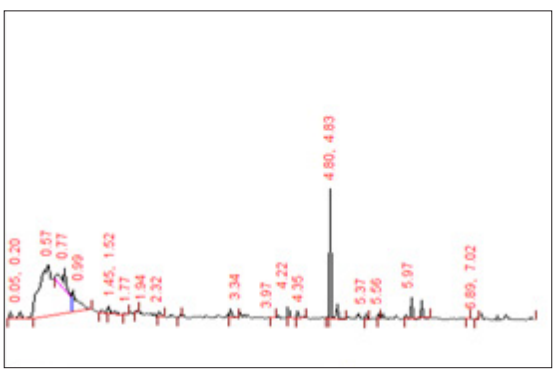

C

Figure 1: Chromotograms showing the recovery of pesticides using ethyl acetate extraction method
a) day 1 (Brinjal), b) day 3, c) day 5

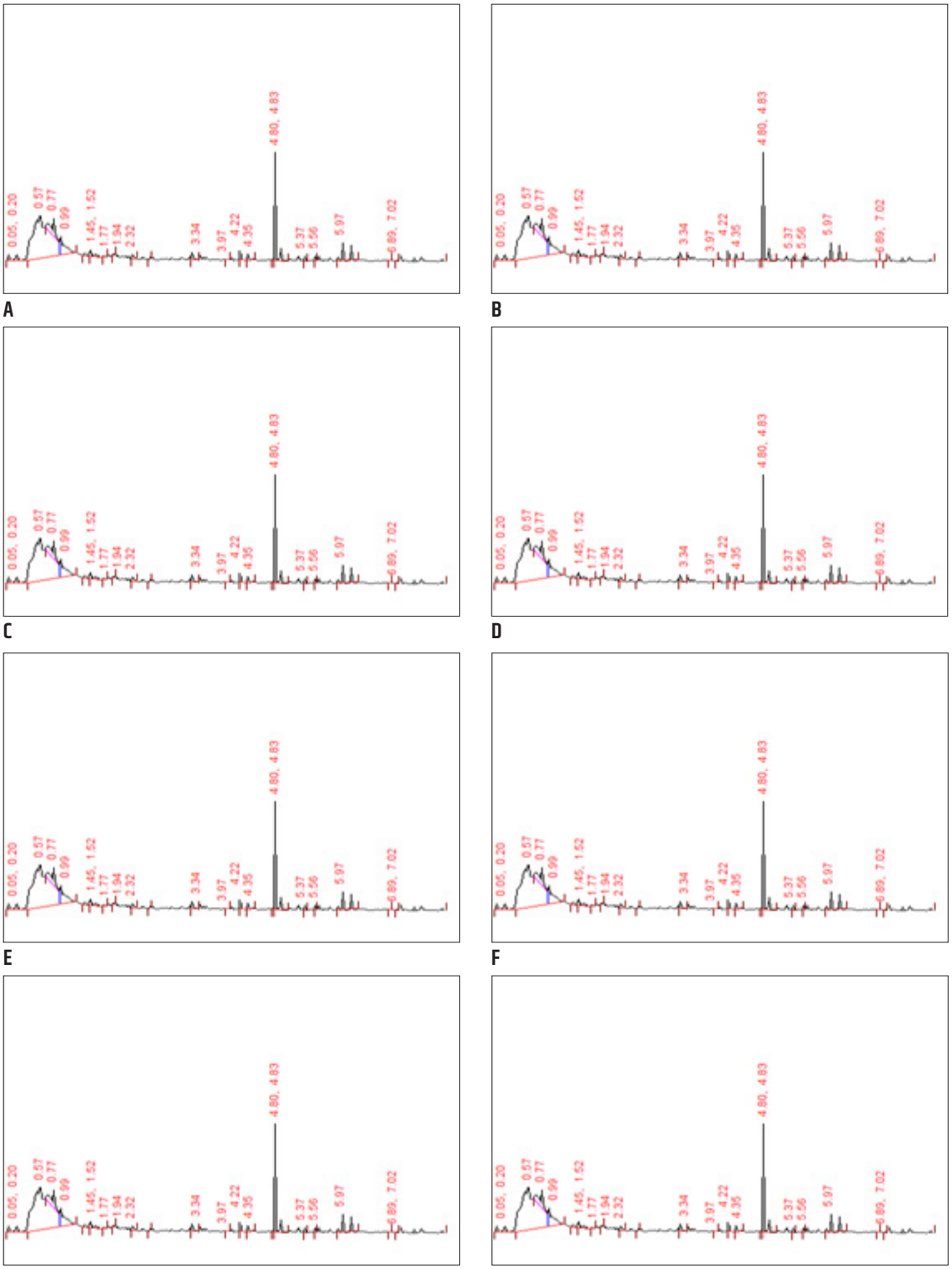

Figure 2: Chromtograms showing the recovery of pesticides using ethyl acetate extraction methods.
a) Day 1 (tomato), b) Day 3 c) Day 5, d) Day 1 (Grapes)
e) Day 3 f) Day 5

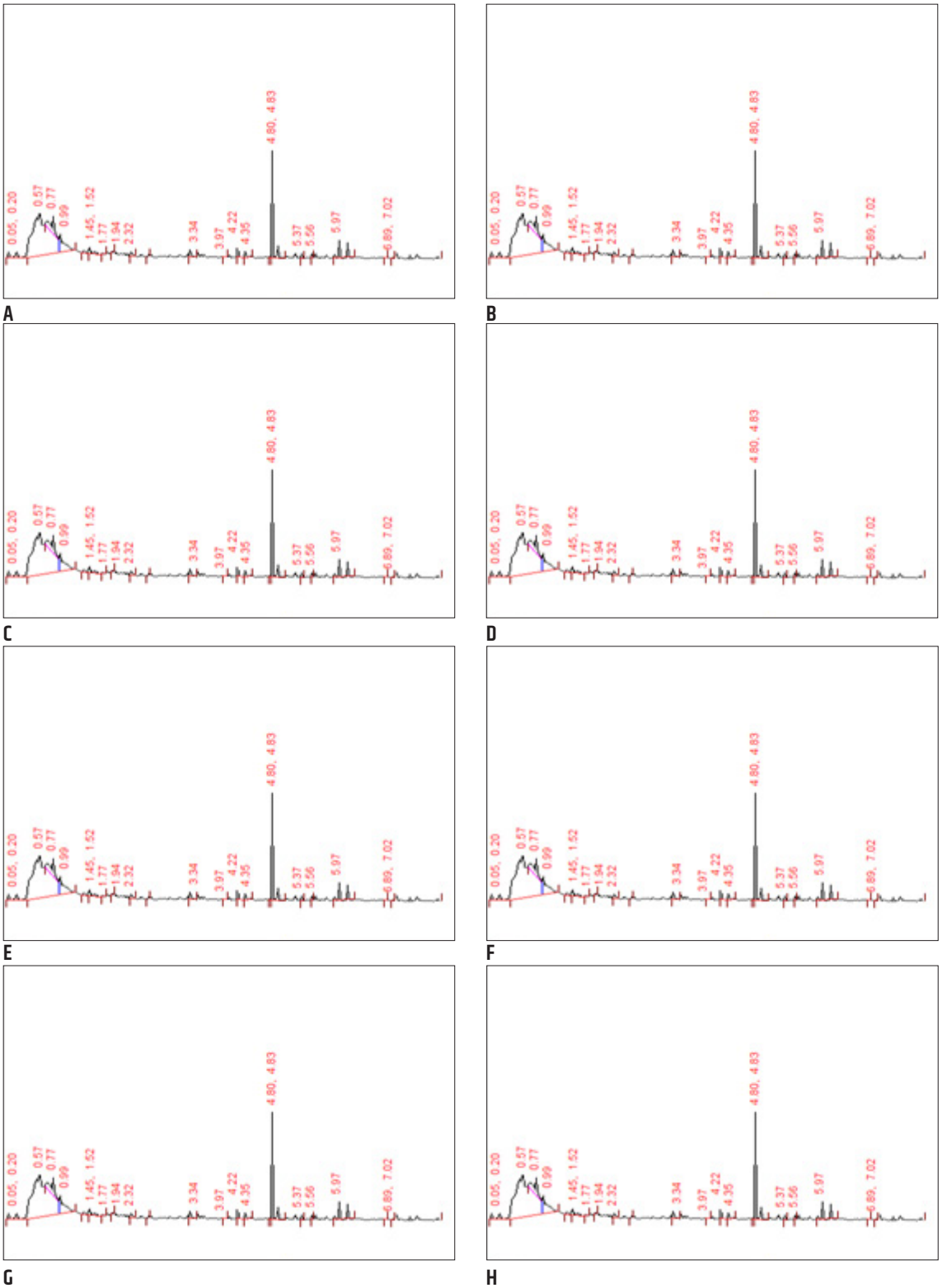

Figure 3: chromotograms showing the recovery of pesticides using acetonitrile extraction method. d) Day 1(Tomato), e) Day 3, f) Day 5

research such as: a) to asses the recovery rate of pesticides at higher concentrations, b) the validation of study by performing on more food matrics and with different analytical methods.

CONCLUSION

A multiresidue approach was used to undertake trace analysis of common pesticides that are commonly used in fruits and vegetables.Using ethyl acetate and acetonitrile, this approach uses a fast and non-selective extraction procedure. The pesticides under investigation were determined using gas chromatography with a flame ionization detector. This research also shows that this procedure is easy, quick, and adaptable to a variety of fruits, and vegetables with just modest amounts of solvent used each sample (10ml ethyl acetate, 7.5 ml acetonitrile). Brinjal, tomato, and grape samples were successfully extracted using the proposed multiresidue approach. Both acetonitrile and ethyl acetate are acceptable solvents for extracting pesticide residues from fruits and vegetables having a wide polarity range.It was proven clearly that Chlorpyriphos was extracted to a better extent by Ethyl acetate and Cypermethrin was extracted to a better extent by acetonitrile. There are numerous cleanup procedures. When compared to other standard multiresidue methods previously employed in the laboratory, the usage of multi-solvent is also a better and easier way. It can be concluded that extraction using acetonitrile gives more recovery that ethyl acetate in specific pesticides and vice versa. The extraction and cleanup processes developed are suitable for a variety of plant materials and can be used with a wide range of multiclass pesticide concentrations. IJFMP

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Conflict of Interest

The authors state that they have no known conflict financial interests or personal ties that could have influenced the research presented in this study.

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ORIGINAL ARTICLE

Digitopalmar Dermatoglyphic Traits: A Tool for Identifying Sports Talent

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ABSTRACT

CONTEXT: The modern sporting world emphasizes the detection and training of the sports talent as early as possible. Dermatoglyphics, the study of patterns in the skin ridges, has been used as a method to identify the medical conditions having a genetic basis. As the dermatoglyphs represent the development of the nervous system, they can be used to identify the innate potential of an individual towards sports.

AIM: This study attempts to compare both the quantitative and qualitative parameters of dermatoglyphics in sportsmen as compared to the controls.

SETTINGS AND DESIGN: A cross-sectional study was carried out at a university in Northern India.

MATERIALS & METHOD: The digitopalmar prints were taken with the help of Canon Lide 300 flatbed scanner and analyzed using SPSS software ver. 20).

RESULTS: A predominance of loop patterns was seen in most of the fingers of the sportsmen. It was found that the a-b, b-c and c-d ridge counts of both the left and the right hands were significantly lesser in case of the sportsmen as compared to the non-sportsmen. Also, the ATD angle of the right and the left hands seem to be greater in the sportsmen as compared to the controls which was not significant statistically.

CONCLUSIONS: It can be concluded that dermatoglyphics can be used as a tool to identify the sports talent by studying the characteristics features of the dermatoglyphics in a sportsman. However, further researches are required to strengthen a better understanding of the factors involved in determining the genetic potential of an individual.

KEYWORDS | digitopalmar, dermatoglyphics, sports talent, tool

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INTRODUCTION

One of the goals of the modern sporting world is the earliest possible detection and development of the sports talent.[1] Sports talent can be defined as the individual who through inherited or acquired properties has a unique disposition for sports performance, above the general population.[2]

In the field of sports, the performance of the sports person depends on numerous factors like physical dimensions, psychology, training, planning, tactics etc. These factors influence but do not modify the inherent sporting ability of an individual determined by the genetics.[1]

Detection of sports talent has been traditionally done through observations of trainers during sports events which can be erroneous in nature due to many confounding factors.[3] This can be eliminated by the use of genetic markers for the early detection of sports talent.[4] Genetic markers are those genes that code a specific feature and manifest on biochemical level, which takes the form of a visible external feature. The examples of these can be blood groups, iris color, dermatoglyphics

and so on.

Dermatoglyphics, which is a study of the patterns of the skin ridges on human palm, fingers, toes and soles, has been used extensively for the purpose of identifying various medical conditions having a genetic predisposition.[1] The unique characteristic of these patterns is that they reflect the normal growth processes of the hands and feet which are congruent with the development of the nervous system because of the common ectodermal origin. These dermatoglyphic grooves or ridges can be classified into loops, arches and whorls which represent them qualitatively whereas, the ridge counts account for the quantitative analysis.[2] Once formed, these are resistant to the environmental changes and thus reflect the growth disturbances that happen before or during their development.[5] Through the use of dermatoglyphics, one can not only determine the sports performance but also help in channelizing the time, effort and money in a right direction.

Martins used dermatoglyphics for identification of genetic physical characteristics of soccer players. They came to a conclusion that dermatoglyphic patterns can be used as a parameter to identify the potential of an individual which can help in selection of sports talent.[2]

Serhiyenko compared the dermatoglyphics of feet for sportsmen and non-sportsmen. They stated that foot dermatoglyphics can be used as a genetic marker for children having inclination to sports, and hand dermatoglyphs are more informative than foot dermatoglyphs for prognosis of motion abilities. They also concluded that future researches need to be carried out for identification of differences of foot dermatoglyphics in sportsmen of different sports.[4]

Tanwar studied the dermatoglyphic patterns in distal phalanges of sportsmen and non-sportsmen and found a significant difference in the patterns between males and females of these categories. They stressed that the general traits of an individual are reflected in their dermatoglyphic patterns and though these are not inherited as simple Mendalian traits, they depend on genetic makeup.[6]

Borin tried to understand the distribution of quantitative dermatoglyphic indicators in basketball players according to their performance as compared to non-players. They stated that some of the fingerprint indicators should be studied between sports that use hand, owing to possible relationship between dermatoglyphics and the prehensile functions of the hand.[3]

Serhiyenko studied sole dermatoglyphics in different sportsmen and concluded that children with athletic inclination have larger quantity of loops on great toes, those with a bent to speed power sports have smaller ridges on both feet and feet dermatoglyphics is a less reliable for prognostication of anerobic-aerobic kinds of sports.[7]

Yadav compared the atd angle between physical education and non- physical education students and came to a conclusion that the atd angle was smaller in physical education students as compared to the non-physical education students, however, this difference was not found to be significant.[1]

The aim of the present study is to find any differences in the digitopalmar dermatoglyphic traits in sportsmen and non-sportsmen in order to validate the use of the dermatoglyphic traits as an indicator for sports talent.

MATERIALS AND METHOD

A total of 142 individuals from Teerthanker Mahaveer University, Moradabad, were approached out of which those who met the inclusion criteria further proceeded for the study (N=84). The inclusion criteria being: individuals participating professionally in sports, having no injury or neurological insufficiency of the hand and those willing to participate in the study. The individuals having a history of genetic disorders like hypertension, cardiac disease, bronchial asthma, etc., were excluded from the study. The control group contained

CATEGORY	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)	Lab Mean (SD)
Sportsmen	37.12 (4.30)	36.68 (4.78)	35.79 (4.97)	36.64 (4.58)	35.81 (4.90)	36.17 (5.05)	46.43 (5.57)	47.62 (5.15)
Non-Sportsmen	37.12 (4.30)	36.68 (4.78)	35.79 (4.97)	36.64 (4.58)	35.81 (4.90)	36.17 (5.05)	46.43 (5.57)	47.62 (5.15)
P-Value	0.000	0.000	0.000	0.000	0.000	0.000	0.600	0.237
Significance	Significant	Significant	Significant	Significant	Significant	Significant	Non-Significant	Non-Significant

Table 1: Descriptive statistics of Quantitative parameters of dermatoglyphs

CATEGORY	L1	L2	L3	L4	L5	R1	R2	R3	R4	R5
Sportsmen	Loop	Loop	Loop	Loop	Loop	Loop	Loop	Arch	Loop	Loop
	40.5%	47.6%	45.2%	38.1%	57.1%	52.4%	42.9%	50%	42.9%	50%
Non-Sportsmen	Whorl	Loop	Loop	Whorl	Loop	Loop	Loop	Loop	Loop	Loop
	42.9%	47.6%	45.2%	38.1%	57.1%	52.4%	42.9%	50%	42.9%	50%

Table 2: Highest Frequency of the qualitative parameters of dermatoglyphs

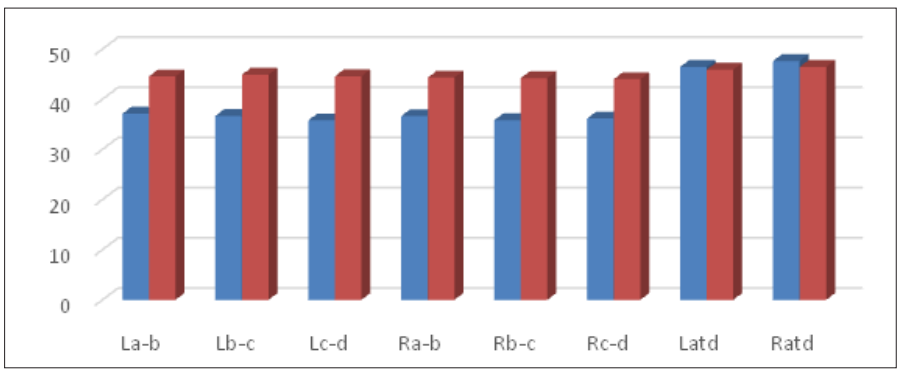

Figure 1: Descriptive statistics of Quantitative parameters of dermatoglyphs

equal number (n=42) of non-sportsmen. Age was not considered an inclusion criterion as the dermatoglyphic traits once developed remain unchanged throughout the life. However, the subjects in the control group were matched as much as possible according to the gender of the sportsmen group because the characteristics of dermatoglyphs vary based on gender.

The purpose and procedure of the study was explained to the participants and a written informed consent was taken from those who were willing to participate. The subjects were made to wash and dry their hands thoroughly before taking the hand prints. A Canon Lide 300 flatbed scanner was used to take the scans of the fingers, thumb and palms individually. The subjects were asked to put the fingers, thumb and palms of both the hands lightly on the flatbed of the scanner without overpressure. The scans were captured and stored for future analysis. An app was used to measure the atd angle from the digital scans of the palms. The method of fingerprint analysis used was that as proposed by Cummins and Midlo (1961). The data was recorded and statistically analyzed using SPSS (version 20) software for the interpretation.

RESULTS

Upon analysis of the recorded data, it was found that the mean age of the Sportsmen was 25.50±5.07 and that of non-sportsmen 21.14± 1.60. There were 35 males and 7 females in the sportsmen group and 29 males and 13 females in the non-sportsmen group. Out of 42 subjects, 37 were right-handed in the sportsmen group and 39 in the non-sportsmen group. In the Sportsmen group 23.8% played cricket, 14.3% volleyball, 11.9% badminton, 11.9% kabaddi and 9.5% constituted the athletes. It can be seen from the Table 1 above that the a-b, b-c and c-d ridge counts of both the left and the right hands

are significantly lesser in case of the sportsmen as compared to the non-sportsmen. Also, the atd angle of the right and the left hands seem to be greater in the sportsmen as compared to the controls which is not significant statistically.

The dermatoglphic patterns which were found to be of highest frequency in the fingers of the two hands are depicted in Table 2. It can be seen that the loop patterns emerge as the patterns of greatest frequency in the fingers of the sportsmen as well as non-sportsmen but with a distinct distribution of the pattern in different fingers.

DISCUSSION

This study was conducted to find the differences in the quantitative as well as qualitative digitopalmar dermatoglyphics of the sportsmen as compared to those of non-sportsmen.

The analysis of the data showed that, the mean age of the Sportsmen was 25.50± 5.07 and that of non-sportsmen 21.14 ±1.60. Here we have not taken age as a classifying feature because once formed these dermatoglyphic patterns do not change but remain same throughout the life. So, these prints do not reflect the changes happening because of the ageing or the growth process and thus are independent of the age.

There were 35 males and 7 females in the sportsmen group and 29 males and 13 females in the non-sportsmen group. The two groups were tried to be gender matched to the maximum possible extent. This was done to eliminate the gender bias in the pattern characteristics which has been confirmed in the earlier researches.

A heterogenous group of sportsmen were taken as subjects who played sports involving strength, agility, flexibility, endurance, prehensile functions, power etc. Similar selection of subjects is seen in the research carried out by Serhiyenko L.P., where the sportsmen were classified into groups dominating speed-power orientation, anaerobic-aerobic abilities and aerobic energy supply in competitions.[7]

The present study reflects a significantly lesser number of the a-b, b-c and c-d ridge counts of both the left and the right hands in case of the sportsmen as compared to the non- sportsmen. The previous studies have taken into account only the a-b ridge count as a quantitative parameter and this is the first study to our knowledge which has tried to correlate all the three ridge counts with the inherent ability of an individual towards sporting events.

When the differences in the atd angles of both the hands were compared it was found that atd angle of the right and the left hands seem to be greater in the sportsmen as compared to the controls which was not significant statistically. This is similar to the study done by Borin, where the atd angle (of both the hands) of the players was found to be greater or equal to that of the non-players.[3]

CONCLUSION

The selection and training of sports talent cannot be based on experience or experimentation, but has to be based on scientific grounds. This selection should take into account the genetic potential of an individual towards the sporting ability. This knowledge in combination with the correct and timely training techniques creates a conducive environment for a sports person to perform at an optimal level. This study highlights the use of dermatoglyphics as a tool to identify the sports talent by bringing out characteristic quantitative and qualitative features of the dermatoglyphs in a sportsman. However, further researches are required to strengthen the better understanding of the factors involved in determining the genetic potential of an individual.

It is proposed to conduct researches involving a specialized sport, to consider differences based on gender, and study other dermatoglyphic patterns not only in hands but feet also. IJFMP

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ORIGINAL ARTICLE

Diatomological Mapping of Water Bodies of Villages of Karnal, Haryana

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ABSTRACT

Context: Diatoms are unicellular, photosynthesizing algae phytoplankton; they are single-celled aquatic algae. Diatoms are one of the largest group of organisms on earth and are found in different shapes and sizes, but diatoms are easily decipherable due to a unique its cell wall structure. The cell walls possess a characteristic silica coating, which is resistant to decay. It helps them maintain their shapes as it contains a large amount of brittle but hard silica, which is hydrated (SiO_2H_2O) and noncrystalline. Diatoms have about 200 genera and 1,00,000 species differing in their structure. Diatoms are found at well-lit places as well as in moist conditions. Hence, they perform photosynthesis. Their small size helps them to penetrate human tissue and hence the forensic significance. Particularly in forensic science diatoms help in identifying the drowned dead body of a person and to distinguish between anti-mortem and post-mortem drowning.

Aim: The present study aims to identify the species of diatoms in different water bodies of Karnal (villages) and to identify the species which are site-specific and are found in a specific water body. The positive identification of site specific diatoms will help us to estimate the site of submersion in case of drowning deaths.

Materials & Method: In the present study we examined diatoms in water bodies located in the villages of Karnal district of Haryana. For this study we collected water samples from 7 different water bodies including canals, rivers, ponds, lakes, and borewells. The samples were centrifuged and diatoms were concentrated. Microscopic slides were prepared and observed under compound microscope at 100X magnification. Diatoms were inspected for their morphological types and for mapping purposes.

Results: Various diatom species including Actinocyclus sp., Cocconeis sp., Rhoicosphenia sp., among others were recovered. Some diatoms were found to be site-specific and others found to be common in all water bodies.

KEYWORDS | diatom mapping, villages, haryana, drowning cases, forensic investigation

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INTRODUCTION

DROWNING IS A FORM OF MECHANICAL asphyxia caused by inspiration of fluid into respiratory tract due to submersion of body (nose and mouth) into water or liquid.[4] When a dead body is recovered from water, there arises a question whether the person was alive or dead before entering the water body. And here lies the importance of the diatoms. The principle behind the diatom test is that diatoms are present in every water body. Diatoms are used to differentiate between ante-mortem and post mortem drowning. If the person had entered the water body while he was still alive and the respiratory system was

functional which lead to the entry of diatoms into the lungs of the person. These diatoms then travel to the distant organs of the body like brain, kidney, bones etc with the flow of blood pumped by the heart.[3] While in case of post-mortem drowning, the person is already dead before entering the water source. Hence prevents the entry of diatoms to the distant organs. On the other hand the decree on tracing accurate place of drowning is an important part of medico-legal investigation. The question about the place of drowning arises when the true drowning tookplace is not clear. Usually, when a dead body rises from the bottom of water source to the surface, it will be seen near to the site where it had actually drowned but water current may soon carry the body to some other place from the original place of drowning. Forensic diatom testing plays an crucial role in the comparison of diatoms isolated from dead body and collected reference water samples to establish whether drowning was the cause of death or not and in establishing the precise site of drowning.[2]

MATERIALS AND METHOD

Haryana: Haryana is a state in northern India that separated from Punjab on Ist November, 1966. Haryana is surrounded by five states namely Punjab, Rajasthan, Himachal Pradesh, Uttar Pradesh and Delhi.[1]

Karnal: Karnal is a part of the National Capital Region (NCR) and situated in the north-central part of Haryana and is the administrative headquarters of Karnal district.

Geographical Area and Location: Karnal is covering an area of 2,471sq.km. The district covers 5.69% area of the state. Karnal District is bordered by Kurukshetra, Jind & Kaithal, Panipat.

Physiographic Divisions: Karnal is a part of Indo-Gangetic plains and is irrigated by a network of Yamuna canals. The district is divided into three regions namely: Khadar, Bangar and Nadrak. The Khadar region are a low-lying river plains lying next to the river. Bangar lies next to Khaddar. Nadrak region lies beyond, with saline water.

Collection of Water Samples:
We collected water samples from 7 different freshwater bodies of villages of Karnal namely Pakka Pull, Karan Tal, Karan Lake, Yamuna river, Atal Park, Kalampura village and Gagsina village. A leak-proof plastic bottle was immersed to the maximum possible depth with its mouth opened. Then, the bottle was dipped in water so that water can enter in it. When the bottle was filled up to the neck it was taken out and closed tightly. The containers were marked and numbers were allotted along with all the relevant details. We collected samples in the month of August 2020.

Laboratory analysis: The present study was conducted in the Biology Division of Forensic Science Laboratory, Madhuban, Karnal (Haryana), India. Centrifugation machine, compound microscope, hot plate were used.
Treatment of water sample: After taking the sample to the laboratory the bottles were kept undisturbed overnight so that diatoms in water sediment at the base of the bottle (being heavier). On the next day, approximately half of the water from supernatant of the bottles were discarded and samples left were mixed well. Clean and sterile beakers were used for the analysis.
Isolation of diatoms:
Clean and sterile beakers, droppers, centrifuge tubes were used to avoid the chances of contamination of the samples. Water samples were then poured into cleaned micro centrifuge tubes (50ml). These tubes were labeled accordingly. The samples were then centrifuged at 2800rpm for 10 minutes. The supernatant was than discarded and the remaining sample was added with water from respective beakers and centrifuged again for 10 minutes at 2800 rpm. The process was repeated 3 times: discard supernatant, add more sample, centrifuge.

SAMPLE NO.	PLACE OF COLLECTION	DATE AND TIME PF COLLECTION	TYPE OF WATER SOURCE	VOLUME OF WATER COLLECTED
1	Pakka pull, (Madhuban)	18 Aug 2020 (2:10 am)	River	1 lt.
2	Kalampura Village	20 Aug 2020 (8:20am)	Canal	1 lt.
3	Gagsina Village	21 Aug 2020 (7:30 am)	Pond	1 lt.
4	Karan Park	21 Aug 2020 (8:20 am)	Pond	1 lt.
5	Yamuna River	23 Aug 2020 (6:15 am)	River	1 lt.
6	Atal Park	25 Aug 2020 (6:30 am)	Pond	1 lt.
7	Karan Lake	25 Aug 2020 (5.30 am)	Lake	1 lt.

Table 1: Sources of water samples

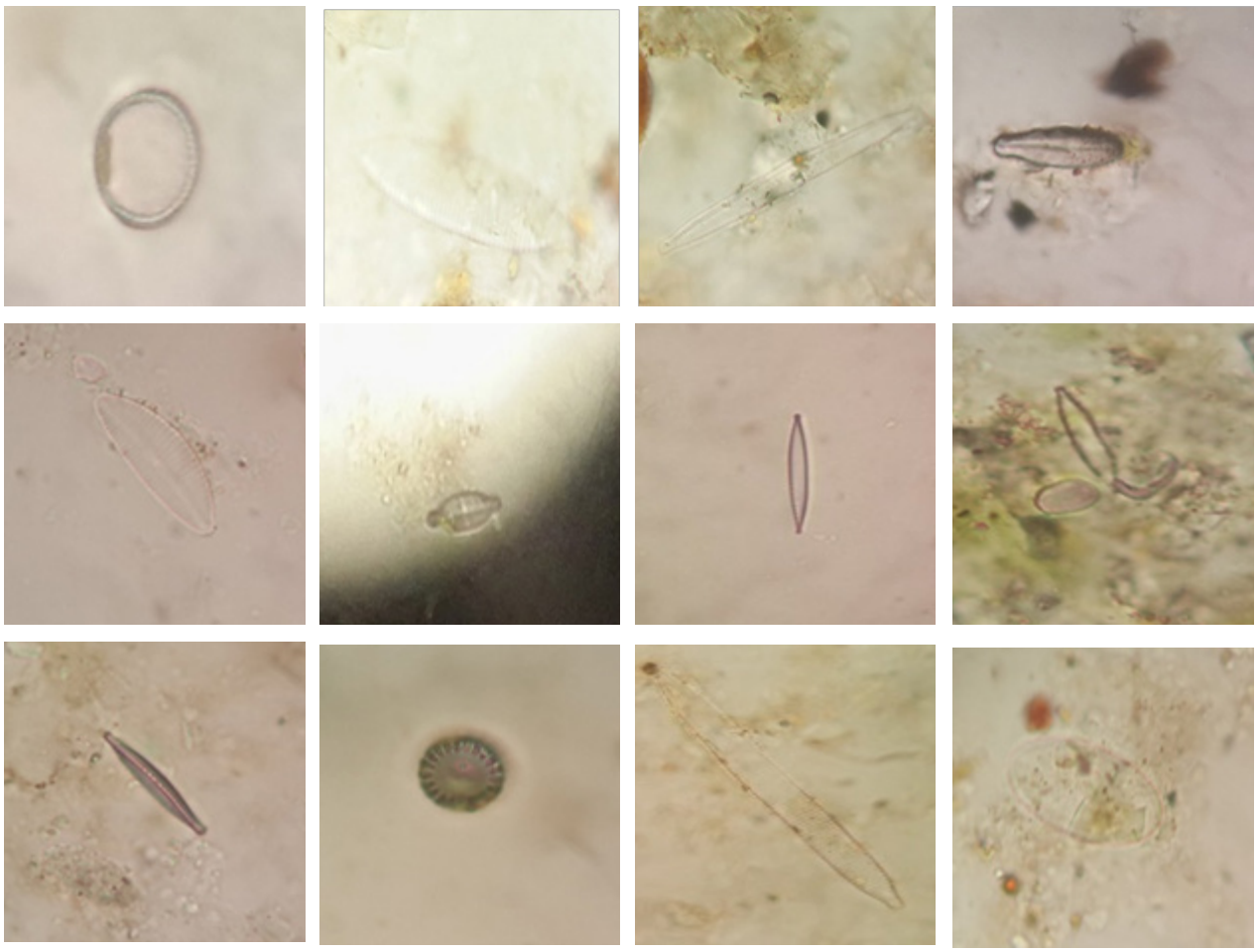

Figure 1: Microscopic view of Diatom species under compound microscope at 100X magnification from left to right - Actinocyclus sp.; Cocconeis sp.; Rhoicosphenia sp.; Opephorepacifica sp.; Cymbella sp.; Placoneis elginensis; Nitzschia sp.; Eucampia sp.; Rhoicosphenia sp.; Cyclotella sp; Navicula viridula; Coconeis peduculus; Cymbella tumida; Rhoicosphenia sp.; Sallaphora popul.

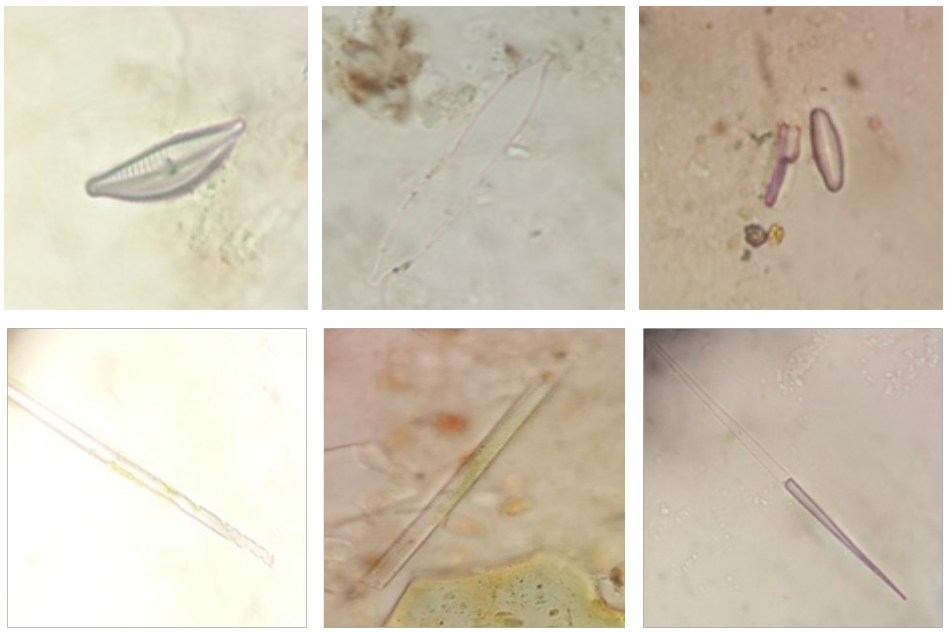

Figure 2: Microscopic view of diatom species which remained unidentified under compound microscope at 100X magnification.

At last, the supernatant was discarded and sediment pellet were used for preparing slides.

Preparation of Slides:

A drop of sediment from base of the tube was placed on cleaned microscopic slide. The slide was placed on a hot plate at 1000 C for 1-2 minutes to remove the extra moisture and then cooled at room temperature and mounted with DPX and cover slip. A drop of oil was placed on the slide and slides were examined at 100X (oil immersion) magnification. Each slide was thoroughly examined.

RESULTS

In the present study 7 water samples were analyzed and different species of diatoms were observed. Some of the species found were common in all 7 water bodies under study and some are common in a few water bodies while others are site-specific as found in the present study.

The results of present study revealed diatoms of *Actinocyclus sp.; Cocconeis sp.; Rhoicosphenia sp.; Opephorepacifica sp.; Cymbella sp.; Placoneis elginensis; Nitzschia sp.; Eucampia sp.; Rhoicosphenia sp.; Cyclotella sp;Navicula viridula; Coconeis peduculus; Cymbella tumida; Rhoicosphenia sp.; Sallaphora popul.* Many of diatoms are found to be common in all water samples while others are found to be site-specific.

DISCUSSION

As shown in the results, the species of diatoms discovered through the present study varies from one water body to another which was supported by earlier studies (Vandna V 2012, 2013). It was also found that diatoms in lake waters were different from diatoms of the canal and other water bodies. In lake waters (sample 7) both of the diatoms discovered belonged to order centrales while the diatoms discovered in park water (sample 6) belonged to order pennales. Mixed type of diatom flaura is found in other water bodies (sample 1,2,3, and 5). Diatoms of *Cymbella sp.; Navicula sp.;*

Nitzchia sp. were commonly found in sample 1, 2, and 3. While the diatoms of other species i.e. *Stephanocyclus sp., Actinocyclus sp., Eucampia sp.,, Rhoicosphenia sp.* were found to be site-specific.

It was also observed that in water bodies examined that a majority of diatoms discovered belonged to order pinnate and some to centrales. Pennates diatoms include *Peronia sp.; Naviculavirdula; Cymbellatunida; Rhoicosphenia sp.; Nitzchia sp.* While *Actinocyclus sp.; Stephanocyclus sp.; Cyclotella sp.* belonged to order centrales. This study was made in the summer season hence many other species can be found depending on the weather conditions and temperature.

Four diatoms remained unidentified, these unidentified diatoms in present study further increases the range of diatomological studies in water bodies of Haryana and also in different seasons.

CONCLUSION

From the findings of this study, it could be stated that many of diatom species are site specific and a database made from these findings is a valuable tool in tracing the exact place of drowning cases and can assist in legal investigation. Diatoms can be extracted from the body of the deceased and can be identified with the water sample in which the body was discovered. This can help in determining antimortem and post mortem drowning and to find out the primary and secondary crime scene. This approach will help in reconstruction of the crime sceneand events. There is a need to further carry out such studies in other water bodies in different season to build up a data base of diatoms. IJFMP

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ORIGINAL ARTICLE

To assess the Anatomical Variations of Lingual Foramen and its Bony Canals Using CBCT in Relation to Age and Gender

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ABSTRACT

CONTEXT: This retrospective study was conducted in the Department of Centre for Advanced Imaging, I.T.S. Dental College. The sampling method was consecutive and the sample size of 100 CBCT scans were included in which 50 scans were of male patients and 50 of female patients. Scans covering mandibular anterior region for preoperative implant placement planning were included. Scans of the patients within the age group of 20-60 years and those who were referred to the department for mandibular anterior region scan and scans done using the same acquisition parameters were included in the study. Different observations were assessed on multiple sections and the section which had greatest dimensions was selected for variables measurement. The effect of patient age and gender on the dimensional measurements of the various anatomical landmark were accessed. **AIM:** To assess the anatomical variations and locations of lingual foramen and its bony canals by using CBCT in relation to age and gender. **MATERIALS AND METHOD:** Through CBCT examinations, 100 patients were carefully examined in the median region of the mandible in order to detect the lingual foramen and their corresponding vascular canals. Their presence, number, position, diameter, morphology and trajectory were established. We also evaluated the effect of patients' age and gender on the dimensional measurements of the anatomical landmark mentioned above. **STATISTICAL ANALYSIS:** The statistical analysis was done using statistical software SPSS version 16.0. comparison of the means of various parameters for categorical and numerical data was done by chi-square and unpaired t test. Inter- observer agreement was assessed with Cohen's kappa test and interclass correlation. **RESULTS:** All of the 100 CBCT scans taken showed the presence of lingual foramen. Of all the participants, 52% of them had two foramina in their images whereas 43% have single foramen and remaining 5% have three foramina. The most common trajectory was downward in 61% of the cases. The mean diameter of lingual foramen canal at buccal end in male and female was 0.42 mm and 0.37mm respectively, average diameter at lingual end in male and female was 0.60 mm and 0.54mm respectively. **CONCLUSIONS:** As the variations were shown in lingual foramen characteristics, is mandatory to be aware of the structures present in anterior mandible to prevent the surgical complications. CBCT appears to be an ideal imaging modality to assess these variations that must be taken into consideration prior to treatment planning. There was also an age and gender related significant difference in the diameter of canal. So, the morphology of lingual foramen and it canals can be used to evaluate the age and gender of an individual which can open a new vista in the field of forensics. **KEY MESSAGE:** CBCT assessment of morphological features of the alveolar bone and locations of nerve canals and foramina in the anterior region of mandible represent useful anatomical information about the inter-foraminal region. This information is a useful guide to the dentist before surgery and also lingual foramen have some morphological variations compared to male and female. This study should be done on larger population in future which can open new vistas in the field of forensics.

KEYWORDS | Cone beam computed tomography, lingual foramen and lingual canal

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INTRODUCTION

It is well known that anterior mandible contains several anatomical landmarks such as intra bony vascular canal, which is named the mandibular incisive canal (MIC) and lingual foramen.[1] Interforaminal mandibular region is considered relatively safe for the dental implant treatment.

Knowledge of lingual foramen and their bony canal dimensions and locations are important to be considered during anterior dental surgery (implant placement, genioplastic or grafting procedures) for avoiding various complications.[2,3] There are many unnamed accessory foramina present in the mandible, especially on the lingual side which are variable in their distribution and may be of significance in relation to the effectiveness of local anesthetic solutions administered for dental procedures.[4]

In Sutton's survey of 300 mandibles, the foramen was present in 85% of the mandibles examined and Shiller & Wiswell found a median lingual foramen in 88.9 % in 126 specimens.[4,5]

Ennis demonstrated a foramen located superior to the genial spines. Suzuki and Sakai mentioned that the bony canals of these foramina coursed perpendicular to the inner surface of the mandible. Their reported frequency was 87% for the foramen supraspinosum, 68% for the foramen interspinosum (superior genial spinal foramen) and 26% for the foramen infraspinosum (inferior genial spinal foramen).[6,7,8] McDonnell *et al.,* reported that the foramina are in the midline on the lingual side of the mandible, at or above the genial spines.[9] Only Gahleitner *et al.,* characterized the diameter of the canals and the distance between their foramina openings and the mandibular border.[10]

The foramina located below the genial spines are denoted as inferior genial spinal foramina and contain sometimes the branches of sublingual artery and vein and sometimes the submental vessels and branches of mylohyoid nerve.[11]

Computed tomography (CT) has the ability to evaluate bony structures on the axial, coronal and sagittal sections. It has low radiation dose and capability of showing coronal, sagittal and axial planes in addition to lower cost for the patient making it a preferred imaging modality for assessment of bony anatomical structures.[12]

Along with the surgical guidance, the assessment of variation of lingual foramen and its canals can also be helpful in the determination of gender and age. Several studies have shown the efficacy of various foramen and canal evaluation in the determination of gender and age.[13] However, to the best of our knowledge no study has been done to evaluate the variation of lingual foramen in relation to age and gender.

Thus, the purpose of this study was to assess the anatomical variations of lingual foramen and location of lingual foramen and its bony canals by using CBCT in relation to age and gender. Their presence, number, position, diameter, morphology and trajectory were established. We evaluated the effect of patient's age and gender on the dimensional measurements of the anatomical landmark mentioned above.

METHODS AND MATERIALS

In this retrospective study conducted at the Centre for Advanced Imaging, I.T.S Dental College, the sampling method was consecutive and the sample size of 100 CBCT scans of which 50 scans were of male patients and 50 of female patients. The data were obtained retrospectively from the data bank of Centre for Advance Imaging, Department of Oral Medicine & Radiology between 2015 and 2017. Ethical clearance was obtained for utilizing the scans for this study. Scans covering mandibular anterior region for preoperative implant placement planning were included. Scans of the patients within the age group of 20-60 years and those who were referred to the department for mandibular anterior region scan and scans done using the same acquisition parameters were included in the study. The scans of the patients with any bone pathology in mandibular anterior region or mandibular fracture or history of trauma, or artifacts or

scans of patients with any genetic abnormality or syndromes affecting the mandibular anterior region were excluded from the study.

All the CBCT scans were taken retrospectively from the CBCT unit (NewTomGiANO) using a standard exposure and patient positioning protocol and analyzed further using NNT software in ambient light on a Dell workstation. All observations were assessed on multiple sections and the section which had greatest dimensions was selected for variables measurement. (Figure 1). Two reference parallel lines were drawn for measurements. One line was drawn passing through the alveolar crest and second one passing through the inferior cortex of mandible. Inter observer reliability was calculated by Cohen's Kappa and inter class correlation was used to check the agreement between the two examiners.

Basic observations used in this retrospective study on sagittal section consisted of (fig. 1):

- Number of lingual foramen and its canals.
- Position of lingual foramen with relation to genial tubercle.
- Trajectory of lingual foramen canal.
- Length of lingual foramen canal.
- Diameter of lingual foramen canal at buccal and lingual end.
- Distance between the terminal end of lingual canal at the buccal and lingual side from the inferior border of mandible and alveolar crest.

The participants were divided into two age groups: Group A (20-40 years) and Group B (41-60 years). Afterwards, the effect of patient age and gender on the dimensional measurements of the anatomical landmark mentioned above was evaluated.

All the data were collected and entered into an Excel sheet. The statistical analysis was done using statistical software SPSS Version16.0. The descriptive statistics, mean and standard deviation of different parameters were calculated. Comparison of the means of various parameters for categorical and numerical data was done by chi-square and unpaired t test. Inter-observer agreement was assessed with Cohen's kappa test score and interclass correlation. The level of significance and Confidence of Interval (CI) were 5% and 95%.

RESULTS

The mean age of the patients was 43.51± 12.9 years. The inter-class correlation coefficient was found to be between 0.838-0.935 which showed a good agreement.

The Cohen's Kappa (*k*) was found to be between 0.849-0.905 for number of lingual foramen, position and trajectory which showed an excellent agreement between the examiners.

Lingual foramen was observed in all 100 patients (100% of the cases). Two foramina were observed in 52% of cases whereas single foramen was observed in 43% of the cases. The remaining 5% of cases had three foramina. On comparing the distribution of number of lingual foramina among males and females, using chi square test, no significant difference was found (Table 1).

Out of 79 foramina found in males, 44 were superior to genial tubercle, 34 were inferior to tubercle and only single foramen was at the level of genial tubercle.In females out of 83 foramina,45 was superior to genial tubercle, 38 were inferior and none were found at the level of tubercle. On comparing the distribution of Position of lingual foramen between males and females, using the Chi-square test, no significant difference was found. (Table 2)

Out of 79 foramina found in males, 48 were downward trajectory, 28 were upward and 3 canals werehorizontal. In females out of 83 foramina, 51were downward trajectory, 30 were upward and 2 canals were horizontal. On comparing the distribution of Trajectory of lingual foramen between males and females, using the Chi-square test, no significant difference was found. (Table 3)

The average length of lingual foramen canal in males was 6.31mm (SD 2.22) and in females was 6.11mm (SD 2.34). On comparing the mean Length of lingual foramen canal between males and females, using the unpaired t-test, no

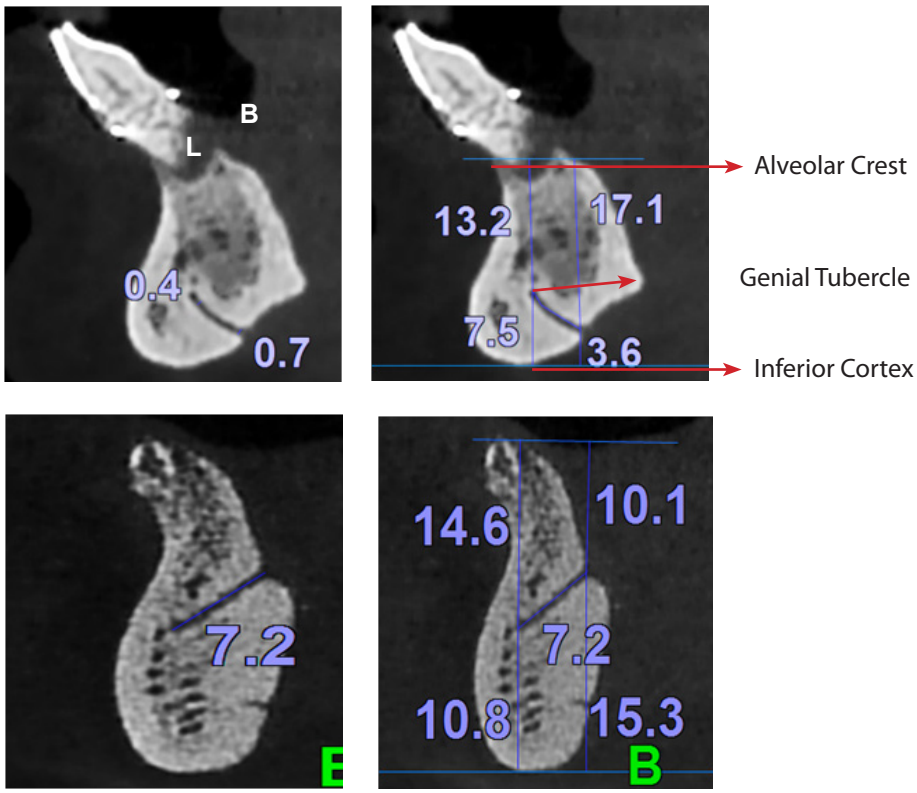

Figure 1 Dimensional measurements on axial sections. **A**. Diameter of lingual canal at buccal and lingual end. **B** and **D**. Distance between lingual foramen to alveolar crest and inferior cortex of mandible at buccal and lingual end. **C**. Length of lingual foramen canal.

No. of lingual Foramen	Gender		Total
	Male	Female	
1	23 46.0%	20 40.0%	43 43.0%
2	25 50.0%	27 54.0%	52 52.0%
3	2 4.0%	3 6.0%	5 5.0%
Total	50 100.0%	50 100.0%	100 100.0%

Table 1: Distribution of number of lingual foramen in relation to gender
Notes: Chi-square test, # Non-significant difference

Position	Gender		Total
	Male	Female	
Superior to genial tubercle	44 55.7%	45 54.2%	89 54.9%
Inferior to genial tubercle	34 43.0%	38 45.8%	72 44.4%
At level	1 1.3%	0 0.0%	1 0.6%
Total	79 100.0%	83 100.0%	162 100.0%

Table 2: Distribution of position of lingual foramen in relation to gender.
Notes: Chi-square test, # Non-significant difference

Trajectory	Gender		Total
	Male	Female	
Downward	48 60.8%	51 61.4%	99 61.1%
Upward	28 35.4%	30 36.1%	58 35.8%
Horizontal	3 3.8%	2 2.4%	5 3.1%
Total	79 100.0%	83 100.0%	162 100.0%

Chi-square value = 0.261 p-value = 0.878

Table 3: Distribution of trajectory of lingual foramen canal in relation to gender.

No. of lingual Foramen	Age Groups		Total
	20-40 Years	41-60 Years	
1	18 38.3%	25 47.2%	43 43.0%
2	27 50.0%	25 54.0%	52 52.0%
3	2 4.3%	3 5.7%	5 5.0%
Total	47 100.0%	53 100.0%	100 100.0%

Chi-square value = 1.673, p-value = 0.433

Table 5: Distribution of number of lingual foramen in relation to age
Notes: Chi-square test, # Non-significant difference

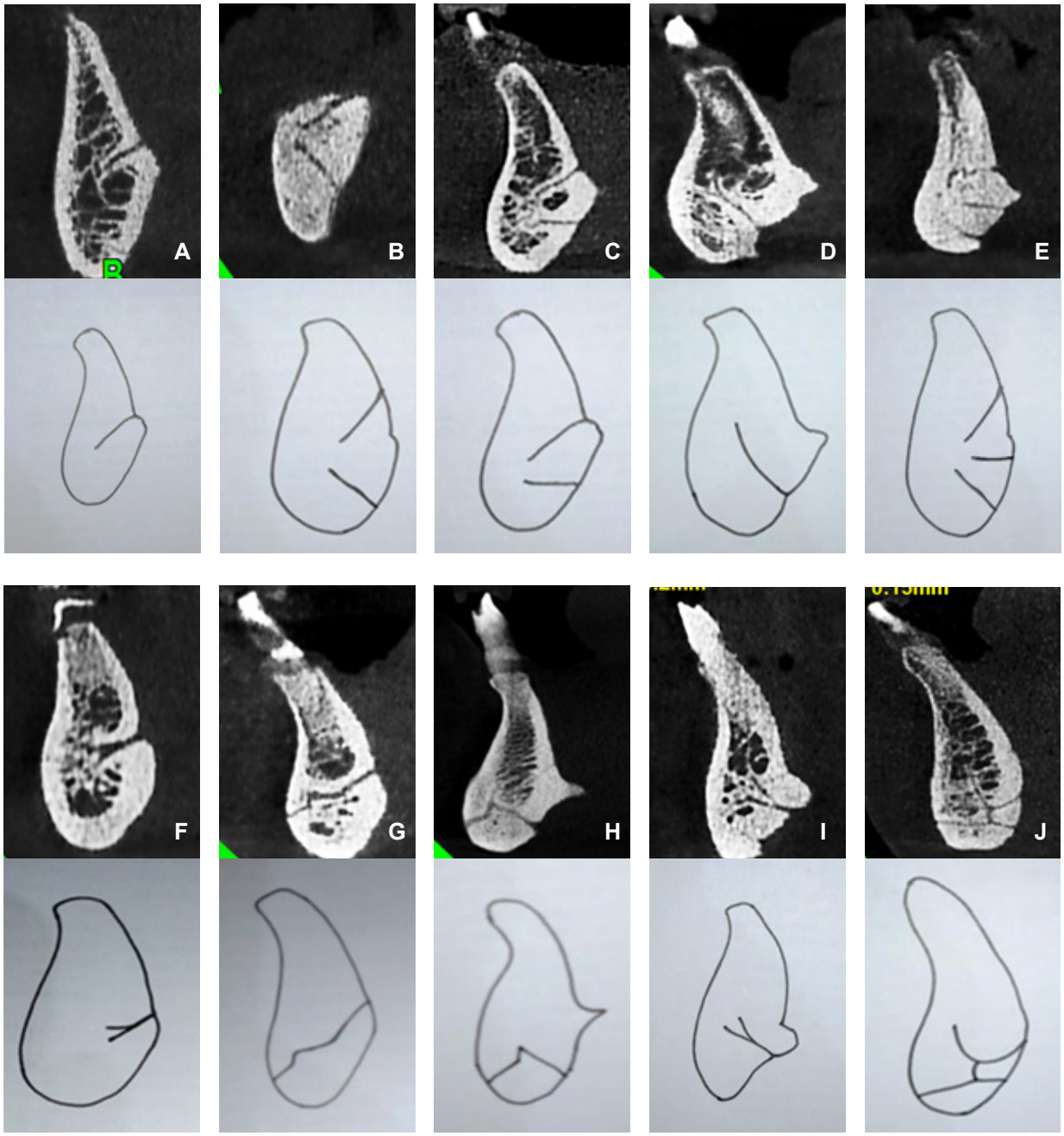

Figure 2: A-J Variations in the morphology of lingual canals in the anterior mandible as observed during review.

LOCATION	GENDER	MEAN	STANDARD DEVIATION	MEAN DIFFERENCE	T-TEST VALUE	P-VALUE
Length of Lingual Foramen Canal	Male	6.31	2.22	-0.20	0.559	0.577
	Female	6.11	2.34			
Diameter of lingual foramen canal at buccal end	Male	0.42	0.15	-0.05	2.113	0.036*
	Female	0.37	0.13			
Diameter of lingual foramen canal at lingual end	Male	0.60	0.25	-0.05	-2.422	0.047*
	Female	0.54	0.21			
BC	Male	18.70	4.62	0.47	0.684	0.495
	Female	18.23	4.04			
LC	Male	17.69	5.28	0.67	0.781	0.436
	Female	16.84	5.72			
BIC	Male	9.84	2.87	1.57	3.564	0.001*
	Female	9.25	2.90			
LIC	Male	10.90	5.06	1.39	2.787	0.046*
	Female	9.51	4.82	-	-	-

Table 4: Variations of lingual canal length, diameter of lingual foramen canal, distance from alveolar crest to lingual foramen at buccal and lingual end, and distance from inferior border of mandible to lingual foramen at buccal and lingual end in relation to gender.

LOCATION	AGE GROUPS	MEAN	STANDARD DEVIATION	MEAN DIFFERENCE	T-TEST VALUE	P-VALUE
Length of Lingual Foramen Canal	20-40 years	5.90	1.85	-0.60	-1.669	0.097
	41-60 years	6.50	2.59			
Diameter of lingual foramen canal at buccal end	20-40 years	0.36	0.11	-0.60	-2.981	0.003*
	41-60 years	0.43	0.15			
Diameter of lingual foramen canal at lingual end	20-40 years	0.54	0.25	-0.60	-2.735	0.045*
	41-60 years	0.60	0.21			
BC	20-40 years	18.83	3.41	1.05	-1.052	0.294
	41-60 years	18.11	5.03			
LC	20-40 years	17.89	5.05	0.72	1.236	0.218
	41-60 years	16.84	5.72			
BIC	20-40 years	8.81	2.90	-0.44	-0.971	0.333
	41-60 years	9.25	2.90			
LIC	20-40 years	9.79	5.00	-0.77	-0.988	0.325
	41-60 years	10.56	4.95	-	-	-

Table 8: Variations of lingual canal length, diameter of lingual foramen canal, distance from alveolar crest to lingual foramen at buccal and lingual end and distance from inferior border of mandible to lingual foramen at buccal and lingual end in relation to age.

No. of Lingual Foramen	Age Groups		Total
	20-40 Years	41-60 Years	
1	18 38.3%	25 47.2%	43 43.0%
2	27 57.4%	25 47.2%	52 52.0%
3	2 4.3%	3 5.7%	5 5.0%
Total	47 100.0%	53 100.0%	100 100.0%

Notes: Chi-square value = 1.673, p-value = 0.433
Unpaired t-test # Significant differences

Position	Age Groups		Total
	20-40 Years	41-60 Years	
Superior	42 53.8%	47 56.0%	89 54.9%
Inferior	35 44.9%	37 44.0%	72 44.4%
At Level	1 1.3%	0 0.0%	1 0.6%
Total	78 100.0%	84 100.0%	162 100.0%

Notes: Chi-square value = 1..116, p-value = 0.572
Unpaired t-test # Significant differences

TRAJECTORY	Age Groups		Total
	20-40 Years	41-60 Years	
Downward	44 56.4%	55 65.5%	99 61.1%
Upward	31 39.7%	27 32.1%	58 35.8%
Horizontal	3 3.8%	2 2.4%	5 3.1%
Total	78 100.0%	84 100.0%	162 100.0%

TYPES OF CANAL MORPHOLOGY	NO OF MANDIBLES MANDIBLES
A	34
B	48
C	3
D	3
E	5
F	2
G	1
H	1
I	2
J	1

Table 9: Ten different types of anatomical variations.

significant difference was found. (Table 4)

The mean diameter of lingual foramen canal at buccal end in males was 0.42mm (SD 0.15) and in female was 0.37mm (SD 0.13). The mean diameter of lingual foramen canal at lingual end in male was 0.60mm (0.25) and in female was 0.54mm (SD 0.21). On comparing the mean Diameter of lingual foramen canal at buccal and lingual end between males and females, using the Unpaired t-test, statistically significant difference was found. [table 4] The mean Diameter of lingual foramen canal at buccal and lingual end was significantly more among males in comparison to females.

The mean distance from alveolar crest to lingual foramen at buccal end (BC) in males was 18.70mm (SD 4.62) and in females was 18.23mm (SD 4.04). The mean distance from alveolar crest to lingual foramen at lingual end (LC) in male was 17.69mm (SD 5.57) and in female was 17.02mm (SD 5.28). On comparing the mean BC and LC between males and females, using the Unpaired t-test, no significant difference was found. (Table 4)

The mean distance from inferior cortex of mandible to lingual foramen at buccal end (BIC) in males was 9.84mm (SD 2.87) and in females was 8.28mm (SD 2.72). The mean distance from inferior cortex of mandible to lingual foramen at lingual end (LIC) in males was 10.90mm (SD 5.06) and in females was 9.5mm (SD 4.82). on comparing the mean BIC and LIC between males and females, using the Unpaired t-test, significant differences were observed.The mean BIC and LIC was significantly more among males in comparison to females. (Table 4)

The distribution of number of lingual foramen was compared between 20-40 and 41-60 years age groups using the Chi-square test. However, no significant difference was observed between the groups. (Table 5)

On comparing, the distribution of Position of lingual foramen between 20-40 and 41-60-years age groups using the Chi-square test, no significant difference was observed between the age groups. (Table 6)

On comparing the distribution of Trajectory of lingual foramen was compared between 20-40 and 41-60years age groups, using the Chi-square test, no significant difference was observed. (Table 7)

The average length of lingual foramen canal in 20-40 years age group was 5.90mm (SD 1.85) and in 41-60 years of age group was 6.50mm (SD 2.59). On comparing the mean Length of lingual foramen canal between 20-40 and 41-60years age groups using the Unpaired t-test, no significant difference was observed between the groups. (Table 8)

The mean diameter of lingual foramen canal at buccal end and lingual end in 20-40 years of age group has been depicted in table 8.

On comparing the mean Diameter of lingual foramen canal at buccal and lingual end between 20-40 and 41-60years age groups, using the Unpaired t-test, the Diameter of lingual foramen canal at buccal and lingual end was significantly more among 41-60 years age group in comparison to 20-40 years age group. (Table 8)

The mean distance from alveolar crest to lingual foramen at buccal end (BC) in 20-40 years of age group was 18.83mm (SD 3.41) and in 41-60 years of age group was 18.11mm (SD 5.03). The mean distance from alveolar crest to lingual foramen at lingual end (LC) in 20-40 years of age group was 17.89mm (SD 5.05) and in 41-60 years of age group was 16.84mm (SD 5.72).

On comparing the mean BC and LC between 20-40 and 41-60 years age groups using the Unpaired t-test, no significant difference in mean BC and LC between 20-40 and 41-60 years age groups was observed. (Table 8)

The mean distance from inferior cortex of mandible to lingual foramen at buccal end (BIC) in 20-40 years of age group was 8.81mm (SD 2.90) and in 41-60 years of age group was 9.25mm (SD 2.90). The mean distance from inferior cortex of mandible to lingual foramen at lingual end (LIC) in 20-40 years of age group was 9.79mm (SD 5.00) and in 41-60 years of age group was 10.56mm (SD 4.95).

On comparing the mean BIC and LIC was

compared between 20-40 and 41-60 years age groups using the Unpaired t-test, no significant difference in mean BIC and LIC between the groups was observed. (Table 8)

In the present study, we found ten different types of anatomical variations of lingual foramen canals morphology which was categorized from A to J. 34 mandibles were type A, 48 mandibles were type B, 3 mandibles were type C, 3 mandibles were type D, 5 mandibles were type E, 2 mandibles were type F, 1 mandible was type G, 1mandible was type H, 2 mandibles were type I, 1 mandible was type J. [Figure 2, Table 9]

DISCUSSIONS

The field of dentistry is consistently evolving with advancements in dental technologies and the procedures like implant, grafting and orthognathic surgeries have gained popularity with increasing success rate. The clinicians should be aware of the anatomy of these vital structures and the variations to prevent any surgical complications.[11]

Many anatomical landmarks in the mandible including foramens and canals exhibit a high degree of sexual dimorphism. In previous studies, various anatomical parameters like the height of mandible, gonial angle and the position of mental foramen, mandibular foramen and mandibular canal have shown significant differences in various age groups and gender.[14,15] To the best of our knowledge there are very few studies in literature that determine the gender and age based on lingual foramen and its canals. In this study, 100 retrospective CBCT scans of anterior mandibular region were evaluated for anatomical variations of lingual foramen and its canals. Furthermore, the age and gender related changes were also evaluated based on the observations like number of lingual foramen and canals, position of lingual foramen, trajectory of lingual foramen canal, length, diameter of lingual foramen canal etc.

The resultsof the present study were consistent with those reported by Tepperet *et al.,* Galhleitner *et al.,* Tagaya *et al.,* Sheikhi *et al.,* McDonnell *et al.,* Denny *et al.,*who suggested that lingual foramen was found in 100% of the cases examined.[16,17,18] However, the results were in disagreement with the Jacob *et al.,* S. Caravilli *et al.,* and Longoni *et al.,* who observed lingual foramen in 82%, 90.35% and 60% of the Computed Tomography (CT) scans examined, respectively.[19,20,21] One possible interpretation of this disagreement is the imaging technique used. CT images with larger slice thickness may limit the accuracy of the measurements. Cone Beam Computed Tomography (CBCT) images obtained with a finer slice thickness is considered to be more reliable and accurate than those with larger slice thickness. The multiple CBCT sections were assessed in the present study with a finer slice thickness from a range of 0.15mm to 2mm due to which minute details on lingual surface of mandible was visualized more clearly than the above-mentioned imaging technique.

According to the present study two lingual foramina were found in 52% of the participants followed by single lingual foramen in 43%. The results were corroborated with those of Shekhiet *et al.,* Aggarwal *et al.,* and Choi *et al.,* Liang *et al.,* conducted a study on dry skull and found 3 foramina only in 4% of the 49 mandibles and our results were in accordance with this study.[23] While a study performed by Abesiet *et al.,* Sheikhiet *et al.,* and Ceena E Denny *et al.,* found 3 foramina in 7.5%, 19.6% and 2% of the cases, respectively.

Most of the subjects in our study were having lingual foramen superior to genial tubercle (54.9 %), followed by 44.4% that were inferior to genial tubercle. The results were in consistence with Sheikhiet *et al.,* Nagar *et al.,* Iulianababiuc *et al.,* Nimje DA *et al.,* and Aoun G *et al.,* who suggested that the most frequent position of lingual foramen was superior to genial tubercle followed by inferior to genial tubercle.[25,26,27,28]

In the present study, 61.1% participants showed downward trajectory of canal followed by 35.8% participants which showed upward trajectory. Our results were similar to the studies performed by Sheikhiet *et al.,* Agrawal PK, and

Liang *et al.,* as their results show that the most common trajectory of lingual foramen canal was downward trajectory.

Our results showed that there was no significant difference in the number, position and the trajectory of lingual foramen canal among various age groups and gender which is in accordance to the studies done by a George Aoun *et al.,* and Denny *et al.*

According to our study the mean diameter of lingual canal at buccal and lingual end was significantly more in males as compared to females. The results were similar to the studies previously performed by He *et al.,* Sekerci *et al.,* and Wang *et al.,* which showed that male subjects had a larger diameter of the lingual foramen as compared to female subjects.[29-31] whereas in the 41-60 years age group exhibited more diameter in comparison to 20-40 yrs age group. Our results were also in favor of Abesi *et al.,* who said that there is significant relation between the diameter of lingual foramen and age group.[24]

Abesi *et al.,* found seven different types of lingual foramen canals morphology which was categorized from A to G.[24] Type B and A were most frequently found canals morphology in our study which is in accordance with the study by Abesi *et al.,*. Another study performed by Sekerciet *et al.,* they found twelve different types of lingual foramen canals morphology which were categorized into I-XII.[29] They found two extra variations in canal morphology as compared to the present study. That is type V (single lingual vascular canal exiting through the labial side of the mandible) and type IX (intersection of two separate canals; one exiting through the lingual cortical plate and one through the labial). These two extra canals variation could be due to the number of CBCT scans assessed. The number of scans assessed were more as compared to our study.

CONCLUSION

Every individual has anatomic variation and different measurements of lingual foramen and its bony canals. These variations must be taken into consideration prior to treatment planning to prevent any surgical complications. The mean diameter of lingual canal and the distance from lingual foramen to inferior cortex of mandible at buccal and lingual end varied significantly in males and females. There was also an age-related significant difference in the diameter of canal.

Hence, the morphology of lingual foramen and its canal can be used to evaluate the age and gender of an individual which can open a new vista in the field of forensics. However, further studies with more sample size are warranted to support this finding. IJFMP

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ORIGINAL ARTICLE

Three-Dimensional Facial Morphometric Analysis: A reliability study Based on Facial Data of North Indian Population

Abhinav Sood[1], Varsha Dogra[2], Gayatri Pathmanathan[3], Aanchal Dwivedi[4]

ABSTRACT

CONTEXT: Accuracy of Non-Invasive structure from motion photogrammetry technique for facial data measurement analysis.

AIM: The purpose of the study was to calculate reliable facial anthropometric measurementsusing 3D photogrammetry and its correlation with direct measurements.

MATERIALS AND METHOD: Three-dimensional facial morphometry was investigated in a sample of 40 males and 40 females with structure from motion photogrammetry technique using specific software. Subjects ranged between 18 to 40 years belonging to the Rajput community in the Arki region of Solan district Himachal Pradesh. The subjects had no history of any facial deformity and had sound dentitions. For each subject, the facial and nasal indexes were measured and compared with previous studies.

STATISTICAL ANALYSIS: The SPSS version 23.0 was used to determine the difference between the mean caliper and mean photogrammetry values.

RESULTS: The study shows that the pervasiveness in both male and female facial types hyper-leptoprosopic (very long face)with a mean facial index of 100.76 and 96.70 respectively. The nasal index calculated for males was 90.04 and for females was 78.96. The mean values were in acceptable agreement with the literature data gathered with direct methods. The results suggest that using SfM photogrammetry for facial measurements is accurate when compared with direct anthropometry.

CONCLUSIONS: The technique permits the non-invasive calculation of 3D linear measurements that could be applied in the field of physical anthropology, medical and forensic science.

KEYWORDS | anthropometry, morphology, three-dimensional photogrammetry

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INTRODUCTION

FACIAL MORPHOLOGY ESTIMATION AND grouping assume a significant part in the face anthropometry of numerous scientific applications. Anthropometry is an efficient and non-invasive method for describing craniofacial morphology but with the advent of more sophisticated technologies, we have seen a shift of gathering 3D data of face from manual to digital methods as the measurements are often made manually, which is a tedious and time-consuming process.[1,2] There are various non-invasive methods for the acquisition of data relating to the shape of a 3D object like laser scanning, structured light scanning, and 3D photogrammetry.[3,4] Toalleviate the potential of these methods, it is essential to have reliable anthropometric data of reference populations, which is made possible through the investigation of a large number of representative samples.[5] Facial information

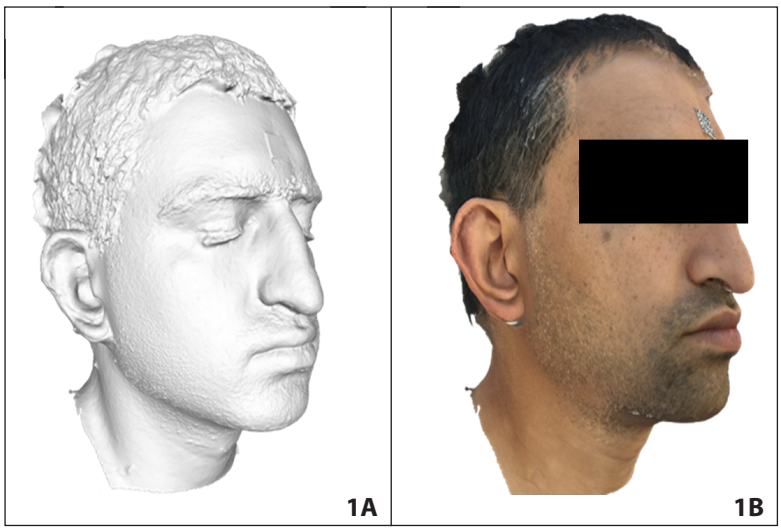

Figure 1: Overview of 3D models. Figure 1A shows 3D generated facial model without texture information and figure 1 B shows the same 3D model with texture information.

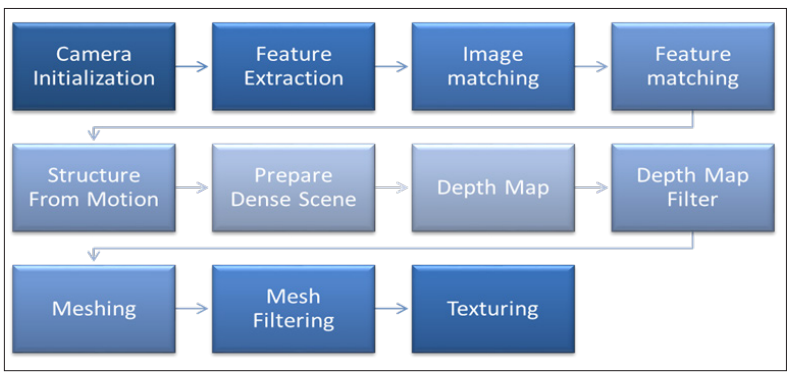

Figure 2: Resulting SfM (Structure from Motion) models were processed in Autodesk Remake 2020.

is ordinarily estimated by anthropometric estimation. The investigation of anthropometry incorporates facial length, facial width, a facial and nasal index which plays an important role in defining facial characteristics.[6,7] As each race has the characteristics that make it unique to other races which help in facial identification in a forensic context. This can then be used for the study of different subjectstoperform repeated measurements of the soft tissue of the face. Photogrammetry is a technique that relies upon photographs to change three-dimensional shapes utilizing the triangulation method to gauge the directions of landmarks on the item to be examined that is the reason it is incorporated inside the non-intrusive procedure. This framework thus requires the distinguishing proof of landmarks that are detectable in each view.[8] Examiners changed and organized this framework with the objective that particular anthropometric estimations could be assessed to a significant degree of accuracy like direct techniques.[9,10] The outcome would be indistinguishable from a 3D surface output (Fig.1) above.

MATERIALS AND METHOD

Three-dimensional facial morphometry was investigated in a sample of 40 males

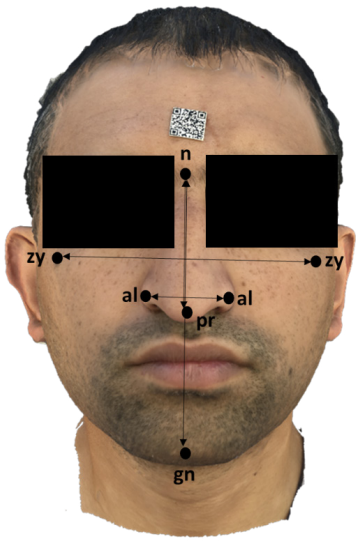

Figure 3: Shows 3D facial model with frontal view and landmarks included in the study for facial measurements.

and 40 females with structure from motion photogrammetry technique using specific software. Subjects ranged in age between 18 to 40 years belonging to the Rajput community in the Arki region of Solan district Himachal Pradesh.The subjects had no history of any facial deformity and had sound dentitions. For each subject, the facial and nasal indexes were measured and compared with previous studies.

The equipment used was a digital single-lens reflex camera without flash (Canon 40D, 10 Mpx) that was mounted on a tripod stand. The photographs were clicked in an open environment in the evening time to avoid any overexposure on the face due to sunlight. The processing of the point cloud and the creation of virtual 3D models were carried out through the use of the SfM technique using Autodesk Recap pro-2020 software. The operation protocol for image processing has been defined in Figure 2 (facing page).

wwMeasurements included in the study are highlighted in Figure 3:

- Facial index (FI) = facial height (n-gn) ×100/ mid face width (zy-zy)
- Nasal index (NI) = nasal height (n-pr) ×100/ nasal width (al-al)
- Here, n- nasion is the root of the nose.
- gn - ganthion is the median point on the lower border of the mandible.
- zy- zygion is the most lateral point on the zygomatic arch.

RESULT AND DISCUSSION

In this study, we have evaluated the facial landmarks obtained using SfM photogrammetry and compared them to manual measurements done with the help of sliding and spreading caliper to access the reliability of our technique. For the assessment, 80 3-D face meshes were created. This 3-D facial data specifies facial traits of the North Indian Rajput community of the Arki region in Solan district of Himachal Pradesh. The result of the study shows that the pervasiveness in both male and female facial types hyperleptoprosopic - very long face - with a mean facial index of 100.76 and 96.70 cm, respectively. The nasal index calculated for males was 90.04 (broad) and for females was 78.96 (medium) as presented in Tables 1 and 2.

The results of the accuracy portion of the study are presented in Table 3. The four variables investigated, demonstrated minimum mean difference of .02 cm in nasal length of male subjects while the maximum difference was observed in nasal width of male subjects

No.	Shape of Face	Range of Face Index	Shape of Nose	Range of Nasal Index
1.	Hypereuroprosopic (very broad face)	< 79.9	Very narrow	< 54.9
2.	Europrosopic (broad face)	80 to 84.9	Narrow	55 – 69.9
3.	Mesoproscopic (round face)	85 to 89.9	Medium	70 -84.9
4.	Leptoprosopic (long face)	90 to 94.9	Broad	85 – 99.9
5.	Hyperleptoprosopic (very long face)	> 95	Very broad	> 100

Table 1: Classification of Face and Nose type.

	Mean (Male)	Min	Max	Mean (Female)	Min	Max
Morphological Facial Length	11.08	10.02	11.8	10.17	8.22	11.7
Morphological Facial Width	11.04	10.03	11.9	10.5	8.9	11.3
Facial Index	100.76	89.65	110.7	96.70	85.65	115.2
Morphological Nasal Length	4.42	3.6	5.2	4.15	3.4	5.2
Morphological Nasal Width	4.04	3.4	5.1	3.29	2.6	4.6
Nasal Index	90.04	75.45	110.88	78.96	64.2	103.23

Table 2: Shows the Mean and Range of meameasurements observed in the study.

Landmark Distances	Obtained by Photogrammetry	Obtained by Sliding Caliper	Difference
n-gn Facial Height	11.08 10.17	11.40 10.30	0.32 0.13
zy-zy Facial Width	11.04 10.50	11.20 10.30	0.16 0.20
n-pr Nasal Length	4.42 4.15	4.40 4.20	0.02 0.15
al-al Nasal Width	4.04 3.29	4.50 3.40	0.44 0.11

Table 4: Inter-method comparison of measured mean values and their difference :

on comparison with direct anthropometry. The mean values were in acceptable agreement with the literature data gathered with direct methods. The results suggest that using SfM photogrammetry for facial measurements is accurate when compared with direct anthropometry.

CONCLUSION

The goal of our research was to create 3D facial data for scientific applications. In this preliminary study, we focused on measuring facial and nasal index for which we intended to reproduce the classification results obtained manually by different authors on similar populations. This 3D facial morphometric study assists in normative and gene studies where such data sets are crucial. IJFMP

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ORIGINAL ARTICLE

Deciphering of secret written content written with various fluids

[1]Mahima, [2] Vikas Bhargav, [3]Anjali Malik

ABSTRACT

CONTEXT: When a hidden or covered message is needed to be conveyed to someone, the skill of secret writing is used as a means of conveyance. It is incorporated to create content undecipherable by the other person even by not hiding the existence of the secret conveyance of the content.

AIMS: The present study has been conducted with an approach of incorporating the secret handwriting on a blank paper using various household invisible inks and retrieved samples were assessed using various deciphering methods.

MATERIALS & METHOD: There are three different categories in which these invisible inks are classified. Invisible inks are fluids that are used for the same and do not come into sight unless it is revealed through any sort of process. These inks viz. revealed by heat, by any chemical reaction and those that are observed under UV light and iodine fuming method

RESULTS: It was observed through analyzing the samples that, heating technique was the best for the secret messages created using biological fluids, as writing became irreversibly visible after the development.

CONCLUSIONS: The results of the study concluded that different physical and chemical methods proves to be an approach for deciphering the various messages written with invisible inks.

KEY MESSAGES: Although the available methods for deciphering the secret written content are abundant nowadays but this task can also be done effectively with commonly available techniques.

KEYWORDS | Hidden message, invisible ink, UV light, iodine fuming

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INTRODUCTION

HUMANS ARE INSTINCT FOR SECRETS AND revealing them to certain people. In history as well certain events can be found involving the art of secret in many aspects. Before the digital era, messages were often hidden with steganographic skills. Talking about technical steganography, writing with invisible ink is the most renowned skill.[1] The art of covered wiring or more technically, hidden writing is generally referred as secret writing.[4] Use of invisible inks is the earliest method of secret writing, others may include the use of codes, marks, latent photographs etc. Other methods require advancements for development procedure.[3] In ancient times, use of certain liquids like lemon juice or milk has proved popular and effective for intentionally hiding the message.[7] Some traces for use of secret inks were seen during World-War II by secret agents;[9] writings were made with common household fluids like lemon juice, baking soda and urine.[8] In general, invisible ink

is a substance used in steganographic schemes so that secret messages can be invisibly written on papers.[6] Invisible inks do not come into sight unless it is revealed through any sort of process. These inks viz. revealed by heat, by any chemical reaction and those that are observed under UV light and iodine fuming method.[1] General household materials, organic fluids which comes under the category of household materials include fruit juices, biological fluids like blood in diluted form, sweat and even urine and the visualizing requirement may be met by means of heat. The principle behind the visualization is the disturbance of fibers caused due to application of the specific ink. These inks change the arrangement of fibers and that can be developed through heating the paper at mild temperature. The ink applied area turns brown faster than the surrounding causing the message to be seen with naked eyes. In addition to organic fluids, invisible inks can be of chemical origin. These inks, on contrary, require treatment with a specific reagent to be developed. Exposure to ultra-violet radiations may be used as one of the development methods but its reliability is comparatively low.[2,6]

METHOD AND MATERIALS

To study the reliabilityof visualization methods for secret writing by various invisible inks, this study was done at Department of Forensic Science, Dyal Singh College, Karnal (Haryana). The inks used were fruits juices (apple juice, sugarcane juice, Bartlett Big juice), chemical fluids (Detergent, soapy water, Dettol) and vegetable juices (tomato, potato, onion) were utilized for preparing secret writing samples. A cotton bud was used as the writing instrument and A4 size blank papers were used for writing samples over them. To prepare the samples for writing, the cotton bud was dipped into particular ink and messages were written over blank sheet of paper. The papers were then marked with the name of ink used for sake of differentiation. The papers containing written messages were allowed to air dry under a running fan. They were then analyzed using different visualizing methods. Those include physical (application of heat and ultra-violet radiations) and chemical (fuming with iodine crystals) method.

DISCUSSION

The mentioned physical and chemical methods were utilized to find out the variations and sensitivity of visualization. The visibility of detergent and dettol by heat, gave positive results, while with UV, it showed a bit irregularity. On the other hand, the soapy water used as invisible ink did not show any kind of visibility by heat and under UV radiations as well. Visibility of all three chemical fluids with iodine fuming gave constant visibility (Fig: 3 & 4).

Varied results were observed for visibility of fruits juices when developed using physical methods. Apple juice, on contrast showed constant visibility (Fig. 1). The surface became brown after heating on hot plate at low temperature. Sugarcane and Bartlett big on contrary were not visualized under short UV light but gave positive results with heat and long UV. Visibility of these juices with chemical was constant.

The visibility of vegetable juices by physical methods gave regular and mostly constant results (Fig. 2) for all three inks that were used. Regular results in terms of visibility were observed when samples were treated with iodine fuming for all the three vegetable juices. The paper bearing the invisible writing became purple-brown in color after treating with Iodine fumes.

CONCLUSION

From the results obtained in this study, it can be concluded that physical and chemical methods can be utilized to develop secretly written messages with different fruit juices, chemical and vegetable juice. The application of heat is the best method among other physical methods for decipherment of these kinds of inks. The advantage being quick and cheap procedure and it doesn't affect the document as well when

SR. NO.	TYPE OF FLUID	INK USED CHEMICAL DEVELOPING METHOD RESULTS		PHYSICAL DEVELOPING METHOD RESULTS		
1	Chemical	Detergent	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
2	Chemical	Soapy water	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
					UV Long	-----
3	Chemical	Dettol	Iodine Fuming	+++++	Heat	-----
					UV Short	-----
					UV Long	+++--

Table 1: Chemical fluids deciphered by physical and chemical methods

SR. NO.	TYPE OF FLUID	INK USED CHEMICAL DEVELOPING METHOD RESULTS		PHYSICAL DEVELOPING METHOD RESULTS		
1	Chemical	Detergent	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
2	Chemical	Soapy water	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
					UV Long	-----
3	Chemical	Dettol	Iodine Fuming	+++++	Heat	-----
					UV Short	-----
					UV Long	+++--

Table 2: Fruit juices visualized by physical and chemical methods

SR. NO.	TYPE OF FLUID	INK USED CHEMICAL DEVELOPING METHOD RESULTS		PHYSICAL DEVELOPING METHOD RESULTS		
1	Chemical	Detergent	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
2	Chemical	Soapy water	Iodine Fuming	+++++	Heat	+++++
					UV Short	-----
					UV Long	-----
3	Chemical	Dettol	Iodine Fuming	+++++	Heat	-----
					UV Short	-----
					UV Long	+++--

Table 3: Vegetable juices visualized by physical and chemical methods

Figure 1: Apple juice after treating at hot plate

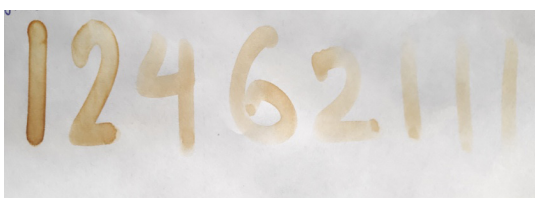

Figure 2: Onion juice after treating at hot plate

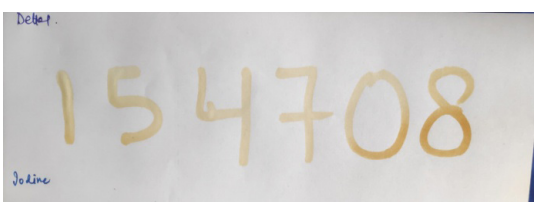

Figure 3: Dettol after treating with iodine fuming

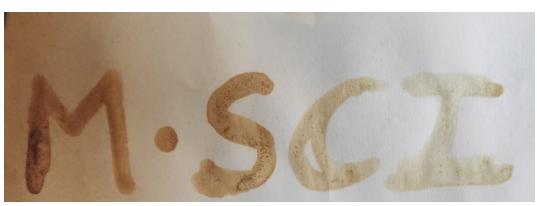

Figure 4: Detergent after treating with iodine fuming

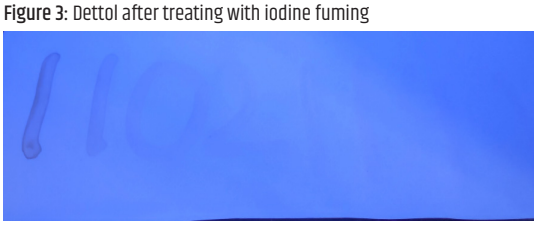

Figure 5: Potato juice under long UV radiation

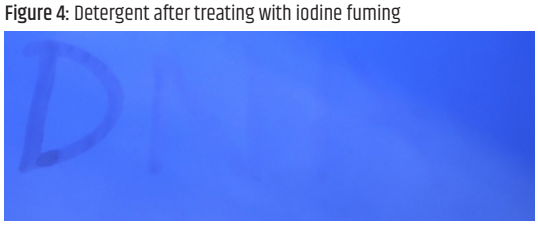

Figure 6: Onion juice under long UV radiation

care is taken while heating. The visualization under UV radiations being a non-destructive method does not give satisfying results in terms of visualization and proved to be time consuming technique. The chemical method viz. iodine fuming does not spoil the documents extensively and gives constant decipherment almost for all the invisible inks. The purple color fades away after sometime therefore it needs to be photographed immediately. However, it requires little more time than heating for development but gives satisfying results. Furthermore, to better understand the visualization of different inks and factors that affect the development procedure over a period of time, studies can be conduct considering a large sample size and more kinds of invisible inks. IJFMP

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ORIGINAL ARTICLE

Evaluation of the Concentration of Heavy Metals in Lipsticks using ICP-OES

[1]Kamna Sharma, [2]Sally Lukose

ABSTRACT

CONTEXT: Lipstick is a universal cosmetic, and it can be colored and textured in different ways. It has a fatty base which spreads easily to the surface. They are applied on a sensitive surface of the human body and finally ingested via skin, hence they must be made to the highest safety specifications. Presently, most of the women and adolescent using lipstick in their daily routine to enhance their beauty.

AIMS: In present study, an attempt has been made to determine the concentration of lead, nickel, and cobalt in lipstick samples.

MATERIALS AND METHOD: The lipstick samples were purchased from online and local markets. The concentration of heavy metals in the samples were determined using ICP-OES.

RESULTS: The levels of heavy metals in the sample brands were found to be significant. The results obtained in the study has been alarming specially in case of lead, which was found to be as high as 15ppm, nickel 5ppm and cobalt 5ppm in different brands.

CONCLUSIONS: The application of lipstick with excessive high content of heavy metals leads to serious health hazards and hence be used judiciously.

KEY MESSAGES: The long term usage of lipstick containing the harmful heavy metals above the stipulated quantity poses a high health risk to users and hence should be used judiciously.

KEYWORDS | lipstick, heavy metals, ICP-OES, toxicity

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INTRODUCTION

Lipstick is a type of cosmetic product used for coloring the lips. It is small stick of wax lip coloring enclosed in a cylindrical case. The basic composition found in lipstick are wax, oil, alcohol, and pigments. Wax enables the mixture to form the shape of cosmetic. Oil such as minerals, castor or may be vegetable oil are added to the wax. Fragrance and pigment along with wide range of other ingredients can also be used to make the product smoother or glossy. In prehistoric time lipsticks were made only available natural resources like fruit and vegetable juices. Evidence and origin of lipstick existed 5000 yrs ago in Babylon, paste made up of crushing precious stones was used (Ullah *et al.,* 2013). The application of same was done by chewing betel leaf in ancient times. The use of lip color was common among Egyptians, Syrians, Persian and Greek. Later in 16th century, it was introduced in England by Queen Elizabeth I.

There are different types of lipstick available in market like sheer, matte, creamy, lip balms, glossy, moisturizing, long wearable and non-transferable etc depending upon the demands of the consumers. The time-to-time variation in the product is incorporated depending upon the latest trends. There are lot of organic ingredients which can be used like Organic waxes, oils and plant butters, such as beeswax,

cocoa butter, mango seed butter, shea butter, avocado butter, coconut oil and avocado oil etc.

It consists of number of toxic chemicals such as methylparaben, polyparaben, Retinyl palmitate, dyes, tocophenyl acetate.

CHEMICAL	USES
Methylparaben	Antifungal and preservative
Polyparaben	Prevents growth of harmful bacteria
Retinyl palmitate	Antioxidants
Dyes	Coloring agents

Table 1: The concentration of lead, nickel, and cobalt in Lipstick brands.

As per US Food Drug and Cosmetic Act defines cosmetics as any articles which are intended to be rubbed, poured sprinkled, or sprayed on, introduced into or otherwise applied to the human body or any other part for cleansing, beautifying, promoting attractiveness or altering the appearance.

MATERIALS AND METHOD

OBJECTIVE

Analysis and estimation of Lead, Nickel and Cobalt in lipstick samples purchased from local markets.

HYPOTHESIS

1. Lipstick samples collected from the local market would contain lead, nickel & cobalt that can cause skin diseases.
2. Quantitative estimation of lead, nickel and cobalt can be done using ICP-OES will give significant results.

SAMPLE TYPE AND SIZE

Lipstick samples were collected from the local markets for the study. In all 10 branded lipstick samples were taken for analysis of heavy metals such as lead, nickel and cobalt.

SAMPLE PREPARATION

All the apparatus were thoroughly washed and rinsed using normal water followed by immersing the same in 5% solution of HNO3 for an overnight, later by rinsing with deionized water before using the same.

1. 1 gm/ 1 ml of lipstick was taken in a beaker.
2. The beaker was then heated in muffle furnace at 450°c.
3. After the sample was turned into ash, the digestion was done.
4. 4. For of the digestion of the sample, hydrochloric acid and nitric acid were taken in proportion of 1:3.
5. 25 ml of acid digestion was added to the beaker and heated on a tripod stand till the solution was clear.

INSTRUMENT USED

The Vista-MPX simultaneous ICP-OES with axially viewed plasma was used for this work. The instrument was fitted with the 3-channel peristaltic pump option for easy introduction of ionization buffer to the sample via a post-pump Y-piece.

WORKING PRINCIPLE

- Firstly, the sample is introduced into the chamber, in a liquid form which is sprayed using nebuliser. Due to the high temperature inside the chamber atomizes and ionizes the sample, creating positively charged atomic ions.
- The larger droplets are then removed from the gas chamber, and the remaining smaller droplets are transferred into the central passage of an argon plasma.
- The droplets are then dried, deteriorated, and dissociated into an individual atom in the chamber.
- These atoms are then converted into cations via interface before they enter into the vacuum system.
- Electrostatic lenses keep the ions focused, as they pass to the final chamber and the outcome were recorded by the detectors It uses a higher thermal energy which discrete the cations from the photons and neutral particles.
- Analyte ions are then separated & scanned using multiplier detector. The spectrometer will measure the spectrum of each ion.

BRAND	LEAD (PPM)	NICKEL (PPM)	COBALT (PPM)
Biotique	4	2	2
Attitude	6	4	3
Colorbar	8	5	3
Coloressence	15	4	5
Loreal	4	3	2
Maybelline	5	3	2
Oriflame	5	2	2
Revlon	7	4	3.8
Signature	4	2	3
Faces	4	2	2

Table 2: The concentration of lead, nickel, and cobalt in Lipstick brands.

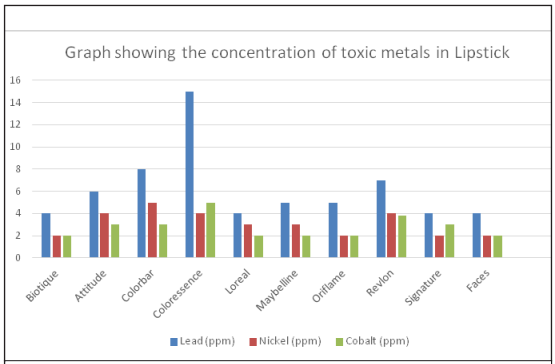

Figure 1: Graphical representation of lead, nickel, and cobalt in lipsticks

- The light intensity on the wavelength is measured and with the calibration calculated into a concentration.

RESULTS

This research was performed in triplicate analysis. The number of selected lipsticks was ten which was collected from the cosmetic shops and local market. The data presented in Table 1 shows remarkably high concentration of lead in all the brands, the least being shared by 4 brands (Biotique, Loreal, Signature and Faces) estimated at 4 ppm. The maximum was observed in Coloressence at 15 ppm. However, Nickel and Cobalt also observed in all the samples with maximum in Coloressence (as shown in table no.1 and graph.1).

DISUCSSION

The metal toxicity has been well-documented from early times in 370 BCE (Palpandi and Kesavan, 2012). The investigation to understand the process of action of heavy metals was identified by Voegtlin *et al.,* in 1923. Studies have also shown that there is a marked difference in the absorption of heavy metals in the skin based upon varied physical parameters. (Lilley *et al.,* 1988). While exposure to heavy metals may take place through various means such as diet, environment and medicines (Adal and Tarabar, 2013), the use of cosmetics have also been identified as one such source. Dermal and oral exposure to these heavy metals can occur from application of cosmetics products such as lipsticks (Sainio, et al., 2000). While the present study has reported significant amount of lead, nickel and cobalt in different brands of lipstick, other studies have also shown significant concentration of heavy metals in different cosmetic products (Al-Dayel, *et al.,* 2011).

CONCLUSION

The study has revealed that high values of lead may be due to the presence of factitious elements in the samples as there are no proper awareness in the production and distribution of these cosmetic products. However, the chances of mixing of sub-standard elements can't be ignored. The result clearly shows that further studies need to be conducted of these toxic metals which are used in cosmetic products. Also, the good manufacturing practice must be followed by the companies. There is need for an assessment of health risk of the individuals from the exposure of cosmetics which are adulterer with heavy metals. It was concluded from the result that most of the brands of were tainted with high concentrations lead. The companies can initiate in minimizing the impurities in products by following good manufacturing practices. These includes testing of ingredients and the finished products to make sure they meet certain manufacturer specifications. The removal of heavy metals from these cosmetic

products is not possible after manufacturing, however if the raw material is carefully chosen while keeping in mind the heavy metal contents, we can surely improve the quality of these products and save our mother nature, the environment and health of humans using these products. IJFMP

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ORIGINAL ARTICLE

Isolation and Identification of Various Types of Microbes Present on Documents and the Inhibitory Effect of Ink on their Growth

[1]Puja Mehta, [2]Sally Lukose, [3]Vandana Prasad

ABSTRACT

CONTEXT: Isolation and Identification of various types of microbes present on documents and the inhibitory effect of Ink on their growth.

AIM: The aim of the experiment is to study the inhibitory effect of ink on certain microorganisms prevalent on cellulose of paper.

SETTINGS AND DESIGN: An experimental setup was designed to study the effect of ink on different microbial growth on cellulose paper. For this purpose, pages old books were used as a sample on which well diffusion method was applied for determining the effect by ink.

MATERIALS & METHOD: Decaying book papers were collected from Ranchi city, Jharkhand, India. From these decaying paper samples four bacterial strains and one fungal strain was isolated and identified as Providentia stuartii, Serratia odorifera, Bacillus megaterium, Pseudomonas antimicrobica, and Aspergillus niger respectively. The effect of ink on these microorganisms were studied by agar well diffusion method.

RESULTS: Black, and blue gel pen inks showed maximum zone of inhibition against Providentia stuartii. Blue ball pen ink also showed maximum zone of inhibition against Providentia stuartii and Serratia odorifera. Red ball point ink showed maximum inhibition zone against Bacillus megaterium and black ball point pen ink showed maximum zone of inhibition for Pseudomonas antimicrobica. **CONCLUSIONS:** With printing ink no inhibitory effect was observed on the bacterial strain and zone of inhibition was completely absent. However, green colored printer's ink showed maximum inhibitory effect on fungus Aspergillus niger.

KEYWORDS | fragile document, decaying paper, preservation, isolation, microorganisms, agar well diffusion method, inhibitory effect, zone of inhibition

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INTRODUCTION

The first paper document was manufactured in 1901 from wood cellulose. This cellulose component of paper was tested against ageing factor, the result showed good stability of paper during the ageing process due to the presence of cellulose. During, mid of 19th century, other factors like: air contamination, SO_2 absorption and its effect on acidity on the paper surface were noticed. Similarly, environmental conditions like effect of temperature on paper during ageing process was also reported in the year 1931. Likewise in the 1960s, a relationship between acidity of paper and its breakdown during ageing process was studied. The study showed that acidity of paper is an important factor to persistence of

the paper.

In detail, cellulose is abundantly available on our planet. Since cellulose comprises of a group of fibrolytic enzyme, it hydrolyzes plant cell wall (made up of fiber) into oligosaccharides, and glucose.[1,2] Thus, hydrolysis process mainly involves three types of cellulase enzymes namely, carboxymethyl cellulase (CMCase), cellobiohydrolase and β-glucosidases or endoglucanase. These enzymes are mostly produced by microbes such as bacteria and paramecia. Despite this, cellulase enzymes are also produced by some plant materials and animal sources. Cellulase enzymes are inducible in nature as they grow on cellulosic materials during its synthesis from microorganisms.

In the current scenario, the cellulase enzymes are mostly used for industrial purposes. Therefore, there are large number of cellulase enzyme producers such as fungi (Cladosporium, Myrothecium, and Penicillium and Cladosporium) and bacteria (clostridium, micrococcus and streptococcus) are the most common producers of cellulase enzymes. However, in late 19th century researchers reported some cellulolytic bacteria namely, Cytophaga and some species of actinomycetes, which secretes strong organic acids and staining pigment that causes destruction of paper surface.[3,4,5] During 20th century, the growth of microbial species like fungi such as Saccharicola, Aspergillus and Trichoderma in humidity on paper surface were reported. Oftentimes, book binding are the first sufferers of microbial growth because they absorb air moisture. Owing its cellulolytic enzymatic property, some filamentous species of fungi is also observed during paper degradation which ultimately leads to the hydrolysis of cellulose fiber. Furthermore, it causes discoloration of ink on the paper in the form of stain or depigmentation. Theses, stains may be of different colors like red, yellow, purple and black.

In order to understand the whole process of microbial growth with reference to ink, the current work focuses on isolation and identification of various microorganisms such as bacteria and fungus on various papers and their growth.

METHODS AND MATERIALS

Since the aim has been identified, a working protocol has been formulated in order to achieve the objectives that have been framed. The materials and methods that have been adopted to achieve the objectives are given below.

Isolation of microorganisms: Book samples were weighed separately and serially diluted and cultured on NAM plates for isolation of bacterial species.

Dilutions of 10-1 to 10-3 were done and the diluents of 10-3 was employed for spreading onto the agar plates that were incubated at 37° C for about 24 hours. Further, Gram's staining and Gram nature of each isolated colonies were studied. Isolation of bacterial species: For the Isolation of bacterial species two books dated 1969 and 1958 as sample 1 and sample 2 respectively were used. Book samples were weighed separately and serially diluted and cultured on nutrient agar medium (NAM) plates for isolation of bacterial species.

Preparation of Media: Nutrient agar medium was prepared for isolation of microbes

- Nutrient agar (NAM)
- Nutrient Broth
- Potato Dextrose Agar (PDA)

Gram's staining: Bacterial smear was prepared on a slide and heat fixed. The cationic crystal violet dye is used to stain the microorganism for one minute. Afterward, it was washed using water and then Gram's iodine was added for upto 1 minute forming a purple complex. Acetone was used to decolorize. The obtained smear was washed again using water and then treated with saffranin for almost 45 seconds. Smear was washed and stained. Microorganisms were then observed under microscope and identified Gram positive (purple) and Gram negative as (red/pink).

Culture Preparation: The isolated microorganisms were cultured onto suitable media and incubated until colonies were

observed on the plates. Using agar plate each colony was firstly cultured, secondly collected, thirdly inoculated in nutrient broth and lastly the cultures were incubated.

Isolation of fungal species: Paper sample was taken from the old books. The paper sample was serially diluted and cultured on PDA plates for the isolation of fungal species.

Microscopic identification of fungus: Slides were prepared by using Lacto Phenol Blue for the microscopic identification of fungal species.

Morphological Characterization of Bacteria: Gram staining of isolated bacteria was done by using light microscope of Olympus Company and the bacteria were observed under 100X.

Biochemical Tests

Biochemical identification of isolates was done as per the procedure given by Aneja (2007).

Sugar Fermentation

By using sugar fermentation test, the strength of microbes for degrading various carbohydrates, glucose, lactose, fructose, sucrose and mannitol can be determined. For performing this, a test tube is filled with10 ml of basal media through inverted Durham's tube and then solution is autoclaved at 121°C for 15-20 minutes. Whereas, 1% concentration of carbohydrate is prepared in distilled water that placed for autoclaved at 10 lbs/inch.[2] Afterward, 10ml of basal media and 1ml of sugar is mixed with loopful organism. For control sample, an un-inoculated tube (without organism) was taken. Then all the final test tubes were place in incubator at 37°C for 24 hours. After incubation process, gas and acid examination was performed on the test tubes. When media coloration changes from purple to yellow, it shows the production of acid in the media, while no change, in color results is negative test. Whereas, gas production shows the accumulation of gas bubbles in Durham's tubes.

Indole Hydrolysis

The indole hydrolysis test is performed by using tryptone broth because it contains large quantity of tryptophan. On hydrolysis, this tryptophan is converted to indole and pyruvic acid in the presence tryptophanase. To perform this, isolated bacteria inoculated in tryptone broth. For control sample an un-inoculated tube (without organism) was taken. The control tubes along with inoculated tubes were placed in incubator at 37°C for 48 hours. After this process, about 5 drops of Kovac's reagent is added in the test tube. If a red layer form at the top of the broth it indicates the positive test and if no change in color observed then it indicates negative test.

Methyl Red Test

In this test, bacterial cultures were inoculated into sterilized glucose peptone, followed by incubation at 37°C for about 24 hrs. Then an incubated Methyl red pH indicator was added to the obtained solution, after addition of indicator if the color changes to yellow it indicates negative test (pH > 6), whereas if color of the media remains red it indicates positive test (pH < 4.4).

Voges Proskauer Test

The bacterial cultures were inoculated into sterilized glucose peptone broth, proceeded by incubation at 37°C for about 24 hours. Afterward, the VP (Voges Proskauer) reagent is added slowly to observe the change in color. The development of ruby pink color, indicates the positive test of VP reagent, whereas no change in the color of media represents negative test.

Citrate Test

This test is used to distinguish enteric bacteria, on the basis of their capability to use citrate, acting as a source of carbon and energy. In organisms, citrase enzymes are present to utilizes citrate. Therefore, this test involves two steps, first: inoculating of microorganism in citrate agar media. Second, addition of bromothymol blue (indicator) in the media. The appearance of green to blue color indicates the positive test, while no change in the coloration represents negative test.

Glycerol Test

This test is similar as the carbohydrate test, where capability of microorganism for degrading carbohydrates is determined. For

this 150ml glycerol broth was prepared and pH was maintained around 7.3 and autoclaved at 121°C for 15-20 minutes. After autoclaving one loopful of bacterial isolates were transferred in the tubes containing glycerol media and kept in incubator for 24 hours at 37°C. If the color of the solution changes from red to yellow, then acid is produced and glycerol test is positive and no color change shows that the test is negative.

Casein Test

Casein is a protein that is responsible for the white coloring of milk. For this test casein media was prepared and pH was maintained around 7.2 and autoclaved. Casein plates were made and streaked with the bacterial isolates and kept in incubator at 37oC for 24hrs. A clear zone around bacterial growth indicates that organism can utilize casein.

Urease Test

This test is performed for determining the capability of microorganisms for degrading urea in the presence of urease enzyme. For performing this, urease broth was prepared, and pH was maintained around 6.2 and autoclaved and loopful of bacterial isolates were transferred in the broth and kept in incubator at 37oC for 24hrs. If the color of media changes, from pink to dark pink the test is positive and bacterial isolates are able to utilize urease.

Starch Hydrolysis

This test is performed for determining the capability of microorganisms to utilize starch, like carbohydrate made by glucose and acting as source of energy for growth. Alpha-amylase is an enzyme used to accomplish starch. For this, starch agar media plates were prepared and streaked with the 5 isolated strains and kept in incubator at 37oC for 24 hrs and after incubation, grams' iodine is added to observe the presence of starch, if the clear zone is formed near the growth that bacterial isolates shows starch hydrolysis test positive, if no zone is formed then that bacterial isolates shows negative test.

Acetate Test

The test is performed to determine whether the organism is able to use acetate as an acting source of carbon. In that situation, breaking of sodium acetate effect the pH of the media which results in shifting of pH toward alkaline and the color of the indicator changes from green to blue.

Lecithinase Test

Egg yolk agar media is used for the determination of lecithinase and lipase enzyme activity. These enzymes (lecithinase and lipase) are most commonly found in microorganisms. The microorganisms which contain lecithinase enzymes, are breaks down into an insoluble phosphorylcholine, which results in the formation of white precipitation. Whereas, microorganisms which contain lipase enzyme, are hydrolyzed the fat of the egg yolk, which produces an iridescent sheen on media colony.

Salt tolerance test

For salt tolerance test, the bacterial isolates were inoculated with higher NaCl concentrations of 7.0%.

Catalase test:

Most of the microorganism uses oxygen to produce H2O2. But H2O2 is toxic for their enzymes. Therefore, to overcome this toxic effect, microorganisms are possessing catalase enzyme. Thesecatalase enzymes convert H2O2 to H2O and O2.

Determination of Inhibitory Effect of Different Inks on Bacterial Growth:

To perform the inhibitory effect of different ink samples against bacteria,well diffusion method was employed. For this, 3 different types of inks were collected i.e. Ball point pen ink, Printer ink and Gel pen ink and from all the 3 types, 4 different colors were used i.e. blue, black, green and red. Bacterial isolates were inoculated into nutrient broth. Anti-microbial activity was performed by well diffusion method against different isolated bacterial strains.

RESULTS

Growth of bacteria on Nutrient Agar after Serial Dilution: After the serial dilution of the paper sample the microbes isolated from book1 (sample1) and the book 2 (sample2) the growth

Figure 1(a): Growth of bacteria after serial dilution from sample1 (Book 1)

Figure 1(b): Growth of bacteria after serial dilution from sample1 (Book 2)

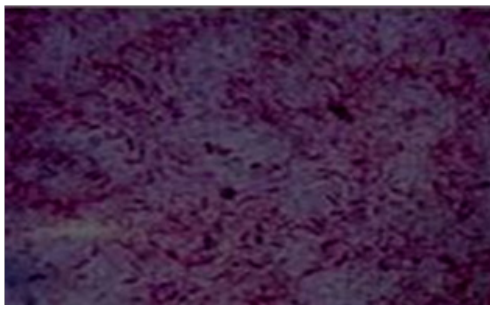

Figure 2(a): Strain 1

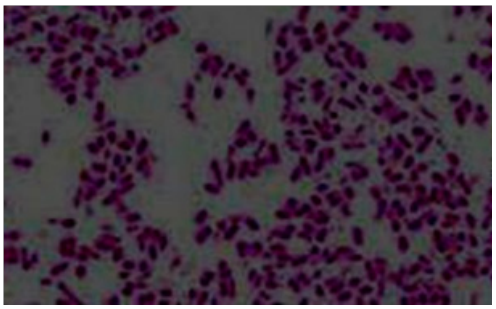

Figure 2(b): Strain 2

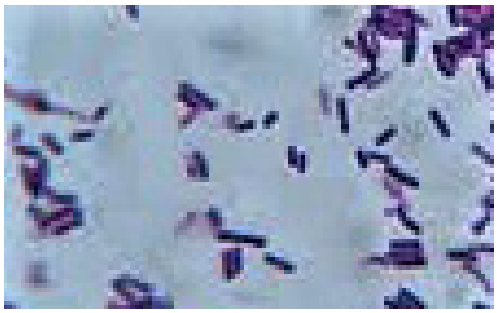

Figure 2(c): Strain 3

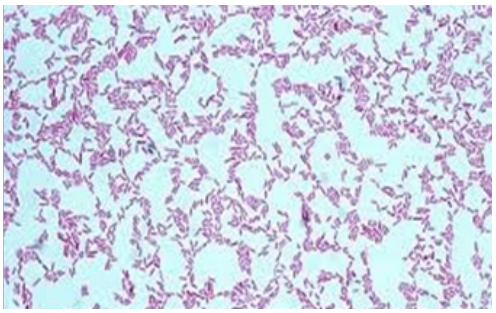

Figure 2(d): Strain 4

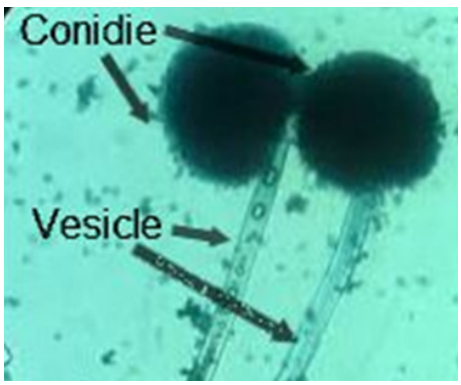

Figure 3: Microscopic view of Aspergillus Niger the entire surface

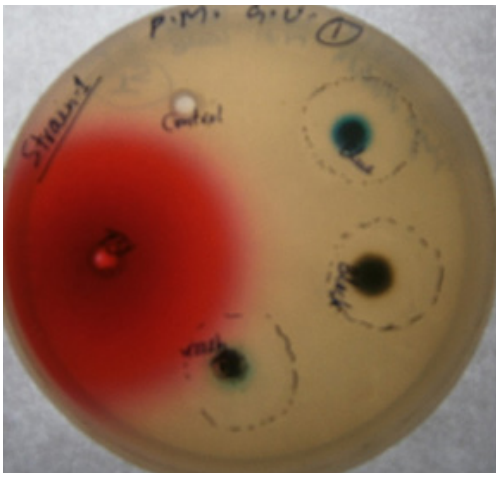

Figure 4(a): Effect of gel pen ink on strain1 well diffusion method

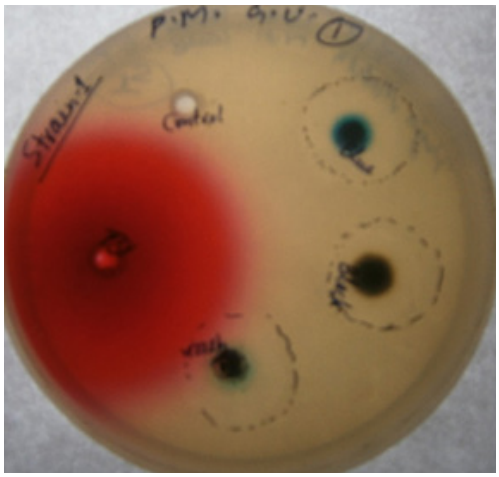

Figure 4(b): Effect of gel pen ink on strain 2 using agar-well diffusion method

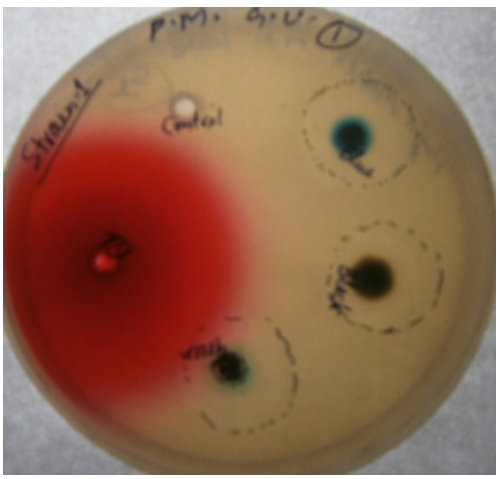

Figure 4(c): Effect of gel pen ink on stain3 using agar- well diffusion method

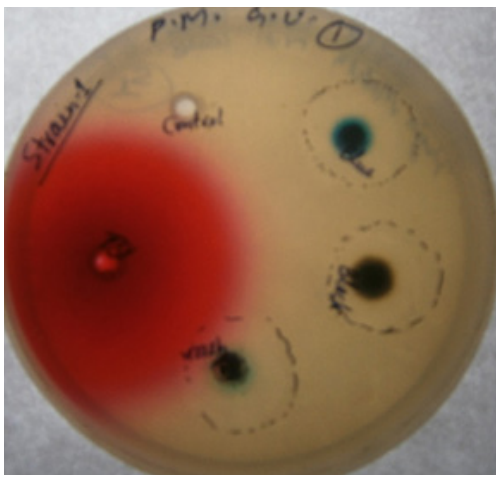

Figure 4(d): Effect of gel pen ink on strain 4 using agar -well diffusion method

of bacteria seen on the nutrient agar plates are as pictured in Fig 1(a): and Fig 1(b).

Morphological characterization of bacterial isolates by Gram Staining: The bacteria grown in the nutrient agar plates in figure 1(a) and 1(b) were then marked as colonies and subjected to Gram staining. These isolated bacteria were then observed under 100X objective lens of the microscope. The observation is as depicted in Fig. 2(a), Fig. 2(b), Fig. 2(c) and Fig. 2(d) which were named Strain1, Strain 2, Strain 3, Strain 4, Strain 5 respectively. Figures are shown below.

Microscopic Identification of Fungus:

Slides prepared by the method mentioned above were observed under the microscope. The slides of different fungal species showed different characteristic features when observed under microscope. (Fig. 3) shows the microscopic characters of different fungal species which were used for further studies. Lacto phenol blue staining of A. niger showed that the colonies were globose, brown to black in color, consists of smooth conidie with transparent vesicle and conidiophores over.

On the basis of biochemical tests along with sugar utilization test results. The detection

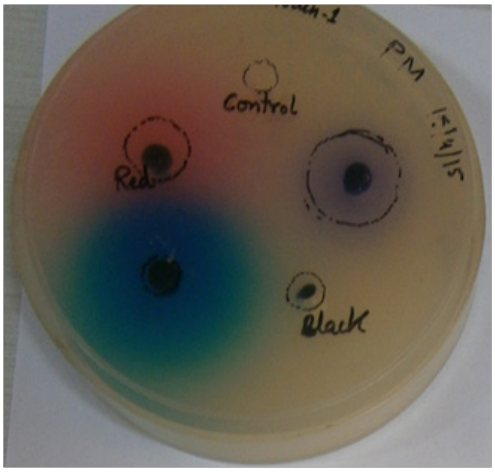

Figure 5(a): Effect of Ball pen ink on strain1using agar- well diffusion method

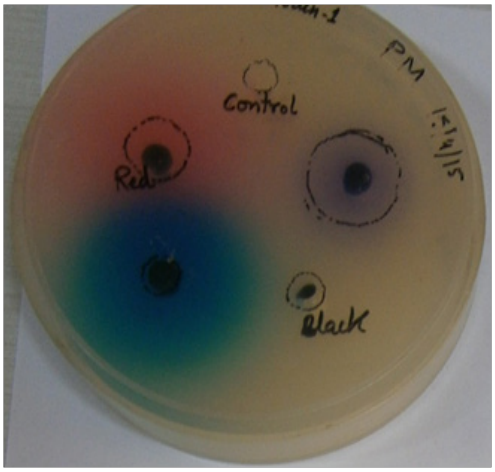

Figure 5(b): Effect of Ball pen ink on strain 2 using agar- well diffusion method

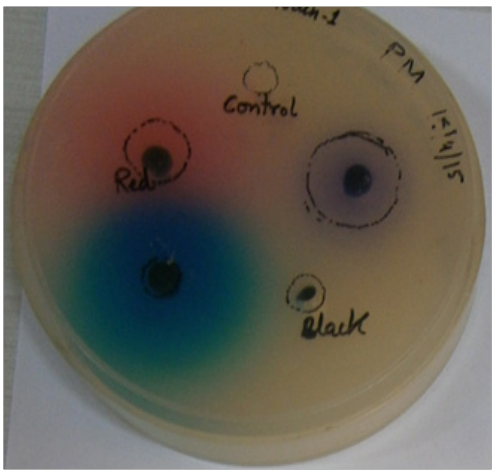

Figure 5(c): Effect of Ball pen ink on strain 3 using agar- well diffusion method

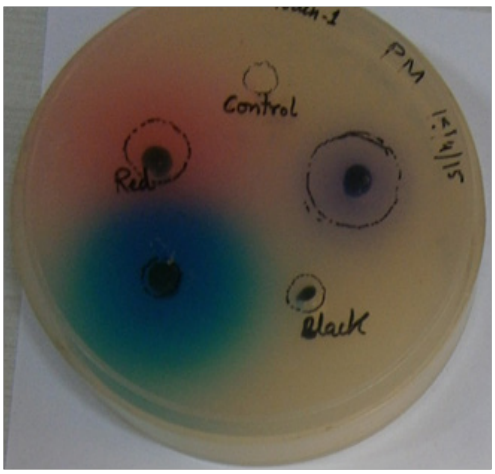

Figure 5(d): Effect of Ball pen ink on strain 4 using agar-well diffusion method

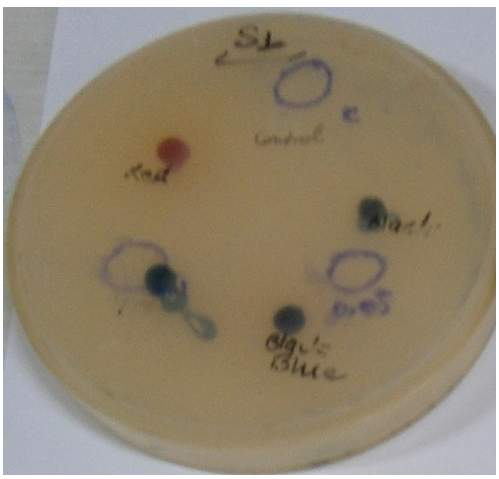

Figure 6(a): Effect of printer ink on strain1 using agar- well diffusion method

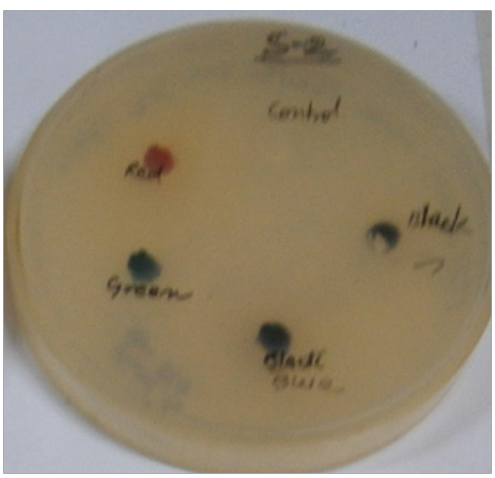

Figure 6(b): Effect of printer ink on strain using agar- well diffusion method

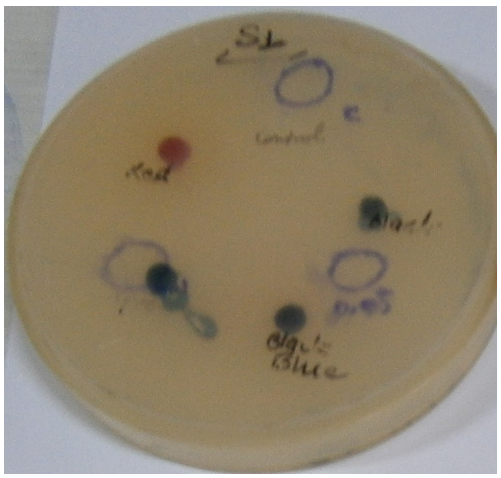

Figure 6(c): Effect of printer ink on strain 3 using agar- well diffusion method

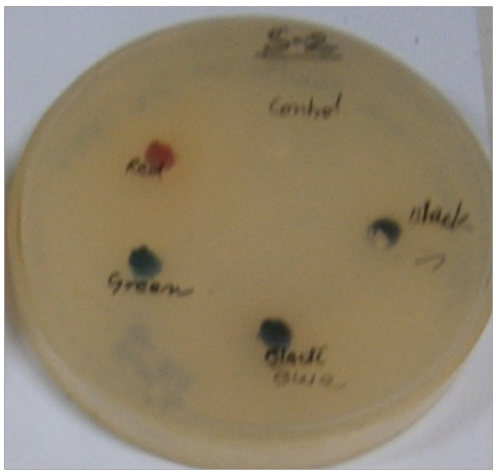

Figure 6(d): Effect of printer ink on strain 4 using agar- well diffusion method

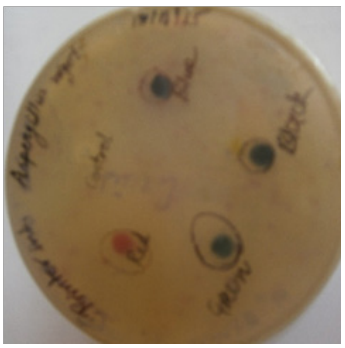

Figure 7:(a) Effect of Printer Ink

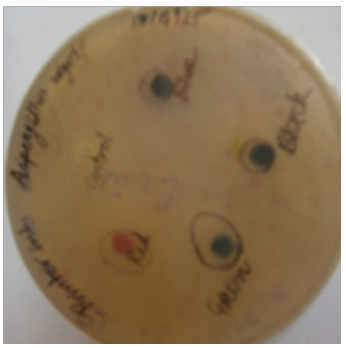

Figure 7:(b) Effect of Ball Pen ink

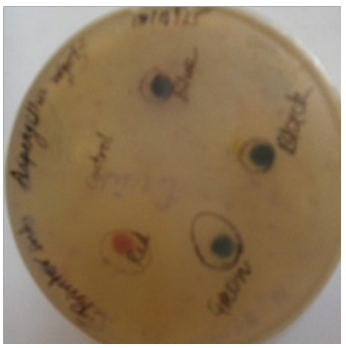

Figure 7: (c)

Sample Bacterial Strain	Morphological Identification	Name of Bacterial Strain after identification
Strain -1	Pink Color, Short rod	Gram -ve
Strain -2	Pink Color, Short rod	Gram -ve
Strain -3	Violet Color, Short rod	Gram +ve
Strain -4	Pink Color, Short rod	Gram +ve

Table 1: Results after gram staining and morphological identification of bacteria in 100x objective lens of microscope

	INDOLE	METHYL RED	VOGES-PROSKAUER	CITRATE
Strain- 1	-ve	+ve	-ve	+ve
Strain- 2	-ve	+ve	-ve	+ve
Strain- 3	-ve	+ve	-ve	+ve
Strain- 4	-ve	+ve	+ve	+ve

Table 2: (a) IMViC Test results.

Name of the Test	Strain-1	Strain -2	Strain-3	Strain- 4
Starch	+ve	+ve	+ve	-ve
Casein	+ve	-ve	-ve	+ve
Glucose	+ve	-ve	-ve	-ve
sucrose	-ve	+ve	+ve	-ve
Lactose	-ve	-ve	-ve	-ve
Glycerol	-ve	+ve	+ve	-ve
Maltose	-ve	+ve	+ve	+ve
Mannintol	-ve	+ve	+ve	+ve
Acetate	+ve	+ve	+ve	+ve
Urease	+ve	-ve	-ve	+ve
Salt tolerance	Low- growth	High -growth	High -growth	Low -growth
Lecithinase	+ve	-ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve

Table 3: Result of different types of Biochemical Test

	Ink	Zone of Inhibition Diameter in CM			
Strain	Sample Control	Blue	Black	Green	Red
S1 Providentia stuartii	0	2.2	2.0	1.8	1.5
S2 Serratia odorifera	0	1.5	1.1	0.9	1.3
S3 Bacillus megaterium	0	1.3	0.8	1.0	1.4
S4 Pseudomonas antimicrobica	0	1.0	1.5	1.1	1.2

Table 4: Zone of Inhibition in Gel pen ink by Well Diffusion method

	Ink	Zone of Inhibition Diameter in CM			
Strain	Sample Control	Blue	Black	Green	Red
S1 Providentia stuartii	0	2.1	0.7	1.1	1.85
S2 Serratia odorifera	0	1.9	1.0	1.7	1.6
S3 Bacillus megaterium	0	2.1	1.3	1.8	1.05
S4 Pseudomonas antimicrobica	0	1.5	0.85	1.9	0.9

Table 5: Zone of Inhibition in Pure Ball Point Pen Ink by well Diffusion Method

	Ink	Zone of Inhibition Diameter in CM			
Strain	Sample Control	Blue	Black	Green	Red
Printer Ink	0	1.2	1.0	1.1	1.5
Gel Pen Ink	0	1.9	1.4	1.55	1.7
Ball Pen Ink	0	2.2	1.5	1.9	2.0

Table 6: Zone of Inhibition when different types of ink on Aspergillus niger.

of unknown bacterial strains is performed by using Bergey's manual.

The results showed that Strain 1 – Providentia stuartii (97% similarity), Strain 2 – Serratia odorifera (88% similarity), Strain 3 – Bacillus megaterium (80% similarity), Strain 4 – Pseudomonas antimicrobica (85% similarity).

Results of effect of different types of ink on document microbes by well diffusion method:

1. Well diffusion method to study the effect of gel pen ink against bacterial isolates:

According to the effect of different colour gel pen ink, zone of inhibition of different diameter were observed after 24hr. of incubation which were described in figure 4(a), 4(b), 4(c)and4(d).

In reference to the above mentioned table, it

has shown that on strain 1, strain 2 and strain 3 blue color gel pen has maximum inhibitory effect, whereas, in case of strain 4 black color gel pen has shown maximum inhibitory effect and blue color has minimum inhibitory effect.

2. Well diffusion method to study the inhibitory effect of ball pen ink against bacterial isolates:

According to the effect of different color ball pen ink zone of inhibition of different diameter were observed after 24hr. of incubation which were illustrated in the following figures 5(a), 5(b), 5(c), 5(d) respectively:

In pure printer ink no inhibitory effect was observed on microbes. 100% Microbial growth was observed on petri plates and zone of inhibition were completely absent.

Effect of different type of ink on fungus aspergillus niger by well diffusion method:

These 3 plates were observed after 48 hours of incubation and different zone of inhibition were observed on different plates which were presented in figure 7(a), 7(b) and 7(c).

Table showed that on fungus blue ball pen and blue gel pen ink has more inhibitory effect followed by green color ink. Whereas, in case of printer ink green color ink has shown maximum zone of inhibition.

DISUCSSION

In a similar study conducted by Kamel *et al.,* [2014], Bacillus subtilis and Acrodictysfimicola[6] were isolated from decaying book and papers collected in Erbil city, Iraq. In the present study 5 strains of bacteria and 2 fungal strains were isolated and identified from book papers dated as old as 1958 and 1969 collected from the market of Ranchi city, Jharkhand, India. In the present study an attempt was made to study the effect of ink on the growth of the above mentioned bacteria Providentia stuartii, Serratia odorifera, Pseudomonas antimicrobica, Bacillus megaterium, Streptomyces species and fungus Aspergilllus niger. A similar study was done by Bragulat et. al, in 1991, reported an inhibitory effect of thirteen dyes on the growth of mycelium fungi (Deuteromycetes and Zygomycetes).[7] Other researchers reported that bacterial contamination of books can be identified by using genera of Pseudomonas, bacillus, micrococcus and clostidium.[8]

As per the literature survey, present study focuses on the isolation and identification of microorganism from decaying papers of books and to study the effect of ink on these microorganisms.[9,10] In this study, variety of dyes including dichloran methylene-blue, aura-mine, phenol-red, rose-bengal and gentian-violet were used. The result showed that the bacterial genera of Providentia, Serratia, Bacillus, Pseudomonas and fungus Aspergilllus niger were isolated. It has been found that species of Aspegillus can degrade cellulose and they are often associated by the holdings of library as found in the study of Konkol *et al.,* in 2009. In present study also Aspergillus was isolated from degraded book samples, so it can be said that Aspegillus degrade the documents. In the present study, the effect of ink was studied by Agar well diffusion method and result of Gel pen ink of blue and black color shows maximum zone of inhibition against Strain S1 – Providentia stuartii. Blue colour of ball pen ink shows maximum zone of inhibition against S1 – Providentia stuartii and S2 – Serratia odorifera , in S3 – Bacillus megaterium red colour ink showed maximum inhibition but in Strain-4 Pseudomonas antimicrobica black colour shows maximum zone of inhibition followed by green colour gel pen ink.In ball pen ink blue coloured ink showed maximum inhibition against S1, S2, S3 and green colour showed maximum inhibition against S4.In pure printer ink no inhibitory effect was observed on microbes. 100% Microbial growth was observed on petriplates and zone of inhibition were completely absent. Against Aspergillus niger inhibitory effect of all colour ball pen ink and Gel pen ink shows maximum result and inhibitory effect of Printer ink was also observed, whereas on bacteria inhibitory effect of pure printer ink was completely absent. On Fungi Aspergillus niger green colour printer ink showed maximum inhibition whereas Blue colour of gel pen ink

and ball pen ink showed maximum inhibition followed by green coloured ink Whereas, in case of printer ink green colour ink has shown maximum zone of inhibition.

CONCLUSION

Paper is a very fragile document and susceptible to attack by microbial and fungal growth. Hence, their preservation and storage is very important. In this regard, inks from the present study have shown that they are significant in inhibitory growth of certain microorganisms. In the present study five bacterial strains and two fungal strain were isolated from two different book samples collected from Ranchi City, Jharkhand (India). The strains which were isolated were identified as:

Bacterial Strains

Strain1 – Providentia stuartii (97% similarity); Strain2 – Serratia odorifera (88% similarity); Strain3 – Bacillus megaterium (80% similarity) ;Strain4 – Pseudomonas antimicrobica (85% similarity)

Fungal Strains

Aspergilllus niger Kavkler et al. in 2011 claim the fungi are the main cause for the degradation of cellulose and cellulose and cellulose containing items. Fungi apparently attack first the cuticle and then penetrate the lumen of the fibre degrading it from inside to out. In the present study, the effect of ink was studied by Agar well diffusion method and result of Gel pen ink of blue and black color shows maximum zone of inhibition against Strain1 – Providentia stuartii. Blue color of ball pen ink shows maximum zone of inhibition against Strain1 – Providentia stuartii and Strain2 – Serratia odorifera , in Strain 3 – Bacillus megaterium red colour ink showed maximum inhibition but in Strain 4 Pseudomonas antimicrobica black colour shows maximum zone of inhibition followed by green colour gel pen ink.In ball pen ink blue coloured ink showed maximum inhibition against S1, S2, S3 and green colour showed maximum inhibition against S 4. In pure printer ink no inhibitory effect was observed on microbes. 100% Microbial growth was observed on petriplates and zone of inhibition were completely absent. Against Aspergillus niger inhibitory effect of all colour ball pen ink and Gel pen ink shows maximum result and inhibitory effect of printer ink was also observed, whereas on bacteria inhibitory effect of pure printer ink was completely absent. On Fungi Aspergillus niger green colour printer ink showed maximum inhibition whereas Blue colour of gel pen ink and ball pen ink showed maximum inhibition followed by green coloured ink. In CFU, the quantitative analysis, maximum growth was observed in case of printer ink and in Pseudomonas strain that means very less inhibitory effect on microbial growth. Hence, based on the present study certain recommendations can be made on the type of ink that is most suitable for writing important documents such as wills which would have a storage value for a longer time. As these type of ink such as blue ball pen ink, green ink also tend to preserve the fibres of the paper cellulose. Care should also be taken that the strains of Pseudomonas should be clenched away from the document as and when possible.

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ORIGINAL ARTICLE

Role of Oxidative Stress Associated Molecular Diagnostic Signatures in the Personal Identification of Rheumatoid Arthritis Patients

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ABSTRACT

CONTEXT: Early interpretation of toxic effect of free radicals leading to cardiovascular diseases (CVDs) in autoimmune disorders is a quench thirst of therapeutic intervention and personal identification. **AIM:** The study aims to evaluate the expression of Nrf2, NQO-1 and LpPLA2 genes along with marker of endothelial dysfunction and oxi-inflammatory stress in active RA patients and to enlighten the assessment of study group markers in personal identification of RA patients for their early therapeutic intervention and prevention of vascular complications.

MATERIALS & METHOD: 64 active RA patients between 35-55 years and 64 healthy controls were recruited from North India region. Using specific primers, mRNA expression of Nrf2, NQO-1 & LpPLA2 genes were evaluated in blood by qPCR. 2 –ΔΔCT method was used to determine the fold change. Brachial artery flow mediated diameter (FMD), total antioxidant activity, malondialdehyde, TNF-α, IL-6 & hs-CRP levels were estimated by using standard methods followed by appropriate statistical analysis of data. **RESULTS:** Increased expression of NQO-1, Nrf2 and LpPLA2 was observed in RA patients along with marked altered levels ($p<0.05$; significant) of oxi-inflammatory markers which may be due to compensatory activation of antioxidant defense mechanism. Remarkably, FMD% was significantly low ($p<0.05$) and inversely associated with the expression of NQO-1, Nrf2 and LpPLA2, which highlighted the culprit effect of oxi-inflammatory stress in inducing altered vascular homeostasis. **CONCLUSIONS:** Thus, combinational analysis of molecular diagnostic signatures associated with toxic free radicals along with FMD measurement exhibits a great promise in personal identification of RA patients for early therapeutic intervention, mainly, by targeting the oxi-inflammatory stress mediated cytoprotective pathway, and thereby, reducing the burden of CVD morbidity and mortality in RA patients.

KEYWORDS | oxi-inflammatory stress, lipoprotein-associated phospholipase a2, keap1/nrf2 endothelial dysfunction, flow mediated diameter

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INTRODUCTION

RHEUMATOID ARTHRITIS (RA) is a multifaceted systemic inflammatory autoimmune disorder, driven by inexorable and stochastic accumulation of damage of biomolecules vital for proper synovial function. The incidence of RA increases during every decade of life and affects the quality and expectancy of human life in developed and developing countries as well.[1] Due to augmented toxic free

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radicals and inflammatory insult with aging process, the joints or bone become damaged causing the physical disability and restricted motion. In addition, in the wake of urbanization, altered metabolic profile, sedentary life habits, increased body weight, aging, smoking, positive rheumatoid factor, disease severity, formation of anti-cyclic citrullinated peptide (CCP) antibodies and genetic factors have a significant impact in establishing CVD risk in RA patients.[2,3] However, the exact clinico-molecular mechanistic pathway underlying this curse is still obscure.

Interestingly, oxi-inflammatory stress in combination with regulation of gene expression involved in cytoprotective pathway has been suggested as a common denominator and contributing factor in inducing endothelial dysfunction followed by atherosclerosis in rheumatological conditions.[4] Recently, a lot of interest has been generated to understand the role of Keap1/Nrf2/ARE pathway in elucidating the relationship between chronic oxi-inflammatory stress and altered vascular homeostasis in active RA patients. This pathway regulates cellular detoxification process and redox status.[5]

Previous studies have considered the role of Nrf2 as a multi-organ protector and its involvement in various diseases associated with oxi-inflammatory stress such diabetes, arthritis, obesity, hypertension and metabolic dysregulation.[6]

NADPH quinine oxidoredcutase (NQO-1) gene is an important downstream target of Nrf2 and involved in cellular antioxidant response by encoding NADPH oxidase, which acts as a potent antioxidant.[7] Thus, expression of NQO-1 may affect the production and removal of reactive oxygen and nitrogen species (RONS). Although, NQO-1 is shown to have an effect on RA pathology in rodents,[8] there is a scarcity of literature on human studies highlighting the role of Nrf2, NQO-1 and Total antioxidant activity (TAA), at a single platform, in maintaining the redox homeostasis.

Apart from Keap1/Nrf2/ARE pathway and TAA, lipoprotein-associated phosholipase A2 (LpPLA2) is a circulating inflammatory biomarker secreted by macrophages and monocytes, and exhibits proinflammatory and proatherogenic role. Independent studies on human and experimental animal models revealed the increased activity of LpPLA2 and expression, as well, in inflammatory conditions.[9] However, not enough literature regarding the expression studies of Keap1/Nrf2/ARE pathway along with NQO-1 and LpPLA2 in active RA is available.

Early pathophysiological alterations in vascular disease arise due to RONS mediated endothelial dysfunction.[10] Interestingly, a repertoire of rheumatic disease researches revealed the importance of non-invasive approach in the detection of vascular health.[11] Recently, the assessment of brachial artery flow mediated dilation (FMD) in RA patients is a subject of research interest for cardio-rheumatologists. Altered expression of genes of cytoprotective pathway, inflammation and endothelial dysfunction may be an interconnected link in accelerated vascular complications; however, the toxic effect of free radical in inducing endothelial dysregulation in rheumatic complications has not been fully elucidated. Therefore, the objectives of present study aimed to enlighten the plausible connecting link between expression of genes of cytoprotective pathway against toxic free radicals and inflammation along with marker of endothelial dysfunction in active RA patients; and to provide a great promise in personal identification of RA patients not only for early interpretation of CVD risk by assessing toxic free radicals mediated destruction but also pave the way of therapeutic intervention as well.

METHOD AND MATERIALS

Subjects

The present study protocol was approved by the research and ethics committee of School of Medical Sciences and Research, Sharda Hospital, India. A total 157 active RA patients

visited outpatient clinic of the hospital with joint complaint over a period of two years (October 2017 to September 2019). In the present case-control study, only 64 active RA patients of either sex belonging to age group 35-55 years were recruited from North India region. 68 patients did not meet the inclusion criteria and 25 patients refused to participate. 64 age-matched healthy individuals were recruited as controls from college staff and their family members, after taking their informed consent.

Inclusion Criteria: Criteria recommended by the American Rheumatism Association were used for the diagnosis of RA.[12] Written informed consent was obtained from all the subjects included in the study. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects The recruited patients had active RA, defined as the presence of at least three of the following criteria: six or more tender joints; three or more swollen joints; ≥30 minutes of morning stiffness and an erythrocyte sedimentation rate of ≥28 mm/h. The number of swollen and tender joints (28 joint count) and patient's assessment of pain on Visual Analog Score (VAS) were registered. Disease activity score-28 (DAS28) were calculated using erythrocyte sedimentation rate.[13] The level of RA disease activity was interpreted as low (DAS28 ≤3.2), moderate (3.2 <DAS28 ≤5.1) and severe disease activity (DAS28 >5.1).

Height was measured by using wall mounted scale where weight was measured with subjects standing barefoot and lightly dressed by using digital weighing machine. The Body Mass Index (BMI) was calculated as [BMI = Weight (kg) / Height (metre 2)]. Blood pressure was measured by mercury sphygmomanometer using auscultatory method. To diminish any confounders developed by other arthritic complications, patients with positive rheumatoid factor were recruited and their disease duration was recorded. However, RA patients with family history of arthritis and hypertension were not excluded. In addition, RA patients who had previously under any medical treatment including supplementation of antioxidants or non-steroidal anti–inflammatory drugs were not excluded from the study if the subject agreed that no supplements or analgesic drug would be taken in the seven days before entry into the study. However, there was no restriction or withdrawal on the conventional anti-rheumatoid drugs treatment.

Exclusion Criteria: None of the patients and control subjects had family history of concomitant diseases, such as diabetes mellitus, hepatitis, renal failure and neurological disorder. In addition, patients with established cardiovascular complications, pregnancy, lactation, obesity (BMI>25), Stage I and stage II hypertension (BP>129/89 mmHg), smoking habit, renal failure, liver disease, hypothyroidism or who did not follow study instructions were also excluded from the study.

Methods

Fasting venous blood sample was collected into EDTA (8ml) and plain (2ml) vials from the study group subjects after confirming their inclusion criteria. Whole blood (2ml) was used for gene expression analysis. For the estimation of study group parameters, plasma and serum were separated from rest of the collected blood sample by centrifugation at 1000g for 15 minutes at room temperature and stored at -80°C until use.

Gene Expression Analysis

Total RNA was isolated from whole blood using TRI-reagent BD from Sigma Chemicals, USA; as per manufacturers' instruction. The quality of RNA was checked by taking the optical density ratio at 260/280; a ratio of 1.8-2.0 was considered adequate. The reaction for cDNA synthesis was carried out using 200U reverse transcriptase (Revert Aid from Thermo Scientific, Inc., USA), 500ng of RNA, 40 U ribonuclease (RNase) inhibitor (Thermo Scientific Inc., USA), 10 mM dNTPs, 100pM

random hexamer and oligo dT in the ratio 1:1 (Sigma Aldrich, India), 4µl of 5 x reaction buffer and the final volume made to 20 µl with diethyl pyrocarbonate (DEPC) treated water. Incubation was carried out at 25ºC for 10 min, followed by 45ºC for 60 min. After that, reverse transcriptase was inactivated at 70ºC for 10 min in a thermocycler (Biorad CFX Connect), and resultant cDNA was stored at -20ºC and used as template sample for qPCR. In order to analyze the relative expression of the genes by real time polymerase chain reaction (RTPCR), specific primers are used.[14] The primer sequences of all the genes are as below:

1) Nrf2 (forward-5'ACACGGTCCACAGCTCATC-3' and Reverse-5'-TGTCAATCAAATCCATGTCCTG-3')
2) NQO-1 (forward-5'GGCAGAAGAGCACTGATCGTA-3'and Reverse-5'-TGATGGGATTGAAGTTCATGC -3')
3) LpPLA2 (forward-5'CCACCCAAATTGCATGTG-3' & Reverse-5'-GCCAGTCAAAAGGATAAACCACAG-3'
4) α-actin (forward 'TCATGAAGTGTGACGTTGACATCCGT-3' and Reverse-5'-CCTAGAAGCATTTGCGG TGCACGATG-3')

For real time PCR of Nrf2, NQO-1 and LpPLA2 along with internal reference gene or housekeeping gene (β-actin), 0.5µl of above mentioned forward and reverse primers, 10µl of Hot-Start PCR master mix (Thermo Scientific Inc., USA), 1µl of diluted cDNA, 1µ l of 1:100 diluted syto9 dye and final volume made upto 20µl with DEPC treated water were used. For all the genes, cycling conditions were the same but annealing temperature was different (hold 95ºC for 4mins, cycling for 35 cycles; 95ºC for 15 sec, annealing at 54ºC temperature for 30 sec and 72ºC for 30 sec). All the reactions were run in duplicates and fluorescence was obtained at 72ºC. ΔΔcT method was used to analyze the relative expression of the gene and 2-ΔΔcT method was used to calculate the fold change.

Biochemical Analysis

Plasma hs-CRP (Calbiotech, USA; sensitivity less than 0.005mg/ml), TNF-α (Diaclone, France; sensitivity less than 8pg/ml), IL-6 (R&D Systems, USA; sensitivity less than 0.7 pg/ml) levels were measured using commercially available ELISA kits, according to manufacturer's instructions. Routine biochemical parameters were assayed in automated analyzer using commercial kits. All these investigations were carried out once at the time of entry into the study. Plasma lipid profile contents (Total Cholesterol, Triglycerides and HDL-cholesterol) were analyzed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). LDL-cholesterol levels were calculated by Friedwald's formula.[15]

LDL-C = TC - [(TG/5)+HDL-C]

Serum MDA levels were estimated by thiobarbituric acid (TBA) reaction.[16] Serum lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid (pH 2-3) and boiled with thiobarbituric acid which reacts with Malondialdehye, forming a MDA-TBA to get pink color. The pink colored complex that occurred was refrigerated to room temperature and measured by using a spectrophotometer at 530 nm.

Plasma total antioxidant activity was estimated spectrophotometricaly by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide, i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of Thiobarbituric acid reactive substances (TBARS). Antioxidants from the added plasma cause the suppression of produc*tion of TBARS. The reaction was measured spectrophotometrically at 532 nm.*[17]

Radiological vascular analysis:

To determine endothelial function, brachial artery flow mediated diameter percent (FMD%) was performed in a subject with overnight

fasting (of at least 10 hours) in the morning, in a quiet and dark room under controlled ambient temperature (20°C to 26°C). After 10 minutes of rest in a supine position, the right arm of the subject was comfortably immobilized in the extending position. Approximately 5-10 cm above the antecubital fossa, ultrasound scanning of brachial artery was performed. After inflation of a cuff to a suprasystolic pressure (40-50 mmHg above systolic pressure) for about 5 minutes, the vessel images were recorded. Post dilation of a cuff, the brachial artery diameter image was taken and recorded for 3 minutes. Brachial artery FMD% more than 10% is considered as normal response whereas FMD% values less than 10% is considered as endothelial dysfunction and subject is susceptible to develop future CVD complications.[2]

Statistical Analysis

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. To compare the parametric data between two groups, paired Student's t test was performed whereas Whitney U test was performed for non-parametric data. The distribution of 't'-probability was calculated depending on 'n' and significance of test was obtained. Correlation studies were carried out to evaluate the relationship between different parameters. For parametric data, Pearson correlation coefficient was used whereas Spearman rho's correlation was used for non-parametric data. 2-ΔΔcT method was used to calculate the fold change in expression and ΔcT was taken as one of the variable to perform correlation studies by using Pearson correlation coefficient. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

RESULTS

Demographic and biochemical profile of the patients:

The anthropometric, clinical and biochemical parameters are shown in Table 1. All the 64 active RA patients were aged 35-55 years and there was no significant difference in mean age, BMT and blood pressure between the patient and control group. Among recruited 64 active RA patients, 67% were females and 33% were males whereas in control group 64% were females and 36% were males. Out of 64 patients, 14 patients were overweight and 48 patients (75%) were pre-hypertensive as per JNC 7th guidelines. However, they were not taking any antihypertensive drug and were being managed by diet and exercise. The ESR level of RA patients was significantly high ($p<0.001$; 28% high) and disease duration was 28.7 ± 3.5 months. RA patient population had a moderate disease activity with a mean DAS28-ESR of 4.27 ± 0.26.

As compared to normal healthy controls, marked occurrence of atherogenic profile along with abnormalities in lipid profile contents were observed in active RA patients (Table 1.0). Plasma total cholesterol ($p < 0.05$), triglycerides ($p < 0.05$) and LDL cholesterol ($p < 0.001$) levels were found to be increased significantly in active RA patients as compared to that of healthy controls Moreover, statistically significant low HDL-cholesterol levels ($p < 0.05$) along with, high atherogenic index (TC/ HDL-C ratio was higher than five) in active RA patients revealed the increased risk of atherosclerotic complication.

Comparative analysis of the markers of oxi-inflammatory and endothelial dysfunction:

The results of present study revealed statistically significant changes in the marker of endothelial dysfunction and oxi-inflammatory stress in the study group patients. The changes in FMD% and plasma TAA along with serum MDA levels in active RA patients and control group were represented in Fig. 1. FMD% value (35.90% low; $p = 0.001$; Fig. 1) and plasma TAA level (31.62% low; $p = 0.004$; Table/Fig. 5) were significantly low whereas serum MDA level was found to be significantly high (29.96% high; $p = 0.002$) in RA patients as compared

to controls. Similarly, plasma hs-CRP (47.05% high; p = 0.000) and TNF-α (42.06% high; p = 0.002) and IL-6 (33.58% high; p = 0.001) levels were found to be increased significantly in RA subjects as compared to healthy controls (Fig 1) which reflect the etiopathological role of inflammation in active RA patients.

Gene Expression Analysis

We observed higher level of mRNA expression of Keap1/Nrf2/ARE pathway and LpPLA2 gene in active RA patients as compared to healthy controls. Taking α-actin as reference gene, it was observed that the fold change in mRNA expression of Nrf2 and NQO-1 in whole blood was 1.7 and 1.9 respectively. Moreover, mRNA expression of LpPLA2 was 3.8 times higher in active RA patients. Interestingly, previous studies revealed that Nrf2 has a role in the maintenance of vascular integrity; we therefore compared Nrf2 expression in active RA patients with normal blood pressure (BP >120/80 mmHg) and pre-hypertension (BP < 129/89 mmHg). Increased blood pressure was found to be associated with 4.5 times higher mRNA expression of Nrf2 in active RA patients.

Correlational analysis of the biochemical parameters

Correlation studies revealed that mRNA expression of Nrf2 was significantly correlated with expression of NQO-1 (r = 0.533, p = 0.05) and LpPLA2 (r = 0.658, p = 0.02), as shown in Figure 2. Remarkably, we observed a negative correlation between Nrf2 gene expression with FMD% and TAA, whereas marker of lipid peroxidation (MDA) and inflammation such as hs-CRP, TNF-α, IL-6 and ESR levels were positively correlated with Nrf2 (Figure 3, p<0.05) which indicates the association of Keap1/Nrf2/ARE pathway induction with oxi-inflammatory stress and altered vascular homeostasis in active RA patients. Similarly, mRNA expression of Nrf2 was positively correlated with VAS pain score, DAS-28 score and disease duration (Figure 3). These results clarify the role of Nrf2 gene expression in the pathophysiological manifestation of active RA most probably by its relation with pain sensation, clinical symptoms with severity of disease.

DISCUSSION

Over the past several decades, a myriad of studies on RA patients emphasized the need of effective diagnostic approach for early interpretation and therapeutic intervention to mitigate the risk of cardiovascular disease (CVD) in RA.[18] Unfortunately, despite massive efforts, scientists have failed to reveal the secrets of CVD complications in RA patients and CVD threat among rheumatic disease is still looming large. Recently, studies focused on regulatory modulation of the expression of genes involved in cyto-protection have received much attraction among cardio-rheumatologists. In this context, the cytoprotective role of Keap1/Nrf2/ARE pathway by means of activating the antioxidant signaling network against oxi-inflammatory stress mediated cytotoxicity has opened new avenues to enlighten the mechanistic pathway of rheumatic diseases & its related drug development. In the present study, we evaluated the expression of genes involved in cytoprotective pathway which include Nrf2 and its downstream target NQO-1. mRNA expression of Nrf2 and NQO-1 were higher in active RA patients. It could be explained on the basis of oxi-inflammatory stress mediated induction of Nrf2/ARE pathway as a result increased expression of Nrf2 gene takes place in combination with reduced ubiquitination and proteasomal degration of Nrf2 which inturn facilitates the enhanced expression of NQO-1 in RA patients.

NADPH quinine oxidoredcutase (NQO-1) is an important antioxidant enzyme which not only contributes significantly in providing protection against augmented oxidative stress but also assigned with multiple protective roles.[7] Higher expression of NQO-1 along with its positively correlation with Nrf2 expression authenticate the contention that activation of antioxidant defence mechanism takes place

in active RA patients. Moreover, DAS28, VAS and disease duration of RA were positively correlated with Nrf2 expression and appeared as independent predictors of cytoprotective pathway activation in RA patients (Figure 3). Similarly, Wruck et al documented the activation of Nrf2 gene in both the joints of antibody induced arthritic mice and RA patients in order to maintaining the cellular defense against oxidative stress.[19] According to Bozbus and Sendur, ozone therapy inhibits chronic inflammation in disorders such as RA. The effect could be attributed to the activation of antioxidant defense through the Nrf2 which is characterized by increased transcription of various antioxidant and phase II detoxification enzymes.[20]

Existence of oxi-iflammatory stress is a common thread which connects RA pathology and cardiovascular complications. Pro-inflammatory cytokines such as IL-6, TNF-α, produced by T-lymphocytes stimulate the release of tissue-destroying matrix metalloproteinases and pro-inflammatory enzymes which eventually lead to degeneration of cartilage extracellular matrix and thereby play a crucial role in RA pathology.[21,22] In addition, oxidative stress triggered by activated neutrophils during inflammatory reactions leads to lipid peroxidation of chondrocytes, mediates collagen degradation, promotes atherosclerotic plaque formation, prostacyclin synthesis, enhancement of cytosolic free calcium and peripheral vascular resistance.[23] Interestingly, alteration in total antioxidant activity and increased levels of lipid peroxides induces the loss of homeostasis in vascular system that eventually elaborates the degeneration of vascular endothelium followed by cardiac complications.[10]

In the present study, plasma TAA levels were decreased significantly whereas serum MDA levels along with markers of systemic inflammation (hs-CRP, IL-6 and TNF-α) were significantly high in RA subjects (Figure 1), which suggest the role of enhanced lipid peroxidation and systemic inflammation along with reduced antioxidant status in the progression and development of CVD complications in RA patients. Correlation studies also revealed that hs-CRP, ESR, IL-6 TNF-α and MDA were positively correlated with Nrf2 expression and TAA was inversely associated with Nrf2 which supports the substantial involvement of cytoprotective pathway activation due to oxi-inflammatory stress in active RA patients (Fig. 2 and 3). Similarly, Saxena et al. estimated the level of C-reactive protein, superoxide dismutase, catalase, glutathione peroxidase and ceruloplasmin in active RA patients and suggested that combined effect of inflammation and free radical generation is involved in the pathogenesis of active RA.[24] Consistent findings have been reported by Dudeja et al.[25] They evaluated the plasma total antioxidant activity along with the markers of systemic inflammation, oxidative stress and metabolic profile in RA patients. In addition to dyslipidemia, they observed a marked reduction in plasma TAA along with enhanced CRP, MDA and synovial IL-6 levels in RA patients. They also suggested that these inexorable alterations contribute significantly to the progression of vascular complications in rheumatic diseases and future drugs of RA could be developed to target the non-traditional CVD risk factors also.

Moreover, LpPLA2, a marker of vascular inflammation, has been found to be associated with cardiovascular complications. It circulates with low density lipoproteins LDL) and high density lipoproteins (HDL) and acts on the oxidized phospholipids, ensuing the production of lysophospholipids and oxidized fatty acids. It has a direct role in the causal pathway of plaque inflammation and implicated in inducing enhanced risk of atherosclerotic event.[9] Sodergren et al. in their cohort of early RA patients from Northern Sweden also observed that the increased concentration of LpPLA2 was associated with enhanced inflammation leading to both subclinical atherosclerosis and disease activity.[26] In the present study, mRNA expression of LpPLA2 was higher in active RA patients and was positively correlated with

PARAMETER	CONTROL GROUP (N=64)	PATIENT GROUP (N=64)	P-VALUE
Age (years)	43.5 ± 5.0	46.4 ± 4.8	0.158
M:F ratio	23/41	21/43	-
Height (meter)	1.58 ± 0.029	1.59 ± 0.030	0.201
Weight (Kg)	59.4 ± 1.6	62.5 ± 2.5	0.069
BMI (Kg/m2)	23.2 ± 1.4	27.6 ± 1.5	0.050
Systolic blood pressure (mm Hg)	109.5 ± 4.58	119.42 ± 5.42	0.042
Diastolic blood pressure (mm Hg)	75.8 ± 3.9	80.5 ± 4.85	0.030
VAS (mm)	0.0	37.08 ± 4.5	0.001
ESR (mm/h)	15.7 ± 2.30	34.4 ± 3.48	0.004
DAS28	0.0	4.27 ± 0.26	0.078
Total Cholesterol (mg/dl)	154.78 ± 7.64	195.36 ± 11.21	0.050
Triglycerides (mg/dl)	107.25 ± 7.9	132.5 ± 9.0	0.071
HDL cholesterol (mg/dl)	44.2 ± 3.15	33.54 ± 3.20	0.004
LDL cholesterol (mg/dl)	91.54 ± 7.67	138.50 ± 7.52	0.001
Atherogenic index	3.47 ± 0.73	5.94 ± 1.52	0.052

Table 1: Anthropometric, clinical and biochemical profile of Patient and Control groups (Mean ± SD)

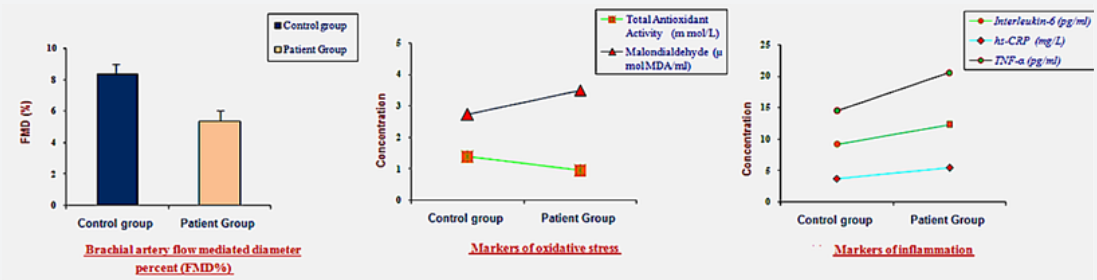

Figure 1: Endothelial dysfunction and oxi-inflammatory markers in study group subjects

where,
*p<0.1: Non-significant,
**p<0.05: Significant,
***p<0.001: Highly significant
BMI: Body mass index;
ESR: Erythrocyte sedimentation rate.
DAS: Disease activity score;
VAS: Visual analogue scale
HDL: High density lipoprotein;
LDL: Low density lipoprotein.

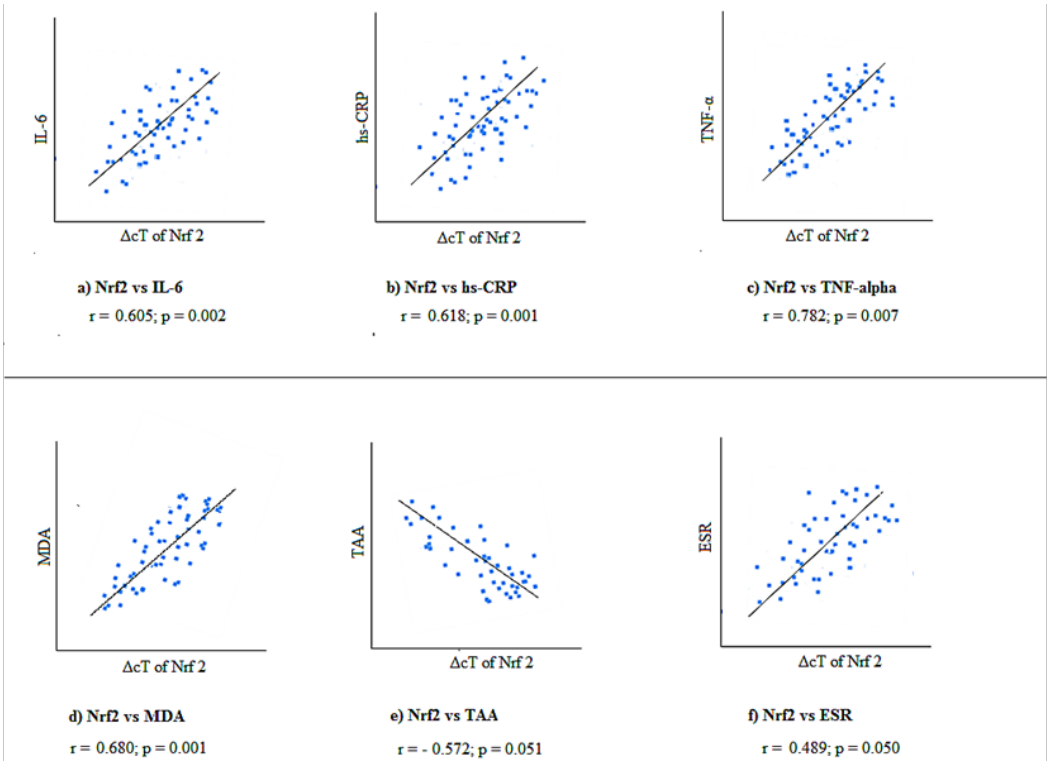

Figure 2: Correlation of Nrf2 expression with markers of Oxi-inflammatory stress in active Rheumatoid arthritis patients

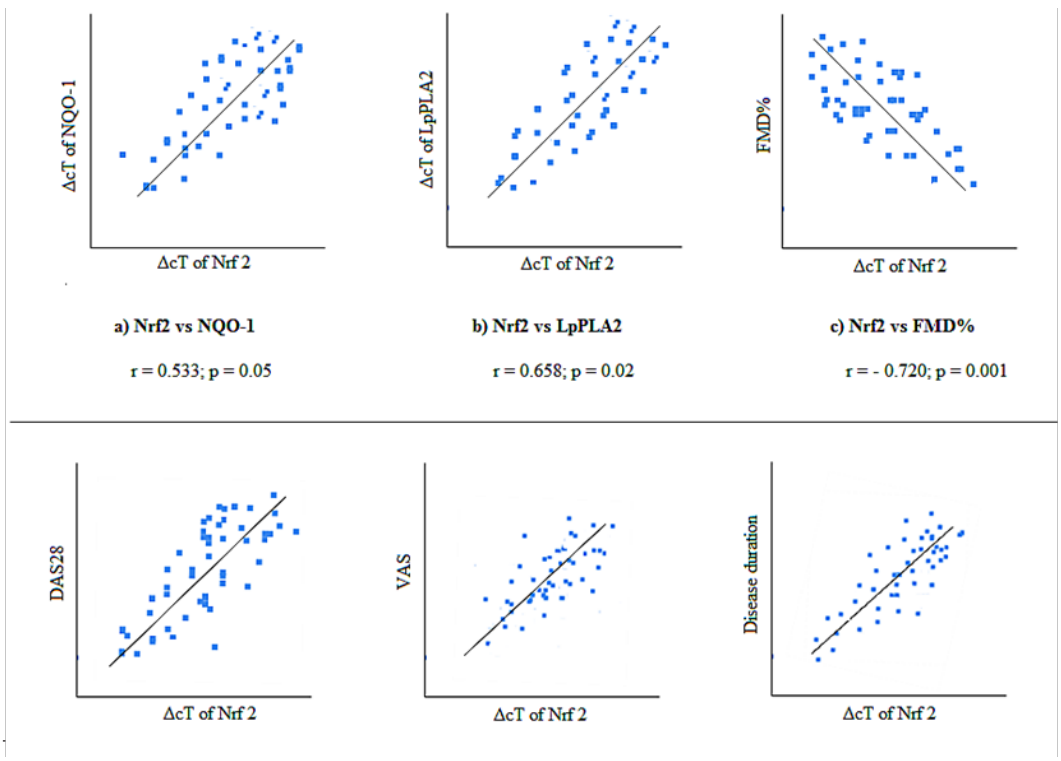

Figure 3: Corelation of Nrf2 expression with several variables in active RA patients.

Nrf2 expressions (Fig. 3) which reflect the role of enhanced inflammation in induction of cytoprotective pathway. Moreover, previous studies pertaining to association of expression of LpPLA2 with hypertension, hyperglycemia, insulin resistance, dyslipidaemia, and abdominal obesity have been documented.[14] These results suggest the regulation of LpPLA2 expression is an attractive therapeutic target candidate as its inhibition not only mitigate the production of pro-inflammatory factors but also its associated consequent sequels such as vascular complication and RA pathogenesis as well.

Our study also revealed that the mRNA expression of Nrf2 was 4.5 times higher in blood in pre-hypertensive RA patients as compare to normotensive RA patients indicating that Nrf2 have some role in endothelial vascular redox homeostasis. Moreover, altered endothelial redox homeostasis as observed in present study and characterized by low FMD% in active RA patients (Figure 1), and its inverse relation with Nrf2 expression (Figure 3) could be explained on the basis of the compensatory vasculo-protective effect of Nrf2 against augmented oxi-inflammatory stress. Similarly, Adawi et al. showed marked reduction in brachial artery flow mediated dilation in RA patients and emphasized the assessment of FMD% as an early predictor of atherosclerosis in RA patients.[2] Matinez-Hernandez et al. also analysed the association between metabolic syndrome (a constellation of cardiovascular risk factors) and genes involved in oxidative stress among Mexican Mestizos. They suggested that NQO-1 gene polymorphism is associated with a high risk of metabolic disorders, including high blood pressure, hypertriglyceridemia and low HDL-c levels.[27] Apart from this, Zakkar et al. reported that activation of Nrf2 reduces the endothelial cell from exhibiting a proinflammatory state at atherosusceptible sites via suppression of p38-VCAM-1 (adhesion molecule) signaling and may provide a therapeutic strategy to halt atherosclerotic complication.[28] Nevertheless, our study revealed the expression of cytoprotective and inflammatory genes along with oxi-inflammatory markers in whole blood in RA patents only. However, the cause and effect relationship between product of these genes and various other markers in synovium needs to be evaluated further to shed more light in personal identification of RA patients.

CONCLUSION

Thus, it can be inferred that higher expression of LpPLA2 and enhanced free radical mediated cellular toxicity leads to the compensatory induction of genes of cytoprotective pathway, i.e. Nrf2-ARE axis in active RA patients. Nevertheless, this study also reveals that induction of cytoprotective pathway has a role in alteration of vascular homeostasis and metabolic profile in RA patients. In toto, it is obvious that assessment of oxi-inflammatory stress markers produced due to toxic free radicals, is responsible for altered gene expression, vascular homeostasis and elevated atherogenic index and thus, used as a tool for personal identification of RA patients to identify the risk of CVD complications. Moreover, Keap1/Nrf2/ARE axis may be an effective "treat to target" approach from a lens of therapeutic intervention strategy in treating RA and its associated complications. Nevertheless, more investigation and continued international collaboration are required to adopt evidence based molecular diagnostic signatures for the personal identification of CVD risk in the patients of rheumatic diseases. IJFMP

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Conflict of Interest:

The authors declare that there is no commercial or financial links that could be construed as conflict of interests.

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ORIGINAL ARTICLE

Comparative Studies on Degradation of Forensic Biological Fluids Recovered from Crime Scene

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ABSTRACT

CONTEXT: Evidence plays crucial role in forensic investigation in solving criminal cases. These evidences should be properly collected and preserved to avoid degradation and loss of evidential value. Hence, this paper describes the experimental study of collected and degraded biological fluids which can be recovered from the crime scene.

AIMS: The study aims to assess the microbial growth on different biological fluids recovered from crime scene in percolation with varied temperature durations. **SETTINGS AND DESIGN:** An experimental setup was designed to study the different biological fluids and their variation of impact on different conditions like type of sample, temperature differences and time of exposure. **METHOD AND MATERIALS:** Biological fluids such as saliva, urine, semen and vomit were collected and used for the study. The identification procedure of microorganisms and extent of degradation was studied by means of physical analysis, bacterial culture, fungal culture, staining for bacteria and fungus, and biochemical testing. Followed by assessment of collected samples inoculated with cotton cloth piece with specified time interval and temperatures. **RESULTS:** Candida albicans and Escherichia coli show maximum profuse growth in inoculated urine samples exposed to temperature 37°C with the time interval of 7 to 27 hours and 20-25°C with time interval of 48 hours. Likewise, Pseudomonas aeruginosa shows maximum growth in inoculated saliva samples exposed to temperature ranging from 20-37°C with time interval of 40 hours. Furthermore, the micrococcus mucillogens, proteus vulgaris and streptococcus pneumoniae show maximum growth in inoculated vomit samples exposed to temperature ranging from 20-37°C with time interval of 7-27 hours. Lastly, Micrococcus mucillogens shows maximum growth in inoculated semen sample exposed to temperature ranging 20-37°C with time interval of 48 hrs. **CONCLUSION:** Forensic biological samples are more susceptible to the contamination by the growth of microorganism because of the nutritive substances present in each fluid. Since compositions of each biological fluid are different, therefore types of microbes growing and their effect on samples will also be different leading to destruction of forensic biological fluid samples. This study reveals the determination of microbes such as a Candida albicans, Escherichia Coli, Pseudomonas aeruginosa, micrococcus mucillogens, proteus vulgaris and streptococcus pneumoniae in urine, saliva, vomit and semen samples. Under ambient-conditions of high temperature and with specific time durations, the growth of microbes was found to be rapid. It is also to be concluded that temperature plays a major role in the preserving the integrity of samples, at high temperatures for more time, the samples will get dry and minimal amount of microorganism will grow. **KEY MESSAGES:** Detection and determination of varied microbial growth on different biological fluids of forensic importance provide prudent information to forensic experts for combating the loss of evidential value for trials and for maintaining proper chain of custody. Hence, the study aids forensic experts to collect and preserve biological fluids with in specific time duration and temperature conditions.

KEYWORDS | forensic microbiology, biological fluids, bacterial culture, biochemical tests

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INTRODUCTION

In the field forensic analysis, one of the main drawbacks has been the quantity of samples collected and their purity. Biological samples are more susceptible to contamination.[1] Biological fluid evidence is easily tampered evidence.[2] Evidences collected from the crime scene plays a vital role in solving criminal case.[3] Especially in the case of biological evidence, they are more susceptible to the growth of microorganisms. For this reason, many times the required results are not given by samples for DNA test and non-DNA tests.[4,5] The growth of microorganism in the biological sample will lead to the loss of integrity of the sample.[6] Microbial growth will lead to the destruction of cells, proteins, metabolic products, drugs and other materials present in it, making the analysis a difficult task.[7,8] This is one of the main drawbacks of forensic biological samples. Usually, the samples are collected long after the occurrence of crime.[9] Because of this, the chances for degradation of fluid evidence gets worse. Microbial degradation can be reduced by collecting samples as soon as possible. The compositions of different biological fluids are different, so the effect of microbial growth also will be different.[10]

The growth of microorganisms in different biological samples makes its detection and analysis a very difficult task. One of the most important sample analysis report that is admissible in court is the DNA analysis report. The permissibility of the DNA evidence before the court of law always depends on its accurate and proper collection, preservation and documentation which can reassure the court that the evidence which has been put in front is reliable. In studies it is shown that microbial genome will interfere with the sample genome and makes the DNA typing method difficult. Sometimes the microbial growth will lead to poor PCR amplification or no PCR amplification.[11] For the detection of body fluids, RNAs of degraded samples are taken and it is being detected by transcriptomic analysis. By massively parallel sequencing technology the sample is detected with least possible sample.[12,13] In sexual assault cases biological fluid evidences have great importance, so the samples should not be degraded. In some countries there are certain protocols and guidelines for evidence management but sometimes it will vary from one region to another in the same country. One of the most common sample collected from the crime scene is blood. Because of improper storage, collection and packaging, many of the tests are not giving appropriate results. Studies done on blood samples shown that with the changing temperature, packaging type and environmental conditions will affect the sample very badly leading to the growth of different types of microbes on it. In this study, it is dealing with the analysis of types of microorganisms growing on different biological samples, the effect of outer environment on sample, the effect of temperature and exposure of time is studied.[12] The study reveals that, according to the changing temperature, time and samples, the microbial growth on samples will differ. Samples which are exposed to high temperatures will suffer less degradation in as it gets dried so fast but samples at room temperature for long duration will suffer more microbial degradation. This is due to less temperature condition will take more time for drying the samples.[15] This study helps us to understand how much the samples are degraded, is the sample suitable for analysis and what time is suitable for sample collection.

MATERIALS AND METHOD

Biological fluids used for this study are saliva, urine, semen and vomit. Methods used for the identification of microorganisms and extent of degradation is done mainly by four methods: physical analysis, bacterial culture, fungal culture, staining for bacteria and fungus, and biochemical testing.[9] After collection of samples, physical analysis of color, texture, coagulation status and smell are done. Five samples are collected with specified time interval and temperatures (e.g. 20-25°C and 37°C). Each

SAMPLE	INDOOR	TEMPERATURE (°C)
Urine	Cloth	20-25 and 37
Semen	Cloth	20-25 and 37
Saliva	Cloth	20-25 and 37
Vomit	Cloth	20-25 and 37

Table 1: Temperature and inoculation surface of samples

biological fluid is inoculated into the cotton cloth piece. The cloth piece is exposed to the environment with different time intervals at different temperatures.

So the sample of inoculated cloth is exposed to the environment for 3 hours (as referred to in the literature), after that the first sample is collected and marked as S1, after 9 hours, S2 is collected, after 15 hours S3 is collected, after 21 hours, S4 is collected, after 27 hours S5 is collected. This procedure is carried out twice for each sample, under two different temperatures (20-25°C and 37°C). For each biological fluid, 40 samples are analyzed, so a sum total of 160 samples were analyzed for this entire study.

When each sample is collected, it is inoculated into the culture media like nutrient agar, blood agar, macconkey agar, peptone water (bacterial culture) and sabourdes dextrose agar (fungal culture) for isolation of microorganism.[13,15] Staining for bacteria and fungus is done by staining methods like gram's staining, lacto phenol cotton blue (LPCB), India ink, and motility test. For identification of organism biochemical testing is done by indole test, methyl red test, voges-proskauer test, citrate test, urease test, nitrate reductase test, catalase, coagulase, triplesugar iron agar test, oxidase test as mentioned in Table 1 above.

The minimum average time for collection of sample after the occurrence of crime is 3 hours.[7] After that first sample is collected and marked as S1, after 9 hours, S2 is collected, after 15hours, S3 is collected after 21 hrs, S4 is collected after 27 hrs S5 is collected. This procedure is done twice for each sample, under two different temperatures 20-25°C and 37°C. For each biological fluid, 40 samples are analyzed, so a total of 160 samples are analyzed for this entire study as mentioned in Figures 1-10.

RESULT

Urine Sample (at 37°C)

1. Sample collected after 6 hours of inoculation is not showing any microbial growth, which indicates that up to6thhour after occurrence of crime the sample is intact.

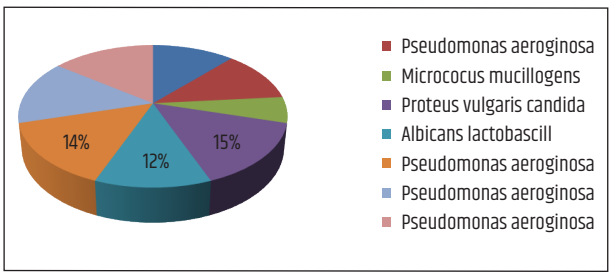

Figure 1: Urine sample (at 37oC)

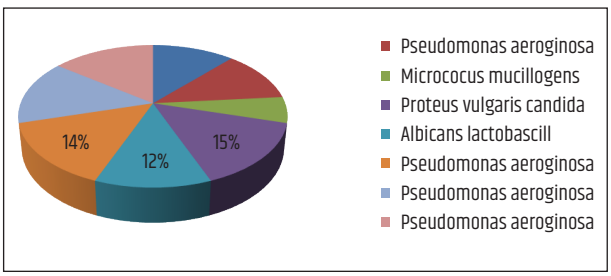

Figure 2: Urine sample (at 20-25oC)

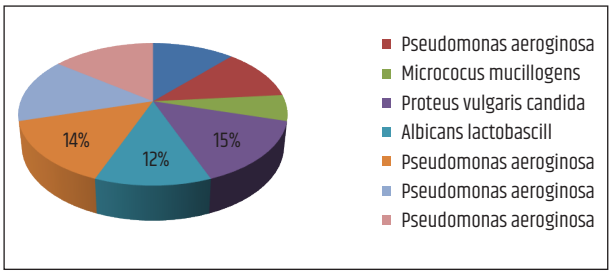

Figure 3: Saliva sample (at 37oC)

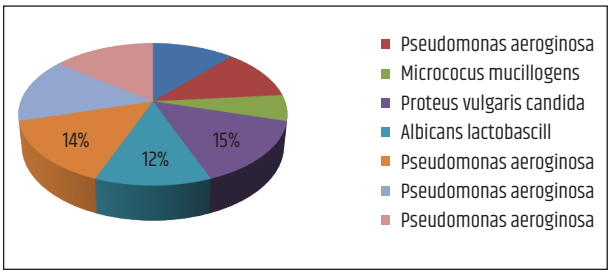

Figure 4: Saliva sample (at 20-25oC)

2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Sample collected after 28th hour is not showing any microbial growth, which indicates two reasons, either the sample is completely dry or the sample is completely degraded.

Urine Sample (at 20-25°C)

1. Samples collected after 3 hours of inoculation is showing microbial growth.
2. Samples collected within 48 hours are showing microbial growth. This indicates that from the time of inoculation till about 48 hours, microorganisms are growing continuously as it takes more time for the samples to dry.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

Saliva Sample (at 37°C)

1. Sample collected after 6hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after occurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Saliva Sample (at 20-25°C)

1. Sample collected after 6 hours of inoculation was not showing any microbial growth, which indicates that up to 6th hour after the occurrence of crime, the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Vomit Sample (at 37°C)

1. Sample collected after 6hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after occurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-

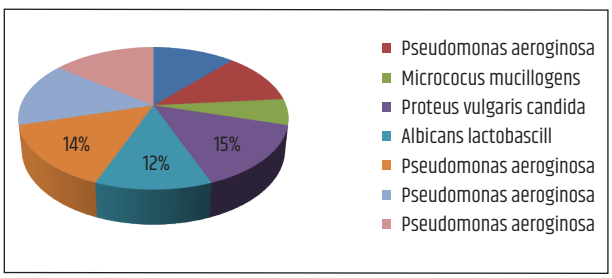

Figure 5: Vomit Sample (at 37oC)

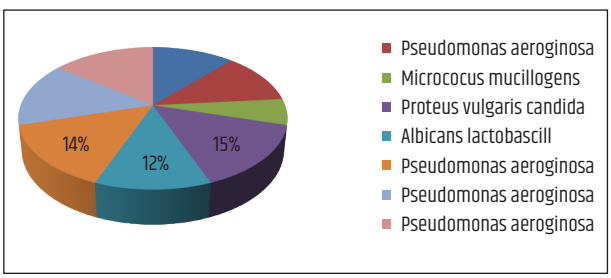

Figure 6: Vomit sample (at 20-25oC)

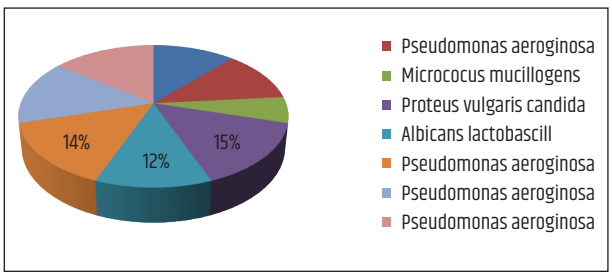

Figure 7: Semen Sample (at 37oC)

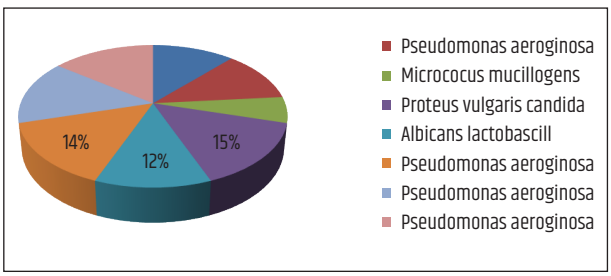

Figure 8: Semen Sample (20-25oC)

organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.

3. Sample collected after 28th hour is not showing any microbial growth, which indicates two reasons, either the sample is completely dry or the sample is completely degraded.

Vomit Sample (at 20-25°C)

1. Sample collected after 6 hours of inoculation is not showing any microbial growth, which indicates that up to 6th hour after the roccurrence of crime the sample is intact.
2. Samples collected in the time interval of 7th to 27th hour, profuse growth of micro-organisms are observed, which indicates that at this time interval is more susceptible to microbial degradation.
3. Samples collected after 28th hour is still showing growth, and after 40th hour there is no growth of micro-organisms in the sample due to drying.

Semen Sample (at 37°C)

1. Samples collected after 3 hours of inoculation is showing microbial growth.
2. Samples collected within 48 hours are showing microbial growth. This indicates that from the time of inoculation till about 48 hours micro-organisms are growing continuously as it takes more time to dry the sample.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

Semen Sample (at 20-25°C)

1. Samples collected after 3hours of inoculation is showing microbial growth in the sample.
2. Samples collected within 48 hours are showing microbial growth. Which indicates that from the time of inoculation till about 48 hours micro-organisms are growing continuously as it takes more time to dry the samples.
3. Samples collected after 49th hours onwards showing no trace of microbial growth.

GRAM STAINING OF MICROORGANISMS IDENTIFED

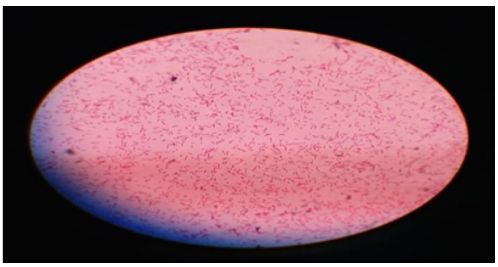

Figure 1: Pseudomonas aeroginosa

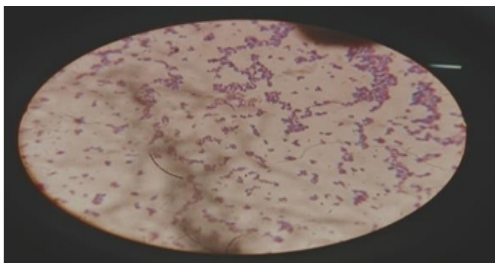

Figure 2: Micrococcus mucillogens

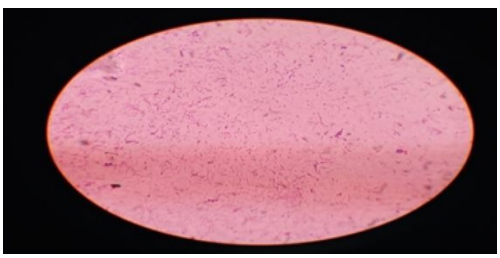

Figure 3: Clostridium tetani

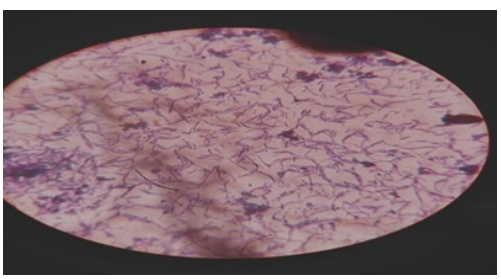

Figure 4: Lactobacillus lactai

Figure 5: Streptococcus pneumoniae

GRAM STAINING OF MICROORGANISMS IDENTIFED

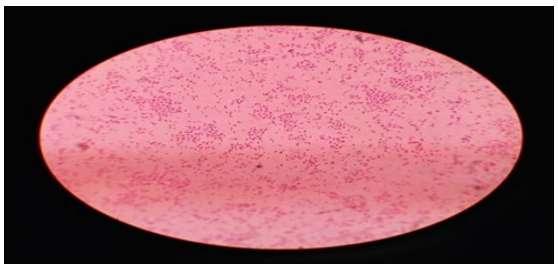

Figure 6: Klebsilella pneumonia

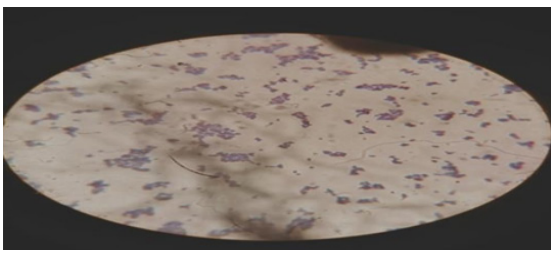

Figure 7: Staphylococcus aureus

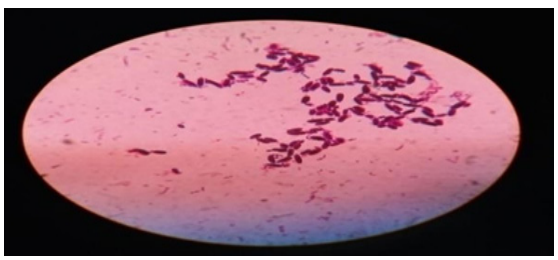

Figure 8: Candida albicans

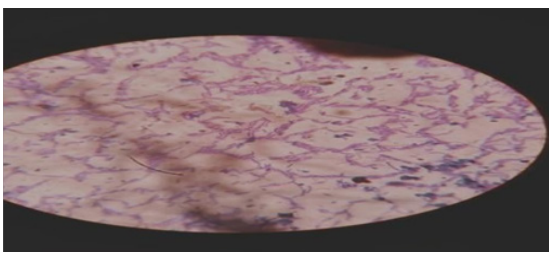

Figure 9: Escherichia coli

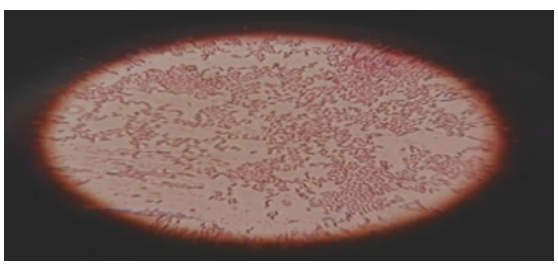

Figure 10: Proteus vulgaris

DISCUSSION

Sample gets degraded at 37°C temperatures for 9-21 hours. Compared 37°C less degradation is seen at room temperature. If the sample is collected before 4 hour from inoculation, the degradation will be less, and more accurate results will result in both temperatures. More contamination is observed in the sample collected from both indoor and outdoor region at the time interval from 9th hour to an average of 30th hour at 37°C 6th to 40th hour at room temperature (20°C-25°C) and showing relevant growth that leads to the loss of integrity of sample. Samples collected after the 31st hour at 37°C, gradually the growth is reducing, which implies that, with the time samples get dry and degradation decreases. Samples collected after 31st hour will be dry but more chances of getting false positive or negative result due to microbial degradation. It is clear that by collecting samples at early stage of crime occurrence the microbial degradation can be reduced so that the integrity of the samples can be maintained. This detailed study of degradation of samples by micro organisms helps in reducing false positive and false negative result to occurrence.

CONCLUSION

In a nutshell, it may be concluded that under ambient-conditions of high temperature and in specific time duration, the growth of microbes were found to be rapid. It is clear that temperature plays a major role in the preserving the integrity of samples. The samples will get dry at high temperature and minimal amount of microorganism will grow. Additionally, within 3 hours of exposure to different temperature and time interval, the microbial grow this minimal.

IJFMP

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ORIGINAL ARTICLE

Forensic Database Management of Diatoms: A Tool for Comparison

[1]Bhagyalakshmi R, [2]Prashant Kumar, [3]Sweta Rai, [4]Anshu Nanda, [5]S N Gimba, [6]Jaskaran Singh

ABSTRACT

CONTEXT: In forensic analysis diatoms plays an important role. Their structure and morphology is widely used to identify the location. Since their species are varied in different geographical locations. Therefore, can be used for diagnosing the suspected drowning and dumping cases.

AIMS: The study portrays the identification of different species of diatoms found in different geographical locations of Punjab.

SETTINGS AND DESIGN: Experimental study design was adopted to isolate and identify the different species of diatoms collected from different district of Punjab.

MATERIALS & METHOD: A total of 36 water samples from 18 different district in Punjab were collected. Each sample was of 500-1000 ml in quantity subjected to proper legend labeling of venue, time and date was procured followed by isolation using Acid digestion method for identification.

RESULTS: Different species of diatoms such as Cylindrotheca Closterium, Triceratium, Hasleastenopterobia, Tabulariavariostriata, Tabellaria, Vulgariabory, Semiorbis, Fragalariforma, Distrionella, Diprorahaenaensis, Hantzschiaamphioxyz, Pinnularia, Peronia fibula, Plagiotropis, Pseudostaurosira, Cyclotella, Melosiraundulata, Aulacoseira, Stenopterobia, Stauroneisaugustilanacea, Craspedastauros, Achnanthes lanceolata were isolated with the help of acid digestion method and were identified by keeping diatoms of North America as a reference standard.

CONCLUSIONS: Diatom species have been identified as indicators of drowning and dumping locations for the sake of criminal inquiry. Morphological analysis aids in the detection of the location where a body is discovered. Hence, site-specific diatoms from the Punjab region can help identify geographical locations.

KEY MESSAGE: In forensic science, diatom analysis is a useful tool for diagnosing suspected death by drowning cases. Diatoms at every few kilometers within a single water body changes thus making it easier as a shred of evidence in the identification of the exact location where the body drowned.

KEYWORDS | diatom, drowning, frustule, trace evidence

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INTRODUCTION

DIATOMS ARE AN IMPORTANT BIOLOGICAL evidence, and gives information of forensic importance. Drowning deaths found to be the third most accidental death known so far. Due to their numerous species they not only help in the direct conclusion of the cause of death, but it can also locate the location of a possible drowning site. The silical framework of diatoms do not decay readily, so they aid in diagnosing heavily putrefied bodies.

Specifically, in drowning cases the utilization of diatom testing from diatomaceous remnants seen on clothing and footwear of victim helps to identify the type of specie. To be more precise the molecular methods like DNA sequencing prompt forensic experts and law enforcement agencies to identify and study the species of diatom with the help of genetic markers.

METHOD

Materials used: Air tight containers, Microscopic slides, Coverslips, Lugol's iodine, Nitric acid, dropper, Immersion oil, Compound Microscope.

Different water bodies of Punjab region such as Patiala Beas, Gurdaspur Beas, Barnala Kotla canal, Nawanshahr Beas, Amrit Sarovar Lake, Ludhiana Sutlej river, Veyin Hoshiarpur, Gurdaspur Chakki, Mohali Sukhna lake, Pathankot Chakki, Muktsar Nangal Hydel, Mansa Manas river, Ropar Nawanshahr, Firozpur Harika Lake, Rupnagar Ropar Lake, Fatehgarh Sahib Sheesh Mahal pond, Kapurthala Kanjili were used for collection of water sample (1500-1000 ml approx.)

Sample collection: For the research of Diatom flora, two water samples were obtained from each selected site, totalling 36 samples from 18 districts in Punjab. Plastic bottles or containers were cleansed with distilled water at least 2-3 times before being collected. After cleaning the bottle, diatom-containing samples were collected in bottles containing 500-1000 ml from the designated sites, and the containers were tightly sealed with appropriate covers and labelled with the date and place of collection. [35]

Extraction and isolation: To extract and isolate diatoms from water samples, first apply 2, 3 drops of 2% Formalin solution to bottles containing water samples to limit diatom growth, then leave it overnight or for 4 hours for settlement. The next day, discard half of the water without shaking it, then vigorously shake it and pour it into a 500 mL beaker. 1-2 drops of Lugol's iodine solution are added, and the beaker is covered with aluminium foil and let to settle overnight. In the beaker containing the water sample, add 4-5 drops of strong Nitric acid (HNO3) to breakdown the organic materials found in the diatom cell wall, which is naturally resistant. These samples were kept undisturbed for two hours. It is then transferred to properly labeled tarson tubes and centrifuged for 10 minutes at 1500 rpm. The supernatant was pipette off leaving behind only a pellet at the bottom of the tube containing diatom frustules. To remove all traces of acid, pellet material was suspended in distilled water and centrifuged twice.[35]

Microscopic Examination: The pellets were placed on a microscopic slide and allowed to dry for a few minutes before being examined using a compound microscope at 10X, 45X, and 100X magnification. This process was repeated for all of the samples for Diatom examination and identification.[35]

RESULTS

After the isolation microscopic examination of diatoms following morphological features were identified. On the basis of identification features the species were named and marked.

Cylindrothecaclosterium

Belongs to Nitzschioid category, Frustules were narrow, elongated with drawn out ends, twisted, and very lightly silicified. Cells are elongated, solitary and are characteristically thin like needle. The valves including the keel and raphe canal wrap around each other forming a twisted frustule.

Triceratium

Cells are attached or free-living. Valve view is triangle in shape and girdle is tapered and oblong, corners are elevated and a projection at the center. Valves are shallow and ornamented with branched spines.

Haslea Stenopterobia

The valves of Stenopterobia are tapered and found to be sigmoid or straight in outline. The raphe is positioned inside a canal with the valve

Water Bodies	Location
Bathinda lake	Bathinda
Buddha nullah	Malwa
Harike lake	Firozpur
Kali Bein	Hoshiarpur
Mansa River	Mansa
Ropar lake	Ropar
Sirhind canal	Chandigarh
Sukhana lake	Chandigarh

Table 1- Different water bodies of the Punjab region were taken into account for sample collection.

margin. The canal is raised onto a keel above the valve.

Tabularia

Valves with lanceolate, acute apices and a central raphe system. Two or three thickened trans apical costae on either side of the valve center form a narrow fascia. The raphe sternum is thickened on one side of the axial area and is fused to the central ribs, give valves a fusiform appearance.

Tabellaria

The valves are elongated and capitate at the ends. The center of the valve is usually wider than the ends. In the center of the valve face, there is a rimoportula. Septae can be seen on copulae in abundance. Pseudosepta may be present as well. Mucilage pads connect zigzag colonies of cells.

Vulgare Bory

Frustules are heterovalvar, that is, raphe is present in one valve, while the other one lacks a raphe. Species of Vulgare are generally small in size, with narrow valves. The shape of the valve differ by species, but the ends may be rounded, capitate or rostrate. Striae usually uniseriate.

Pinnularia

Frustules of pinnularia are large, about 300um long and alveolar shaped striae. Internally, striae are confined in chambers. The elongated lines crossing the striae clearly shows the openings of chambers. The raphe found to be straight or fused. Raphe is enlarged and bent externally.

Fragalariforma

Valve margins vary in shape, being lanceolate, elliptical, or linear. Frustules are rectangular in shape when viewed from the girdle. The center section of valves is either very narrow or missing. Striae are made up of separate uniseriate aerolae.

Peronia fibula

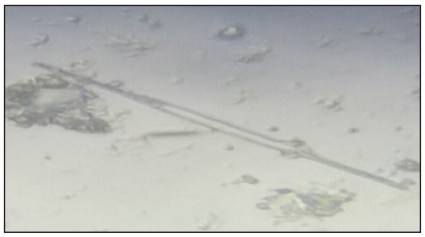
Peronia is heterovalvar and heteropolar. Valves have a tapered, wider headpole and a round footed pole. The headpole is round, while the footpole is narrow and rounded. Heterovalvar valves in which one valve has a raphe that elongates from each pole.

Plagiotropis

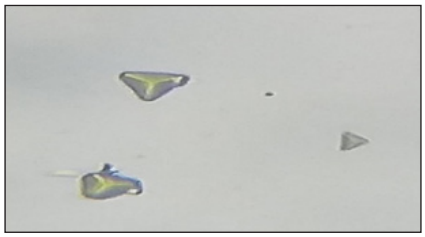
The valve margins are lanceolate in shape with narrow poles. Valve is folded on both sides. The raphe is positioned within a keel and itself raised above the valve face. The central area is variable in shape and axial area is narrow.

Pseudostaurosi Rabraevistriata

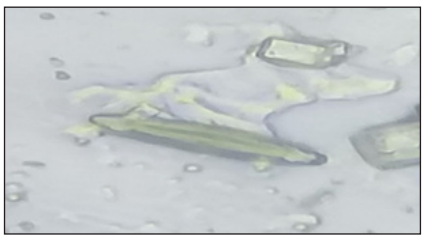
Valves are elliptical in smaller specimens and valve ends are round in shape. Face of the valve is flat and a gradual progression between the mantle and the valve face. The axial area is broadly lanceolate. Frustules are rectangular in shape connected by spines. Striae are small, visible, extending into the mantle, oval to round



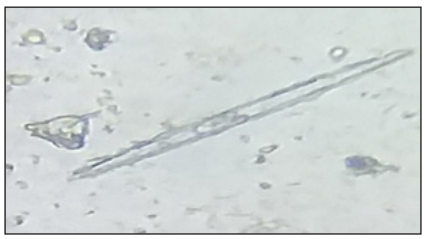
Cylindrotheca Closterium
Site 1: Patiala Beas



Triceratium
Site 2: Gurdaspur Beas



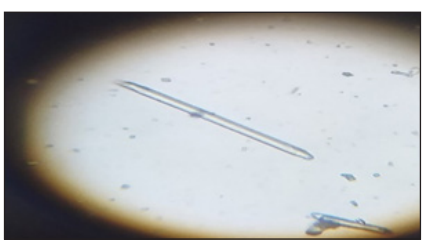
Haslea stenopterobia
Site 3: Barnala Kotla Canal



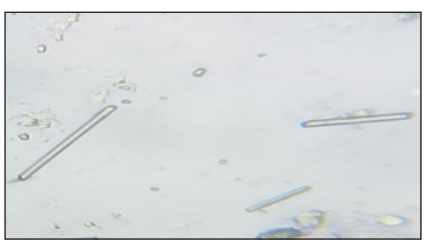
Tabularia variostriata
Site 4: Nawanshahr Beas

Tabellaria
Site 5: Amrit Saravor Lake

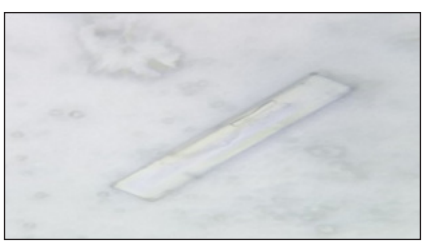
Vulgare bory
Site 6: Ludhiana Sutlej



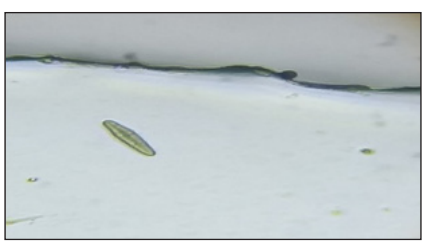
Pinnularia
Site 7: Veyn Hoshiarpur



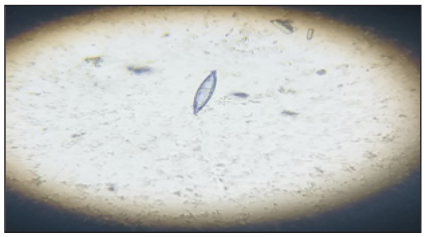
Fragalariforma
Site 7: Veyn Hoshiarpur



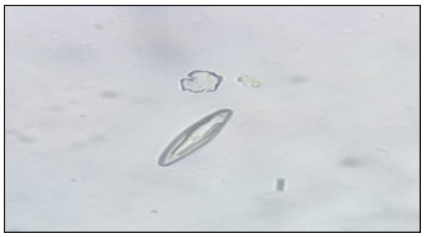
Cyclophora tenuis
Site 8: Gurdaspur Chakki



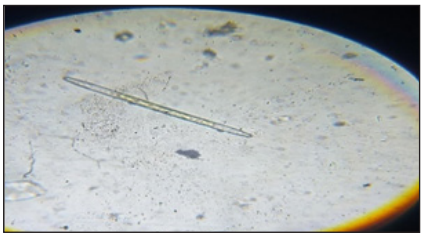
Peronia fibula
Site 9: Bathinda Sirhind Canal



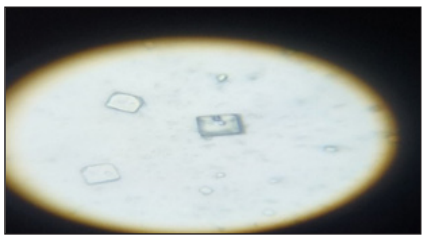
Plagiotropis
Site 9: Bathinda Sirhind Canal



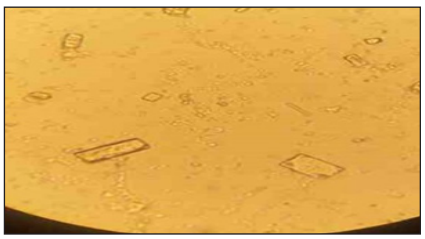
1) Pseudostaurosira
Site 10: Mohali Sukhna lake



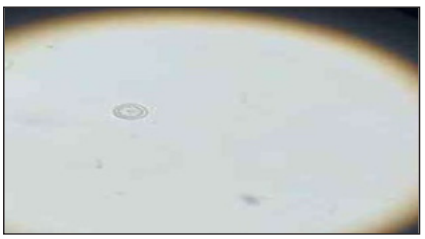
2) Craspedastauros
Site 10: Mohali Sukhna Lake



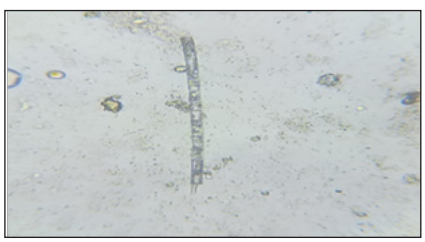
1 Triceratium favis
Site 11: Pathankot Chakki



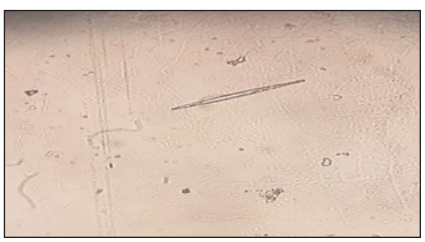
2 Melosira undulate
Site 11: Pathankot Chakki



Cyclotella
Site 12: Mukstar Nangal Hydel



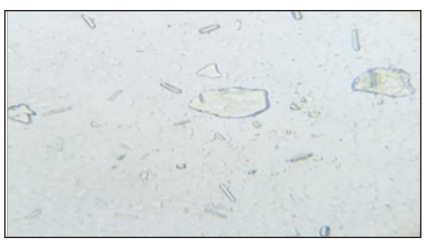
Aulacoseira ambigua
Site 13: Mansa Manas River



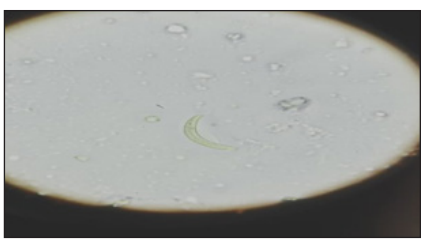
Achnanthes Lanceolata
Site 14: Ropar Nawan Shahr

Stenopterobia
Site 15: Firozpur Harika Lake

Stauroneis augustilanacea
Site 16: Rupnagar Ropar Lake



Diprora haenensis
Site 17: Fategarh Sahib Sheesh Mahal pond



Semiorbis
Site 18: Kapurthala Kanjili

aerolae, parallel to radiate near the valve's center to mildly radiate towards the valve's end Costae is a broad term. Except at the apices, spines can be seen along the valve face edge and are always found on the striae. The valve mantle has apical circular fields with round poroids close to the valve face. Copulae (girdle view) are open and lack perforations.

Craspedastauros

Valves of Craspedastauros are linear, with a slenderedstauros or fascia. Frustules are narrow in girdle around the central stauros. Cells often present in girdle view, because of the extensive girdle. Numerous girdle bands are present.

Distrionella

Cells are attached or free-living. Valve view is triangle in shape and girdle is tapered and oblong, corners are elevated and a projection at the center. Valves are shallow and ornamented with branched spines.

Melosira Undulate

It has large, cylindrical shaped frustules linked in chains. Valves and mantles are distinctly enhanced with striae and rimoportulae. A ring of evenly positioned rimoportula encloses near the mantle valve. The mantle is thickened unevenly.

Cyclotella

Valves are large with a tangentially undulate valve face. Various central fultoportulae are present. Multiseriate striae are present.

Aulacoseiraambigua

Valves are 3-12um in diameter, and 5-15um mantle height. The ratio of the height of the mantle to diameter of the valve is greater than 1. The helical rows of the mantle aerolae are curved. Spines are positioned at the pervalvar costae ends. Linking spines are and short and triangular.

Achnanthes lanceolata

Frustules possess 2 valve in which raphe is present in one and other lacks raphe. Hence it is heterovalvar. It possess very small and tapered valves. Valve shape may differ depends on species. May be round or rostrate.

Stenopterobia

The valves of Stenopterobia are tapered and found to be sigmoid or straight in outline. The raphe is positioned inside a canal with the valve margin. The canal is raised onto a keel above the valve.

Stauroneisaugustilanacea

Stauroneis has solitary cells. It has two chloroplasts, on each side against the cingulum. Valves are lanceolate to linear to elliptic lanceolate. The central area is an eminent trans fascia known called a "stauros". The typical fascia elongates to the margin of the valves.

Semiorbis

Valves are thick and transverse. Costae is present externally between the striae. The costae often ends in spines at ventral and dorsal margins. Raphe, which is small is present adjacent to the poles. The proximal raphe ends are on ventral margin.

Diprorahaenaensis

Genus Diprora is monotypic (contain a single species). The taxon is somewhat similar to marine taxon, hyalinella. Frustules typically with a concave and convex valve. Valves are round with broad ends. The smallest valves are nearly circular. Presence of single row of pores along the valve margin. Frustules are joined into filaments. The valve filament is extended to form apical prows, a feature visible in girdle view.

Hantzschia amphioxyz:

The ratio of the height of the mantle to diameter of the valve is less than 1. The helical rows of the mantle aerolae are elongated.

CONCLUSION

In present study, a total of 22 species were identified from different locations of Punjab. The genera identified are Cylindrothecaclosterium, Triceratium, Hasleastenopterobia, Tabulariava -riostriata, Tabellaria, Vulgariabory, Semiorbis, Fragalariforma,Distrionella,Diprorahaenaensis, Hantzschiaamphioxyz, Pinnularia, Peronia fibula, Plagiotropis, Pseudostaurosira, Cyclotella, Melosiraundulata, Aulacoseira, Stenopterobia, Stauroneisaugustilanacea, Craspedastauros, Achnanthes lanceolata. These species have been identified morphologically

using Diatoms of North America as a reference. Diatom species have been identified as indicators of drowning and dumping locations for the sake of criminal inquiry. Morphological analysis aids in the detection of the location where a body has been discovered. It can also be useful to identifying the site based on the morphological characteristics of the Diatom Flora; site-specific diatoms from the Punjab region aid in the recognition and identification of the site in the event of drowning or dumping. This study is extremely useful for medico-legal applications, such as unclaimed bodies in cases of disputed drowning and dumping, as well as location correlation and identification in cases of uncertainty.[8]

The major job of forensics is to ascertain the cause of death, such as whether the deceased drowned or if the body was put into the water after death. However, because of variables such as chewing by fish or worms in the water, as well as corpse decomposition, dead bodies in water often lack the typical signs of drowning, making drowning diagnosis extremely difficult.

The diatom test is based on the fact that when a person drowns, diatom enters the lungs through inhalation of any liquid. They may penetrate the wall of lungs, and if the cardiovascular is efficient it will carry them to other internal organs (heart, kidney, liver, etc.) where they will remain. Although some diatoms may still be present in the lungs if a person is already dead before entering the water, they will not be present in any other internal organ, and the cause of death may be something other than drowning. Examination of lungs and other internal organs for the presence of diatoms therefore yield supporting evidence for drowning, if diatom valves are found.[28]

Diatoms are naturally occurring, so its persistence and transfer of particulates to an evidential surface such as clothing or footwear, can impart valuable evidence to differentiate between suspect or victim and a crime scene. Diatom utility in anthropogenic by products such as pesticides, filters, paints and construction materials, mining of fossil diatom deposits, presents additional probability for presence and transfer of diatoms in Forensic samples.[20] IJFMP

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ORIGINAL ARTICLE

Development and Visualization of Latent Fingerprints by Using Talcum Powder

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ABSTRACT

CONTEXT: Fingerprints are used as reliable evidence in criminal and civil investigations all around the world. There are several methods available for detecting latent fingerprints. Powder dusting is one of the most used methods. The current research began with the need to solve a challenge that arose from an everyday actual forensic work. The latent print on diverse surfaces created for this investigation was developed using talcum powder. Talcum powder is easily available at home and can used by Investigating Officer if fingerprint powders like charcoal powder, aluminum powder, gray powder, fluorescence powder, magnetic powder and others are unavailable.

AIMS: The aim of this research was to see how effective talcum powder can be as a low-cost, non-toxic fingerprint powder, especially in areas where conventional powders are scarce.

MATERIALS & METHOD: 20 samples of latent fingerprints were developed from varied surfaces using a camel hairbrush and powder-dipping techniques. Moreover, adhesive tape was used to lift and collect the fingerprints on to fingerprint cards.

RESULTS: Twenty substrates with diverse surfaces, color, and nature had been chosen to deposit fingerprints, and their development efficiency as investigated using talcum powder. The majority of the fingerprints that were created had big contrast and transparency.

CONCLUSION: The talcum powder can be an effective and inexpensive substitute for other fingerprint powders, particularly in the case of shortage of other powders.

KEY MESSAGES: The non-toxic talcum powder approach is simple to use, inexpensive and effective. The results of this investigation demonstrate that, with a little bit of fingerprint expertise and training, police officers can utilize a readily available product like talcum powder to identify latent fingerprints.

KEYWORDS | fingerprint, talcum powder, personal identification, latent print, forensic analysis

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INTRODUCTION

FINGERPRINTING IS OFTEN THE MOST SUCCESSFUL technique for identifying a person out of all the methods of personal identification known to date. It used all over the world as an infallible form of identification.[1] One of its advantages is its simplicity, as fingerprinting requires minimal equipment. A fingerprint is an imprint made by a human finger's friction ridge.[2] Because of their uniqueness, competence, and consistency over time, fingerprints utilized in criminal investigation.

There are three types of fingerprints, based on its prevalence at the scene of crime. The latent fingerprint found to be present on all

surfaces that a perpetrator may come in conflict with and touch. Paper, polished surfaces, glass, metal panels, doorways, and glass are examples of these surfaces.[3] Friction ridge skin on a surface leaves a latent imprint at the scene of a crime by mistake or by coincidence. This type of fingerprint hidden and go unnoticed. A patent fingerprint is a mark made composed of fluid, lubricant, paint, or grit that is visible to the naked eye.[4] Fingerprints made on sticky paint, paraffin, plaster, resin, soapy, or bitumen known as plastic fingerprints. Humans can easily see plastic prints, and they do not require any further processing to make them apparent. These prints may be present on porous, semi-porous, and non-porous surfaces etc. Experts utilize a powder and brush method on non-porous surfaces, which lifted using clear adhesive tape. On diverse substrate types, latent finger print deposits react differently.[4-5] Furthermore, certain detecting algorithms work on particular surfaces but not others. As a result, while choosing a series of fingerprint detecting algorithms for a certain set of conditions, the surface type is a key factor.[6] Powder selected for development method should be fine-grained, squishy, and greasy. For fingerprint development, police investigators implement a variety of powders, including black powder, gray powder, silver powder, bright powder, magnetic powder and fluorescent powder.[7] There are varieties of techniques that can be use on such surfaces. Over the last two decades, fingerprint powdering has remained largely unchanged as a detection method. There are various powders and brushes to choose from, and the decision is usually dependent on personal preference, availability, and experience. Magnetic powders applied using a magna brush have long been thought to be the least damaging, whereas aluminum flake powders have long been thought to be particularly effective. Magnetic powders have become more sensitive because of the recent creation of iron flake powders. A variety of luminous powders is available for multi colored surfaces. Because latent prints are not visible to the naked eye, they require specific development tools or improvements in order to seen. New ways for detecting latent fingerprints had developed, although the most basic approach for detecting latent prints was powder method.[8] The power sticks to oil, perspiration, or other substances left in the fingerprint if dusted over the region impacted by the fingerprint. Since the early 1900s, this powder method had successfully employed[9-10] various fingerprint powder formulas employed at this time, with each formulation containing a dye for contrast and a resin substance for effective adherence.

MATERIALS AND METHOD

The latent prints developed using a variety of techniques, including black powder, magnetic powder, iodine fuming, cyanoacrylate fuming, and small particle reagent method, silver nitrate method and ninhydrin method.[10]

Sample Fingerprint Preparation

Sample preparation done by preparing fingerprints for each porous surface (colored paper and door slag paper) and non-porous surfaces (glass bottle, metal, printer, doors, window, marble tiles, transparent plastic, plastic plate, bike, chairs, CD surfaces, mobile etc.). Latent fingerprinting obtained by means of plain impressions; the fingerprint printed evenly on several substrates as mentioned above. Complete latent print quality checked and latent prints on all surfaces left at room temperature. Then, within 2 to 5 hours, check again. Each sample was taken in a sebum (oil)-rich state, mostly from the skin, hair, behind the ears, and forehead.

Materials

- Fingerprint brush (camel hair brush)
- Fingerprint powder (talcum powder)
- Camera

Formula of Talcum Powder:

Talcum powder is a hydrated magnesium silicate mineral that has the chemical formula H2Mg9 (SiO3)4 or Mg3Si4O10 (OH)2. It ranges in hue

from white to gray and has a greasy texture. The chemical structure of talc is shown below in Figure 1.

Sample Collection

Twenty samples were collected from various surfaces including bike, car, glass, mirror, chair, wood, almirah, window, door etc. A criminal can easily deposit his latent fingerprints on these surfaces.

To develop fingerprint we can use two different methods as given below:

First Method

- Apply talcum powder lightly on the latent fingerprints by spraying to make it visible; using a soft camel hairbrush light strokes should made.
- Brush motion performed along the flow of ridges once the shape of a pattern observed. This aids in the removal of surplus powder stuck between the ridges without causing damage to the ridge.
- With the aid of a camera, a developed fingerprint captured.

Second Method

- Powder applied to a surface by dipping a brush into a powder container, trapping powder on the developing surface.
- When powder brushed over an area with a latent print, particles stick to the oily deposit.
- Remove any excess powder from the region around the fingerprint pattern.
- When the patterns standing out against the backdrop and captured using a camera.
- Lift the fingerprint using transparent adhesive tape after pattern formation and save it on a fingerprint card for future use.

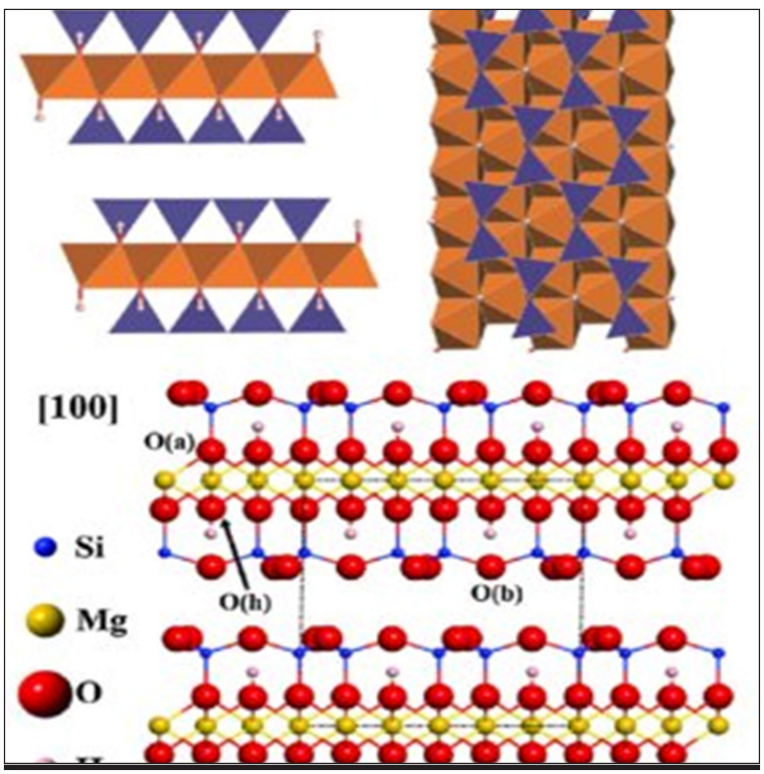

Figure 1: The chemical formula for Tallcum powder

RESULTS AND DISCUSSION

The shape of the fingerprints generated, color contrast on the surface, and contrast effect on the test time span were all investigated using manual identification of the findings of the creation of visible latent fingerprints. Brushing powder onto a print surface is a simple and straightforward method, but it has the drawback of destroying the ridge features of brushes that exposed to the surface, which have mold crushing capabilities. The surface area and color contrast provided by talcum powder affected by size of the powder. Talcum powders such as Johnson & Johnson, Eve body powder, Nivea Pure, Spinz, Yardley London, and others are available on the market. When compared to coarse powder, fine-grained powder produces superior results.

The goal of this research was to offer a readily available household product like talcum powder for developing latent prints on non-porous and porous surfaces and to investigate the impact of talcum powder on the working area.

The ridge quality of the latent fingerprint created with talcum powder is excellent. On various surfaces such as colored paper, metal, glass, steel, marble tiles, and so on, talcum powder produces a superior effect. Even several days after contact, talcum powder has shown to be very efficient in producing an outstanding latent print.

The results of developing latent fingerprints with Talcum powder on various surfaces are displayed above. The majority of the surfaces investigated have latent fingerprints that can be successfully produced with fingerprint powder.

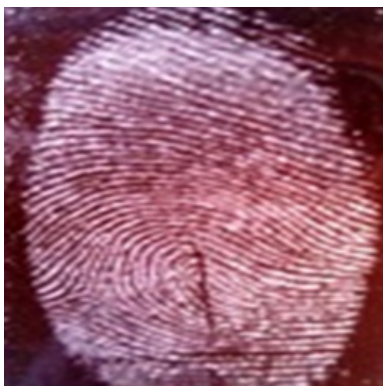

Figure 1: Finger print on a bike

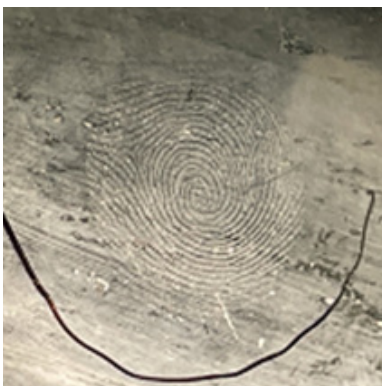

Figure 2: Finger print on glass bottle

Figure 3: Finger print on water bottle

Figure 4: Finger print on a plastic plate

Figure 5: Finger print on a stapler

Figure 6: Finger print on ornament

Talcum powder interacts with sweat on the skin to form a distinct ridge pattern. Figure 1 depicts a fingerprint's ulnar loop pattern on the bike's surface. Figure 2 shows a simple whorl design on a glass bottle. Figure 3 shows a talcum powder-created radial loop design on a water bottle. Figure 4 shows a composite loop and whorl design on a plastic plate. Fig. 5 shows a plain whorl design with a slight ridge visible on the stapler due to talcum powder. Figure 6 shows a palm fingerprint on an ornamental metal object. The fingerprint developed on the Canon printer shown in Figure 7. Figure 8 depicts the radial loop pattern on the mirror surface. We can gather fingerprints from many surfaces, such as metal, glass, and doors, using talcum powder as illustrated in Figures 9 & 10.

Figure 7: Finger print on Canon printer

Figure 7: Finger print on steel article

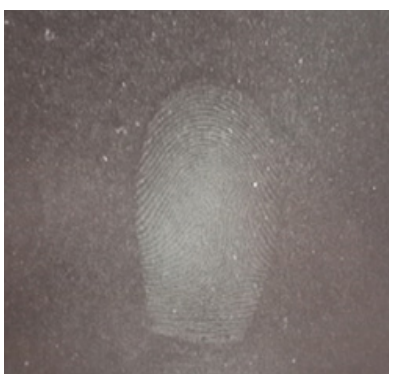

Figure 9: Finger print on miror

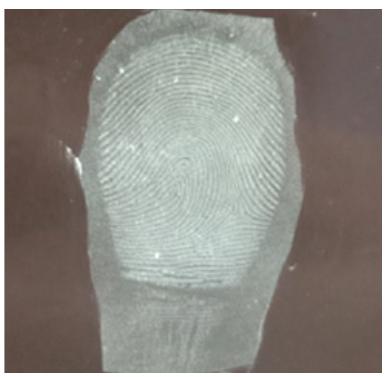

Figure 10: Finger print on door

CONCLUSION

According to the findings of this investigation, the creation of latent fingerprints was achieved successfully using the talcum powder dusting method. Talcum powder is economical since nearly all of it is recovered and reused. It is also simple to use and less cumbersome. Talcum powder, unlike certain chemicals and black powder, does not pose a carcinogenic danger. People can use talcum powder if a police officer is not present at the site of the crime, or police officers can utilize this approach on their own in the absence of a forensic fingerprint kit or specialist. IJFMP

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ORIGINAL ARTICLE

Study of Predominant Lip-Print Patterns in University Students of Faridabad, Haryana

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ABSTRACT

CONTEXT: Cheiloscopy is the study of lip print patterns and can aide in identification and gender determination of the individuals.

AIM: The current study was aimed at knowing different lip groove pattern distribution, their relation with gender identification in the studied individual.

SETTINGS AND DESIGN: Lipstick used from middle line of lips and then to lateral sides. Then prints of both lower and upper lips were collected together on cellophane tape.

MATERIALS & METHOD: The samples collected randomly in this study belonged to 53 healthy selected individuals who were mainly of student population aged between18-25 of both sexes of North - West Faridabad region. Every lip print was divided into 4 Quadrants (QI, QII, QIII, QIV) according to the method given by Santos.[1] Examination of lip prints were done under direct light using magnifying hand tool. Y. Tsuchihashi and K. Suzuki[2] method was then applied for classifying the lip print samples into four different lip prints.

STATISTICAL ANALYSIS USED: The statistical package for social sciences 16 (SPSS 16) was used for the analysis of the data.

RESULTS: The dominant pattern was found to be Branched (Type II) among quadrants I, II and III while Intersecting pattern (Type III) was found highest in quadrant IV. Vertical type lip pattern was least dominant and found only on 1.9% and 5.5% in quadrant I and quadrant II respectively of study population.

CONCLUSIONS: Identification of lip print patterns can be used as tool among the subjects of this region.

KEY MESSAGE: Lip prints presence on scene of crime can confirm the presence of that specific individual on the scene, thus allowing to link crime scene with the suspect, victim and individualizing the suspect.

KEYWORDS | lip prints, identification, crime scene, quadrants, cheiloscopy

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INTRODUCTION

In cases of civil, mass disaster, criminal categories identification of unidentified deceased individual becomes very difficult. There are various techniques and methods available which are being used in Forensic science for the purpose of human identification. The most commonly used technique includes fingerprints, dental records, DNA profiling etc. However, identifying a person using their lip prints is a new and interesting field. But the research show the presence of lip prints on a scene and their utilization as an evidence for identification of criminal or personal identification is very less because the lip prints

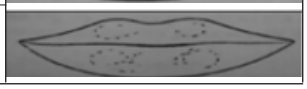
CLASSIFICATION	CHARACTERISTICS	SHAPE
Type 1	Clear vertical lines that run all over both lips	
Type 2	Partial or incomplete lines	
Type 3	Branching lines or fork shaped	
Type 4	Intersecting lines which are also branched	
Type 5	Reticulate	
Type 6	Mixed or lines which cannot be classified in the mentioned patterns	

Figure 1: The Objective of the present is to see the differences in the dominant lip characteristics for identification of the lip pattern in selected study group and to scrutinize the differences and find the prevalence of dominant pattern in the various quadrants of male and female.

cannot be found easily in every crime on the scene.

Lip prints are generally found on food article like apple, pear, bread, utensil, clothing, paper, skin etc. Lip prints can also be found on glass tumbler, butts of cigarette, and adhesive tape. Methods like photography, direct visual examination allow much accurate and fast observation of even minute details which are needed for lip prints investigation. Lip prints presence on scene of crime can confirm the presence of that specific individual on the scene, thus allowing to link crime scene with the suspect, victim and individualizing the suspect.

Lip prints found on scene of incidence are not usually well defined and clear so to establish the identity of the person becomes difficult but this point cannot curtail the fact about uniqueness characteristic of lip print as important physical evidence. Even then, the significance of studying lip prints in depth and establishing further information and data related to it will surely be helpful in establishing the role of lip prints as evidence for forensic purposes.

Suzuki and Tsuchihashi's[2] in 1970 gave a system of classification popularly known as Tsuchihashi classification of lip prints. It's the most commonly known and applied method till date.

MATERIALS AND METHOD

The present study was conducted at College of Traffic Management, Institute of Road Traffic Education (CTM-IRTE), Faridabad and Manav Rachna Dental College, Manav Rachna University, Faridabad, Haryana.

The specimens collected in this study were of 53 individuals who were selected randomly from student population aged between18-25 at Faridabad. Valid Consent of participation was taken from all the candidates. Any candidate having any disease, deformity, inflammation or previous history of any cosmetic surgery of lips was excluded.

Every Individual lips were cleaned first then application of lipstick was done properly taking into the consideration the importance of uniformity of lipstick on lips. Lipstick used was without gloss, without presence of metallic substance and dark in color and was applied from middle line of lips and then to lateral sides. Then prints of both lower and upper lips were collected together on cellophane tape. The tape was gently pressed against the lips of subject and then pasted on white sheet. The lip prints

Figure 2: Four Quadrants (QI, QII, QIII, and QIV) were made to divide every lip print sample earlier done Santos in 1967 1.Examination of samples were done by using magnifying lens under direct and focused light on them.

were also recorded by applying direct light pressure against the folded white sheet which were then stored properly with proper measure so as to avoid contamination and alteration of prints. Method given by Ahmed S.A. et.al3 was used for recording and analysis of lip prints.

Before analysing the lip print pattern in the collected samples, all the samples were divided in 4 quadrants i.e. 2 quadrants on each lip and were allotted digit Q1,Q2, Q3, Q4 in clockwise manner starting from subjects 'upper right'

Statistical Analysis:

In the study 53 individuals were studied. Lip print samples were studied from all four quadrants giving 212 samples and four pattern types were analyzed (Type I-Type IV). Irrespective of the gender the overall frequency of most repeated pattern found in the population is in the order T2>T3>T4>T1.

Out of total 53 subjects who participated in this study the dominant pattern as found is Branched (Type II) among quadrants I, II and III while Intersecting pattern (Type III) was found highest in quadrant IV. Vertical type lip pattern was least dominant and found only on 1.9% and 5.5% in quadrant I and quadrant II respectively of study population while in QIII and QIV Reticulate pattern was the least dominant pattern. Reticulate pattern was present in all quadrants with almost same frequency. Branched and Intersecting pattern were present in more than 70% of study population in QI and QII (Table 1).

Branched type pattern was more dominant in Quadrant I in both female and male subject. Vertical pattern type wasn't present in female and is found only in one male. The distribution was not found significant may be due to the due to the variation in the number of samples of male and female (Table 2).

Branched type of lip pattern was found dominant in Quadrant II in female followed by Intersecting and reticulate patterns while in male the dominant pattern was intersecting followed by branched. Vertical type pattern was not present in female and is found in three male. The distribution of pattern in both the gender was found significant (Table 3).

Branched type of lip pattern was found dominant in Quadrant III in female followed by Intersecting patterns while in male the dominant pattern was intersecting followed by branched. The distribution was not found significant may be due to the due to the variation in the number of samples of male and female (Table 4).

Vertical type pattern was found dominant in Quadrant I in female followed by Intersecting pattern while in male Intersecting pattern

PATTERN	Quadrant I		Quadrant II		Quadrant III		Quadrant IV	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Vertical (Type I)	1	1.9	3	5.6	11	20.4	14	25.9
Branched (Type II)	29	53.7	21	38.9	16	29.6	11	20.4
Intersecting (Type III)	12	22.2	18	33.3	16	29.6	20	37.0
Reticulate (Type IV)	11	20.4	11	20.4	10	18.5	8	14.8
Total	53	100.0	53	100.0	53	100.0	53	100.0

Table 1: Lip print pattern frequency in the North-West Faridabad population in various Quadrants

Table 2: Summary of the patterns of lip print in Quadrant (Q1) with regard to pattern distribution among both the gender

GENDER	Quadrant I (Q1)				
	Vertical	Branched	intersecting	Reticulate	Total
Female	0	17	8	8	33
Male	1	12	4	3	20
Total	1	29	12	11	53

Chi-Square Tests for Quadrant (QI)			
Pearson Chi-Square	Value	df	P Value
	2.425	3	0.489

Table 3: Summary of the patterns of lip print in Quadrant (Q2) with regard to pattern distribution among both the gender

GENDER	Quadrant I (Q2)				
	Vertical	Branched	intersecting	Reticulate	Total
Female	0	15	9	9	33
Male	3	6	9	2	20
Total	3	21	18	11	53

Chi-Square Tests for Quadrant (Q1)			
Pearson Chi-Square	Value	df	P Value
	8.643	3	0.034

Table 4: Summary of the patterns of lip print in Quadrant (Q3) with regard to pattern distribution among both the gender

GENDER	Quadrant I (Q3)				
	Vertical	Branched	intersecting	Reticulate	Total
Female	0	15	9	9	33
Male	3	6	9	2	20
Total	3	21	18	11	53

Chi-Square Tests for Quadrant (Q1)			
Pearson Chi-Square	Value	df	P Value
	.510	3	0.034

Table 5: Summary of patterns of lip print in Quadrant (Q4) with regard to pattern distribution among both the gender

GENDER	Quadrant I (Q4)				
	Vertical	Branched	intersecting	Reticulate	Total
Female	0	15	9	9	33
Male	3	6	9	2	20
Total	3	21	18	11	53

Chi-Square Tests for Quadrant (Q1)			
Pearson Chi-Square	Value	df	P Value
	8.643	3	0.034

was most common followed by Branched lip pattern. Reticulate and Branched lip pattern are least found in male and female respectively. The distribution was not found significant may be due to the variation in the number of samples of male and female (Table 5).

Dominant pattern in females was found as Branched type then follows intersecting pattern and reticulate pattern. Vertical pattern was least present in all quadrants in female. In males Branched and intersecting pattern were most dominant followed by Vertical and Reticulate pattern. In pooled data Branched pattern was found in maximum number of subjects followed by Intersecting pattern. Vertical pattern was least common among the participants (Table 6).

DISCUSSION

The data and research studies used in lip print as a mode of identification is very less and this current study was done so as to give more details and reference for the lip prints use, this study showed the distribution of lip print pattern in the population of university students of Faridabad. To our knowledge, this is the first study done in North-West Faridabad for identifying the lip pattern distribution.

It was also found in the current study that Type III (37.5%) and Type II (37.5%) were found to be equally dominant in males whereas pattern Type II (36.5%) was found to be predominant in females. Ahmed S.A. *et al.,*[3] in their study found that dominant pattern in males was Type I (28.3%) and in females the dominant pattern was Type III (26.9%) which is similar to the present study. The study of Badiye *et al.,*[4] (2015) found that Pattern Type III (35.75%) and Type I (29.75) were most reoccurring in women and men respectively.

Sultana Q et.al5 found in a study that the pattern Type III (40%) is more reoccurring in males similar to present study and Type I (54%) was more predominant in females followed by other patterns which is different from our finding the difference may have come due to geographical location of he studied subjects.

In a study conducted by Karki RK6, pattern Type I and I' were most reoccurring in male (29 cases total) and very rare in females. In conjunction to present study findings, Type II pattern was more common in females as 32 cases (42.5%) cases were found with this pattern.

In the study it was also found that no lip patterns were completely identical in nature as discussed by Suzuki et al.[2] During collection of lip prints, it was noticed that lip prints may vary in appearance depending upon collection method and direction, application of pressure.

A study by Vahanwala SP et al.[7] concluded that lip prints of two different individuals are not identical in every aspect hence proving uniqueness of these print in individual by considering labial wrinkles and grooves as characteristic features.

Basheer S *et al.,*[8] (2014) also observed gender wise difference among lip print patterns with Type II as the most prevalent in males and Type IV being more dominant in females.

In a study done by Augustine J et.al9 lip print pattern comparison between both the gender showed Type III pattern as most reoccurring pattern in both females and males accounting for 47.78 % 49.15% of all the given patterns respectively which agrees with the current study in case of females and males Type II and Type III both are equally dominant.

In a study conducted by Nagrale N et.al10 Type I, I', II pattern were more reoccurring in females while type III and IV lip patterns were more prevalent in male, while Type II was found present in both the gender, which is similar with the current study, in the fact that Type II is the most reoccurring pattern. In contrast to present study Randhawak et al.[11] found Type I (32.33%) pattern as more predominant.

Thomas, BS12 Type I pattern was more reoccurring in all regions of lips in both the gender, leaving only lower middle region where pattern Type I' was more prevalent. Ishaq N et al.[13] did a study where the most reoccurring pattern in females was Type II, while Type III as more prevalent among males which is similar as in the current study.

Table 6: Summary of patterns of lip print in Quadrant (Q4) with regard to pattern distribution among both the gender

GENDER	Quadrant I (Q4)				
	Vertical	Branched	intersecting	Reticulate	Total
Female	0	15	9	9	33
Male	3	6	9	2	20
Total	3	21	18	11	53

Chi-Square Tests for Quadrant (Q1)			
Pearson Chi-Square	Value	df	P Value
	8.643	3	0.034

Kaur R *et al.,*[14] in their study findings found, Type I (35.5%) lip pattern was most reoccurring, then followed Type II (26.1%) and Type III (16.3%). Type I (37.9%) lip pattern was most prevalent in males, then followed Type II (19.2%) and Type III (18.4%). Therefore, the most prevalent pattern for both the gender in this study was Type I which also differs with the current study.

CONCLUSION

Lip prints presence on a scene of crime can confirm the presence of that specific individual on the scene, thus allowing to link crime scene with the suspect, victim and individualizing the suspect.

The dominant pattern was found to be Branched (Type II) among quadrants I, II and III while Intersecting pattern (Type III) was found highest in quadrant IV. Vertical type lip pattern was least dominant and found only on 1.9% and 5.5% in quadrant I and quadrant II respectively of study population while in QIII and QIV Reticulate pattern was the least dominant pattern.

In Female, for all quadrants the dominant pattern was Branched type which is followed by Intersecting pattern and reticulate pattern. Vertical pattern was least present in all quadrants in female. In males Branched and intersecting pattern were most dominant followed by Vertical and Reticulate pattern.

Identification lip print can be used as tool among the subjects of this region. Further analysis should be done on large population sample of different region which would be more accurate for comparing, creating a database so lip prints might serve as a significant source of information which can be used for crime solving, personal identification and population study. IJFMP

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ORIGINAL ARTICLE

Identification and Characterization of Counterfeit Kohl Samples using Sophisticated Analytical Techniques

[1]Abhinav Sood, [2]Varsha Dogra, [3]Gayatri Pathmanathan, [4]Aanchal Dwivedi

ABSTRACT

CONTEXT: This research paper is divided into three parts: (a) packaging analysis (b) chemical analysis to determine the composition (c) contamination analysis of questioned kohl cosmetics.

AIM: The present work deals with the comparative analysis of authentic and questioned cosmetic products using different analytical techniques.

SETTINGS AND DESIGN: The study examined 24 samples of two different kohl brands named B1 and B2. Four genuine samples were used as a benchmark to check the variability and relevancy of the obtained results. 20 questioned samples were named C1-C10 of first brand (B1) and C11-C20 of second brand (B2). The references were named GA1-GA2 (authentic samples of brand 1) obtained from the original website of the first brand and as GB1-GB2 (authentic samples of brand 2) purchased from the original website of the second brand. Packaging, chemical and contamination analysis were done on questioned, and authentic samples of two dissimilar brands of kohl cosmetics. **MATERIALS & METHODS:** Chemical analysis was done by utilizing sophisticated techniques such as FTIR, 1HNMR, and EDX. Additionally, contamination analysis was performed on the questioned samples by employing optical microscopy.

STATISTICAL ANALYSIS: The SPSS version 23.0 was used to determine the difference between the mean elemental composition of authentic and suspected counterfeit samples. **RESULTS:** Through the first-line investigation of the hologram on the samples, it was detected that out of 20 samples, 17 samples contained damaged or scrambled holograms (B1) or just a silver tag (B2). The micro-text was not detected in these samples compared to genuine samples (n=4). In terms of chemical analysis using EDX, the presence of palladium, cadmium, and mercury were detected in all samples. NMR delta values for both the authentic and questioned samples were different which concluded that the molecular structure and composition of both samples were dissimilar and consisted of different elements. Optical microscopy affirmed the presence of E. coli in two samples.

CONCLUSION: From the analysis, it was observed that the visual comparison with authentic sample is the first step to detect counterfeit packaging but due to the adaptation of new printing technology by the counterfeiters, they can easily replicate authentic product packaging including security features such as barcodes. Therefore, it is essential to analyze the sample through chemical investigation to check the product in detail. The study was performed on a limited number of samples and therefore encourages chemical and packaging profiling of counterfeits on a bigger scale.

KEYWORDS | counterfeits, cosmetic products, analytic strategy, composition analysis

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INTRODUCTION

COUNTERFEITING IS A SERIOUS PROBLEM THAT has magnified over the past decade as the cosmetics we use on our skin if not made legitimately can hurt our wellbeing.[1] With no valid verification of vendors by the organizations and proficient-looking sites, the counterfeit cosmetic business has amplified in recent years.[2-4] Packaging is usually accompanied by security features as a visible indicator which if absent or altered, indicates as evidence that the product has been tempered or completely a substandard product.[5] Counterfeiters are focused on getting maximum profit from minimum investment and as replicating packaging is potentially expensive to do, therefore, it's much harder to replicate the exact quality, weight, and feel of a paper or contents on the label including security features such as holograms and barcodes.[6-8] Holograms and barcodes frequently give clear first-line authentication and have turned out to be extremely difficult to duplicate precisely. [9-11] Unlike medicines that include active substances with pharmacological importance, and are distributed and controlled by pharmacies, cosmetics do not have any such type of pharmacological use and can be sold without any restraints.

The current market for cosmetics in India is US$6.5 billion and is expected to reach US$ 20 billion with a Compound Annual Growth Rate (CAGR) of 25% by 2025. On the other hand, the global cosmetic market is expected to reach US$450 billion with a CAGR of 4.3% by 2025.[12] In India, under the Drugs and Cosmetics Act, 1940 and Rules, 1945 and labeling declarations by the Bureau of Indian Standards (BIS), the cosmetics products are regulated. Under Schedule 'S' of the Drugs and Cosmetics Rules 1945, BIS sets the standards of cosmetics for the products listed. BIS has provided the specification for skin creams and lipsticks in the Indian Standards (IS) 9875:1990 and 6608:2004, respectively. [13]

Kohl or eye pencils are popular eye cosmetics being widely used across Asia, Africa, and the Middle East. Since ancient times, kohl has been believed to protect the eye from different eye diseases. Kohl is described as an ultra-fine powder consisting of various ingredients such as herbs, pearls, gemstones, galena, etc., for the prophylaxis and cure of eye ailments.[14,15] Today, the ingredients have changed considerably and different eye cosmetic brands employ various components to make their products more consumer-friendly by giving diverse features to it such as by making it water-proof, various color options, etc. But counterfeit manufacturers rip off these brands to manufacture copycats and produce the duplicate version of the eye cosmetics, thus creating problems to consumers as well as to authentic cosmetic companies. Counterfeit cosmetics are sold at huge discounts, thus harming the image of authentic proudcts and causing huge loss for original cosmetics companies.

One may need to investigate the counterfeit products in great detail to differentiate them from genuine products. The investigation should be carried out by sophisticated techniques in a dedicated laboratory. Numerous analytical techniques are available for the detection of counterfeit cosmetics. Some of these methods are non-invasive while others involve chemical analysis that is capable of examining the active ingredients and impurities in counterfeit cosmetic products. The presence of heavy metals like zinc and lead found in the vast majority of cosmetic products are of insignificant amount, but if the amount increases even a little above the approved level, they can harm the entire body.[16-17] According to a few reports, counterfeit cosmetic products contain heavy metals in lipsticks, kohl, eyeliner, etc, and the presence of heavy metals in cosmetics like lipsticks and kohl, heavy metals get absorbed or enter the human body through the mouth and eye. There have been some studies on contaminated eye cosmetics like kohl which have shown ingestion of lead up to 15% in adults and 41% in children.[18,19] Currently, counterfeiters use a small percentage of active ingredients in their illegal preparations therefore, detection should not only be qualitative but also quantitative.

In the present study, an investigation has been

done on questioned kohl samples which were bought from third-party sellers. Two different brands of kohl/eye pencils (n=12/brand), which were bought from different genuine sources, and third-party sellers (total 24 samples) were analyzed. Analysis was done in three steps: in the first step, packaging analysis was performed by checking the seal, holograms, and barcodes. Physical analysis revealed that out of 20 questioned samples bought from third-party sellers, 7 samples from brand 1 and 8 samples from brand 2 were found to be suspect. In the second step, chemical analysis was done by utilizing sophisticated analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), Proton nuclear magnetic resonance (1HNMR), and Energy Dispersive X-Ray Analysis (EDX). In the third or final step, the analysis was done by employing optical microscopy which confirmed the presence of E. coli. The schematic representation of the work carried out is given in Figure 1. From the chemical and contamination analysis of kohl samples selected from two different brands, it was found that counterfeit samples contained heavy metals which are harmful to the human body, and in addition, microbial contamination was also detected that could cause different diseases.

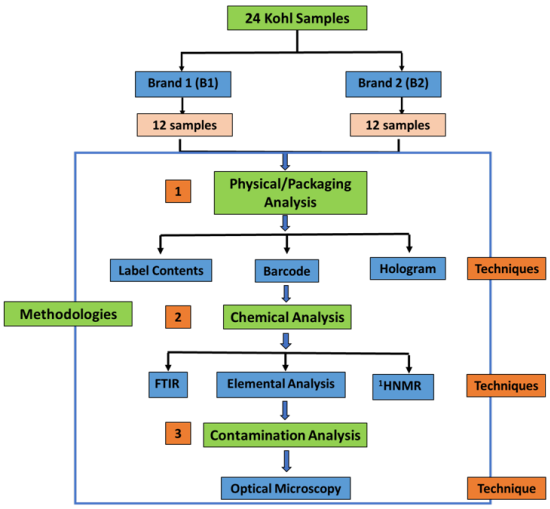
MATERIALS AND METHOD

Materials:

Nutrient broth, Nutrient agar, and Safranine dye were obtained from HiMedia Laboratories Pvt. Limited, (Mumbai, India). Ethanol (99.9% purity) was obtained from Changshu Yangyuan Chemical Co., Ltd, (Suzhou, China). E. coli (MTCC 40) was obtained from IMTECH Chandigarh, India. All the chemicals were used as such without purifying any further.

Samples:

(a) *Authentic/genuine products:* Four genuine samples were utilized as a control for the investigation. These products were ordered online from original websites and these were employed as a control to compare the results to other samples obtained from suspected third-party sellers.



These four samples were from the two different brands named as B1 and B2. Two samples of brand B1 and B2, separately utilized as control, were employed to check the variability of the genuine products and also to assure the relevancy of the obtained results. The references were named as GA1-GA2 obtained from the original website of the first brand and as GB1-GB2 purchased from the original website of the second brand. The naming of the brands was done as such because of confidentiality reasons. The kohl product was selected for the examination because of its frequency to be counterfeited.

(b) *Counterfeits:* Twenty suspected counterfeits of the kohl product were examined in the study which was bought from third-party sellers, local vendors, local and street shops. The counterfeit samples were named as C1-C10 for the first brand and C11-C20 for the second brand.

Methodologies:

Packaging Analysis: The authentic and questioned cosmetic products have been purchased online from genuine e-commerce websites and suspected third-party sellers, respectively. The questioned cosmetic products were bought after reading critics' reviews in which the quality of the product has been questioned because of the torn packaging, smell from the cosmetics, irritation after application, and finally having massive discounts up to 79% which seemed too good to be true. The visual

comparison was performed between genuine and suspect kohl cosmetic packings.

The packaging was analyzed considering the keypoint i.e. authentication of the hologram analyzing concealed features, for example, scrambled pictures, micro-text, and UV-sensitive inks, the barcode using the mobile app (QR & Barcode Scanner by Gamma Play), content on the label, spelling mistakes or misprint, presence/absence of words on the product body, repeated letters usually at the end of the word, tags, modifications that appear to be typos, and poor printing. All the samples that were bought online were scanned first using a barcode scanner mobile app.

Chemical analysis: The determination of elemental composition in counterfeits is important since it enabled us to determine the level of toxicity of the counterfeits. The chemical composition of the questioned and authentic product was confirmed by EDX using Phillips XL 30 EDX unit. The phase transition temperature for both questioned and authentic products was estimated using Differential Scanning Calorimetry (DSC) on DSC-Q20 (TA Instrument, USA) using an aluminum cup with T zero hermetic lids. Measurements were performed in the dry phase. Perkin Elmer- FTIR Spectrophotometer-100 was used to obtain the FTIR spectra; samples were prepared as pellets operated in the spectral domain of 50-600 and 400-4000 cm-1 at a resolution of 4 cm-1. Bruker Avance 400 MHz was utilized to obtain the 1HNMR spectra. Tetramethylsilane was utilized as an internal standard and the solvent used was deuterated chloroform.

Contamination analysis: Leica DM 3000 optical microscope was utilized to check the microbial contamination in questioned samples.

Culture preparation to assess the contamination in questioned products:

(a) *Bacterial culture preparation:* Nutrient Broth (NB) is a basic medium, used for the general cultivation of bacteria. NB comprises 5.0 g/L peptic digest of animal tissue, 1.50 g/L yeast extract, 5.0 g/L sodium chloride, and 1.50 g/L beef extract. The nutrient broth was weighed and autoclaved at 121oC and 15 psi. The questioned sample was used as an inoculum in the broth and incubation was given for 24 h at 37oC.

(b) *Microbial contamination analysis in questioned products:* To check the microorganism contamination or growth on questioned kohl samples, the tip of questioned kohl was cut using a sterile cutter and dipped in autoclaved water for 10 minutes. Further, this water was used as an inoculum in nutrient broth to grow the contaminants and which additionally employed to prepare slides. Staining was done using safranine dye 0.5% w/v.[20]

RESULTS

Packaging Analysis:

Most of the cosmetic products available in the open market are easily replicated. Packaging is one of the key components that different companies use to show the reliability of their products. To analyze the packaging of different samples, various parameters were taken into consideration such as contents on the label, hologram investigation, and barcode reading.

Label Content:

Spelling mistakes, misprints, presence or absence of words on packaging, repeated letters usually at the end of the word, tags, modifications that appear to be typos, and poor printing were found on the packaging of most samples bought from street vendors.

Whereas in the case of samples bought from genuine websites (n=4), quality inks were used for printing of the packaging. It was noted that the packaging of questioned products had poor quality of inks that were fading off.

Holograms:

The hologram is the most reliable sign of authenticity in which a 3-D image is seen and is readable on the product surface. The visualization is done by keeping the hologram at a particular angle/space to maintain the white light on it and situated on the watcher's side of the 3D image. Through the first-line investigation of the hologram on the samples, it was found that out of 20 samples, 17 samples contained damaged or fake holograms (B1) or just a silver tag (B2). The absence of micro-

text was also detected in these samples when compared to genuine samples (n=4).

In conclusion, it was observed that the advancement in optical technology and a combination of opaque and translucent inks has not only enhanced the appeal of holographic effects but also made it difficult to replicate and produce the exact copy of hologram by the counterfeiters.

Barcode:

Barcodes are divided into 1D, 2D, and 3D categories. The 1D barcodes consist of a series of bars, 2D barcodes comprise of dots and squares, and 3D barcodes use the same basic principle as 1D (linear) and 2D barcodes but are engraved on the product. Examined samples contained only 1D barcodes, to authenticate the cosmetic samples, a barcode reader mobile app was utilized i.e. QR & Barcode Scanner by Gamma Play. All the samples bought online were scanned using a barcode scanner app on mobile. Few of the questioned cosmetic products having 1D linear barcodes that did not scan or had no online data about its origin were red-flagged but on most items, counterfeiters did a good job duplicating the 1D linear barcode on the packaging thus the scanning app showed results of that product from the web to original barcodes which are manufactured and fed information regarding its SKU (Stock Keeping Unit) and it was concluded that one positively can't know a counterfeit item from a 1D barcode scanner. More chemical analysis was performed to investigate all the questioned samples (n=20).

Chemical analysis: The determination of elemental composition in counterfeits is important since it enables the determination of the level of toxicity of the counterfeit. Many technologies are available for that purpose; for example, Energy Dispersive X-Ray Spectroscopy (EDX), Proton Nuclear Magnetic Resonance (1HNMR) technologies, etc.

EDX:

Energy Dispersive X-ray analysis (EDX) is a widely used non-destructive elemental analysis method. The elemental analysis of all samples was confirmed by EDX. The examples were then exposed to morphological perception and basic investigation through filtering electron microscopy/vitality dispersive X-beam examination (SEM/EDX) with no coatings.

Figure 1 represents the elemental composition (EDS) of the authentic kohl samples i.e. GA1 and GA2 of first brand(B1), confirming the presence of carbon, iron, oxygen, silicon, and nitrogen with an average weight percentage of 53.44%, 24.67%, 17.91%, 2.39%, and 1.58%, respectively (Fig. 1a and Table I). Whereas elemental analysis of 10 questioned kohl samples indicated the presence of carbon, silicon, iron, and oxygen with an average weight percentage of 88.07%, 0.82%, 0.69%, and 10.03%, respectively (Fig. 1b and Table II). The presence of palladium, cadmium, and mercury was also detected in all the samples with an average weight percentage of 0.01%, 0.10%, and 0.26% in trace amount indicating the harmfulness of counterfeit kohl samples C1-C10 of first brand (B1).

The elemental analysis of the authentic kohl sample GB1 and GB2 of the second brand (B2), confirmed the presence of carbon, iron, oxygen, silicon, and nitrogen with a weight percentage of 65.39%, 14.42%, 14.86%, 1.80%, and 3.54%, respectively (Fig. 2a and Table III). Whereas, elemental analysis of questioned counterfeit kohl samples confirmed the presence of carbon, silicon, iron, and oxygen with the average weight percentage of 74.30%, 1.70%, 1.44%, and 22.35%, respectively(Fig. 1b and Table IV). The presence of palladium, cadmium, and mercury was also detected in all questioned counterfeit samples with an average weight percentage of 0.02%, 0.07%, and 0.11% respectively in trace amount indicating harmfulness of questioned kohl sample B2. This comparison study confirmed that all the questioned kohl cosmetics of both brands i.e., B1 and B2 which were purchased from suspected third party sellers on 70% discount contained very harmful heavy metals that could cause harm.

FTIR

Fourier transforms infrared (FTIR) spectroscopy is a rapid analytic technique that can identify

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	62.73	53.44 ± 2.00	71.66
Iron K	K-series	28.96	24.67 ± 1.00	7.11
Oxygen K	K-series	21.03	17.91 ± 0.05	18.03
Silicon K	K-series	2.81	2.39 ± 0.42	1.37
Nitrogen K	K-series	1.86	1.58 ± 2.00	1.82
	Total	117.39	100.00	100.00

Table 1: Average elemental composition of authentic kohl sample GA1 and GA2 of first brand (B1) (mean= ± S.D., n = 2).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	88.07	88.07 ± 4	91.60
Silicon K	K-Series	0.82	0.82 ± 1	0.36
Iron K	K-series	0.69	0.69 ± 2	0.15
Oxygen K	K-series	10.03	10.03 ± 2	7.83
Palladium L	L-series	0.01	0.01 ± 0.23	0.00
Cadmium L	L-series	0.10	0.10 ± 0.48	0.03
Mercury M	M-series	0.26	0.26 ± 0.39	0.02
	Total	100.00	100.00	100.00

Table 2: Average elemental composition of questioned counterfeit kohl samples C1-C10 of first brand i.e. B1 (mean= ± S.D., n = 10).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	73.15	65.39±4	78.36
Iron K	K-series	16.12	14.42±3	3.72
Oxygen K	K-series	16.62	14.86±3	13.37
Silicon K	K-series	2.01	1.80±1	0.92
Nitrogen K	K-series	3.96	3.54±3	3.63
	Total	111.86	100.00	100.00

Table 3: Elemental analysis of authentic kohl sample GB1 and GB2 of second brand (B2) (mean= ± S.D., n = 2).

Element	Series	unn. C [Wt%]	norm. C [Wt%]	Atom. C [at%]
Carbon K	K-series	74.30	74.30 ± 4	80.60
Silicon K	K-Series	1.70	1.70 ± 1	0.79
Iron K	K-series	1.44	1.44 ± 2	0.34
Oxygen K	K-series	22.35	22.35 ± 5	18.19
Palladium L	L-series	0.02	0.02 ± 0.6	0.00
Cadmium L	L-series	0.07	0.07 ± 0.4	0.01
Mercury M	M-series	0.11	0.11 ± 0.1	0.07
	Total	100.00	100.00	100.00

Table 4: Average elemental composition of questioned counterfeit kohl samples C1-C10 of first brand i.e. B1 (mean= ± S.D., n = 10).

Sl. No.	Functional Groups	Authentic Sample of GA1 & GA2	Questioned Sample C1-C10 of first brand
1.	Alkane (C-H)	2916, 2958 stretching (strong)	2915, 2958 (strong)
2.	Alkene (C=C)	1634 weak stretching	-
3.	Alkyne	2165 medium stretching	-
4.	Aromatics	1733 bending Weak	-
5.	Aldehyde	-	1738
6.	Nitrosamine	1462	1462
7.	Halogen Group	556	543, 378 and 718
8.	Alkyl Amine	1049	-
9.	Alkyle Ketone	1258	-

Table 5: FTIR spectra values of authentic samplesGA1 and GA2 and Questioned samples i.e.C1-C10 of first brand B1.

Sl. No.	Functional	Authentic Sample B2	Questioned Sample B2
1.	Alkane (C-H)	2915 (strong), 2962 stretching	2915 (stretching prominent) 2966 stretching
2.	Aromatics	1466	1462 strong
3.	Aldehyde	1748 stretching	1738 strong stretching
4.	Alkyl amine	1060 prominent	1017 (for amines)
5.	Alkyl ketone	1258 prominent	-
6.	Aromatic Compound	2915 (strong), 802 prominet bending	-
7.	Halogen Compound	534 prominent, 706	722
8.	Nitrosamine	1466 weak	1462 prominent
9.	carboxylic acid	2855 stretching	2848 stretching
10.	Ester carbonyl	-	1172

Table 6: FTIR spectra values of Authentic samples(GB1 and GB2) and Questioned samples (C11-C20)of second brand i.e. B2

Value	Authentic Samples (GA-1 & GA2)	Value	Questioned Sample (C1-10)
1.12, 1.17 1.24	Amino, Alpha-methylene	0.9	Primary aliphatic RCH3 Protons of $CH_2CH_2N(CH_3)_3$
7.20	Aromatic	1.28	Methylene protons
		2.31	Benzylic 2.33 2.36
		4.00	Fluorides R-CH_3-X
		7.29	Aromatic

Table 7: 1HNMR δ value of authentic sample (GA1-GA2) and questioned samples (C1-C10)of first brand (B1).

Value	Authentic Samples (GA-1 & GA2)	Value	Questioned Sample (C1-10)
0.13-1.6 1.6 hydrocarbon chains	Methylene proton, terminal methyl groups of 2	0.88 0.98 1.10	Primary and secondary aliphatic chain
7.1-7.4	Aromatic	2.29	Benzylic
-	-	3.97-3.98	RCH-X, Alpha to halogen (C is attached to chlorine) alkyl halyde
-	-	7.2	Aromatic

Table 8: 1HNMR δ value of authentic sample (GA1-GA2) and questioned samples (C1-C10)of first brand (B1).

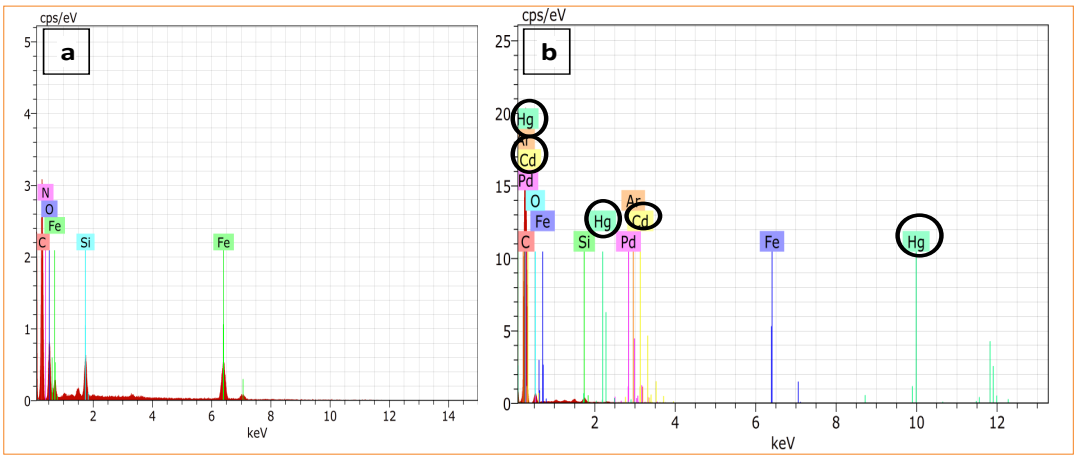


Figure 1: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C1-C10).

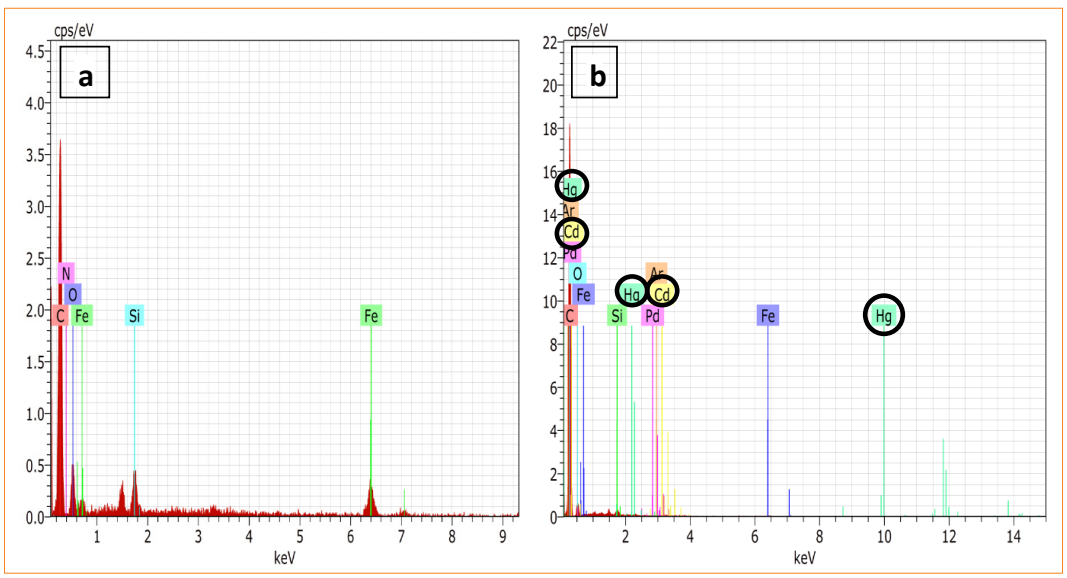


Figure 2: Combined result of elemental analysis of (a) authentic kohl sample and (b) questioned kohl sample C11-C20 of second brand

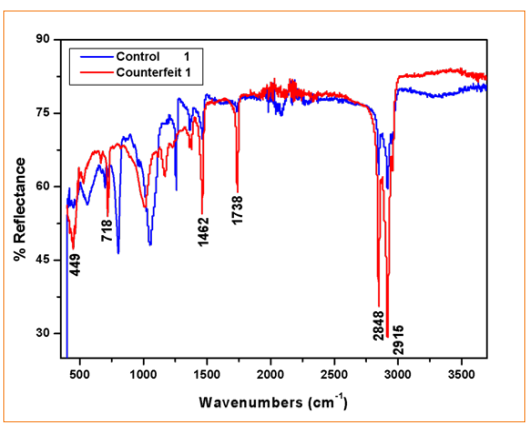


Figure 3: FTIR spectra of Authentic sample (GA1-GA2) B1 and Questioned samples (C1-C10) of first brand B1.

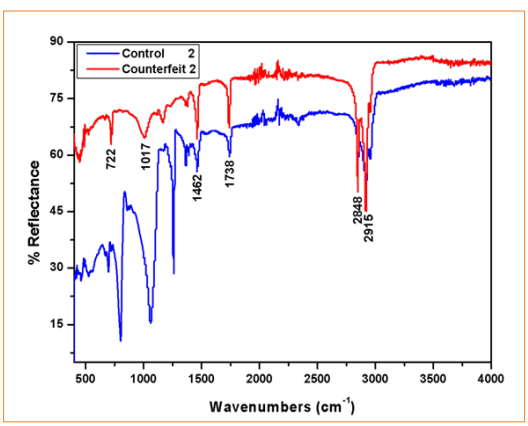


Figure 4: FTIR spectra of authentic sample (GB1 and GB2) and counterfeit samples (C11-C20) of second brand

METALS	BRAND B1 (ppm)	BRAND B2 (ppm)
Pd	100±3	200±2
Cd	1000±3	700±1
Hg	2600±2	1100±2

Table 9: Concentration of heavy metals found in the kohl samples (i.e.B1 and B2) (mean= ± S.D., n = 10)

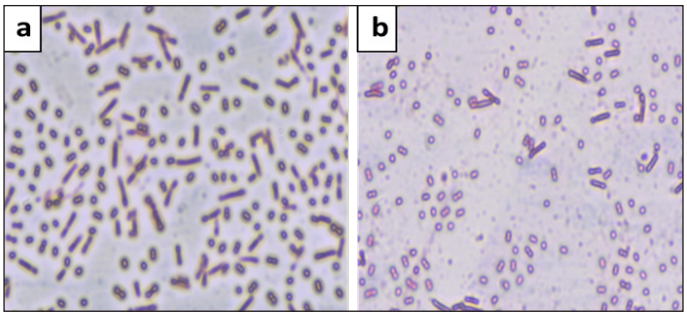


Figure 7: Contamination analysis of two different questioned kohl samples (a) Presence of E. coli observed in C3 sample of first brand (b) Presence of E. coli observed in counterfeit sample C14 of second brand.

organic and inorganic materials in a short time. FTIR offers a simple, rapid, and non-destructive technique for the identification of counterfeit cosmetics. The composition of the authentic and all the counterfeits showed high similarity with the chemical groups. However, counterfeit cosmetic samples showed lower absorbance intensity.

The elemental analysis of the authentic kohl samples from GB1 and GB2 of the second brand (B2), confirmed the presence of carbon, iron, oxygen, silicon, and nitrogen with a weight percentage of 65.39%, 14.42%, 14.86%, 1.80%, and 3.54%, respectively (Fig. 2a and Table III). Whereas, the elemental analysis of questioned kohl samples confirmed the presence of carbon, silicon, iron, and oxygen with the average weight percentage of 74.30%, 1.70%, 1.44%, and 22.35%, respectively (Fig. 1b and Table IV). The presence of palladium, cadmium, and mercury was also detected in all counterfeit samples with an average weight percentage of 0.02%, 0.07%, and 0.11% in trace amount indicating harmfulness of questioned kohl sample B2. This comparison study confirmed that all the questioned kohl cosmetics of B1 and B2 which were purchased from suspected third party sellers on 70% discount, contained very harmful heavy metals that could cause harm to the human body.

FTIR analysis was employed to check the profile of the samples and also to compare the authentic and questioned samples. FTIR assisted in identifying the organic as well as inorganic components present in the questioned sample. FTIR spectra of the authentic samples (GA1 and GA2) gave a peak at 2916 and 2958 cm-1 which corresponds to CH2 stretching vibration. The peak at 1634 and 2165 cm-1 indicated stretching of the alkene and alkyne group, respectively. The peak at 1733 cm-1 corresponds to the aromatic group. The peak at 1049, 1258, 802, 556, and 1462 cm-1 confirmed the presence of alkyl amine, alkyl ketone, aromatic compound, halogen compound, and nitrosamine, respectively (Fig. 3).

In comparison, the questioned samples confirmed many prominent peaks and also indicated the absence of many functional groups such as alkene, alkyne, alkylamine, and alkyl ketone. FTIR spectra of the questioned sample gave peaks at 1738 and 1462 cm-1 which correspond to the aldehyde and nitrosamine functional group, respectively. The peaks at 543, 678, and 718 cm-1 indicate the presence of the halogen group (Fig. 3).

FTIR spectra of the authentic samples (GB1 and GB2) gave the peaks at 2915 and 2962 cm-1 which corresponds to alkane chains present in the sample. The peak at 1748, 1060, and 1258 cm-1 correspond to aldehyde, alkylamine, and alkyl ketone, respectively. The peak at 1466 and 802 cm-1 confirmed the bending of the aromatic group. The peak at 534 and 706 indicates the presence of a halogen compound. A weak peak was also found in 1466 cm-1

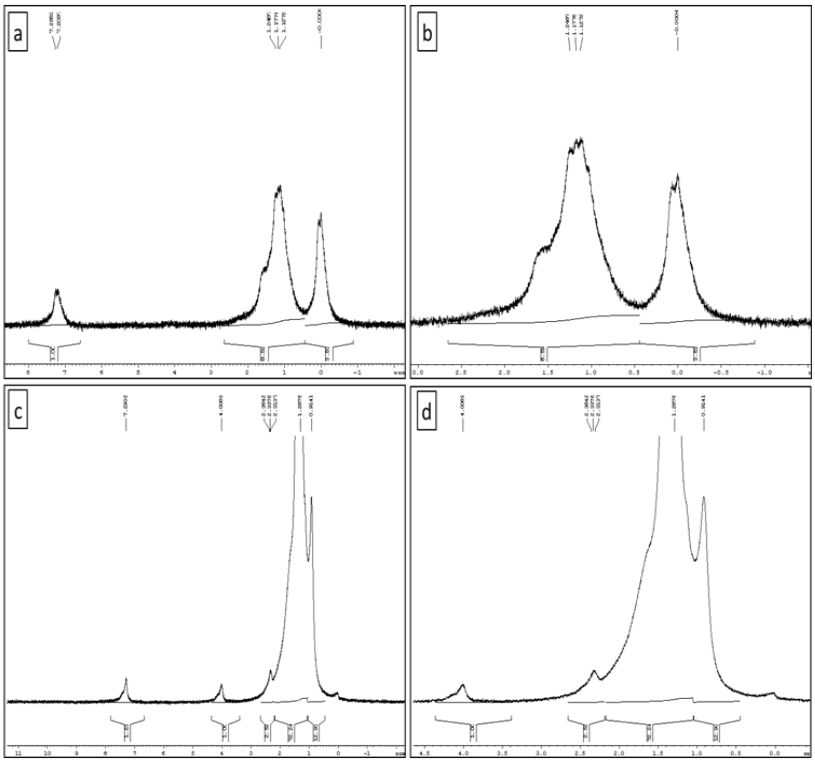


Figure 5: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C1-C10).

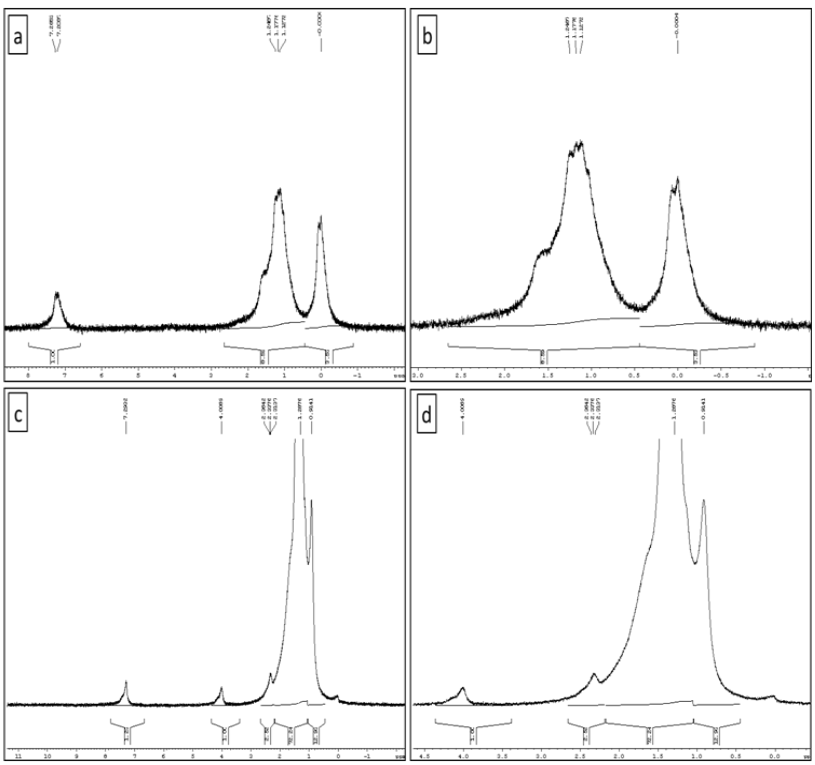


Figure 6: Combined results of elemental analysis of (a) authentic kohl sample (GA1 and GA2) of B1 (b) Questioned kohl samples (C10-C20).

relates to a nitrosamine. The peak at 2855 cm-1 corresponds to a carboxylic acid (Fig. 4).

In comparison, the FTIR spectra of all the questioned samples (C11-C20) confirmed the absence of the alkyl ketone group. The peak at 2915, 1462, and 1738 cm-1 correspond to the alkane chain, aromatic, and aldehyde group, respectively. The peak at 722, 1462, and 2848 cm-1 indicate the presence of halogen compound, nitrosamine, and carboxylic acid, respectively. A peak at 1172 cm-1 corresponds to ester carbonyl which was not detected in the FTIR spectra of the authentic sample (Fig. 4).

HNMR

Nuclear magnetic resonance spectroscopy or (NMR spectroscopy) determines the physical and chemical properties of atoms or the molecules in which they are contained. 1HNMR provides useful information for resolving the structures of closely related compounds.

The 1HNMR spectra obtained for authentic samples (GA1 and GA2) of B1 reports signal at δ=1.12, 1.17, and 1.24 ppm which was assigned to alkyl-methylene protons. The signals obtained at δ=7.20 and 7.26 ppm were assigned to the aromatic proton present in the sample (Fig. 5a & b).

In comparison, 1HNMR spectra obtained for all the questioned samples (C1-C10) confirmed a peak at δ=0.9 ppm which was assigned to primary aliphatic RCH3. A peak at δ=1.28 corresponds to methylene protons. A peak at δ=4 ppm was obtained which was allocated to R-CH3-X (X=halogen, O). The peak at δ=2.31, 2.33, and 2.36 ppm were assigned to benzylic protons, and a peak at 7.29 ppm was assigned to aromatic, a proton is on the phenyl ring (Fig. 5c & d). From 1HNMR, it was found that both the samples (authentic and questioned) have different molecular structures and protons are allocated in different positions as confirmed from the spectra. It was also confirmed from 1HNMR spectra of first brand that questioned samples contained many elements which were noted to be absent in the authentic samples. All the peaks obtained in the 1HNMR spectra of authentic and questioned samples are provided in Table VII.

In authentic samples of B2, 1HNMR spectra confirmed the peak at δ=0.13 & 1.6 ppm which were assigned to methylene protons. A peak at 7.29 ppm was assigned to an aromatic compound, a proton is on the phenyl ring (Fig. 6a & b).

In comparison, 1HNMR spectra of questioned samples of second brand (B2) obtained peaks at δ= 0.88 and 0.98 ppm which was assigned to primary aliphatic RCH3. The peaks at δ= 1.10, 1.25, 1.41, and 1.61 were assigned to peak secondary aliphatic R2CH2. A peak at δ=2.29 ppm was assigned to benzylic protons. The peaks at δ=3.97 & 3.98 ppm were obtained which was allocated to R-CH3-X (X=halogen, O). A peak at 7.2 ppm was assigned to an aromatic compound, a proton is on the phenyl ring (Fig. 6c & d). All the peaks obtained in the 1HNMR spectra of authentic (GB1-GB2) and questioned samples (C11-C20) are provided in Table VIII. NMR delta values for both the authentic and questioned samples B2 are different therefore it is concluded that the molecular structure and composition of both samples are dissimilar and consist of impure elements.

Microbiological contamination analysis: Different questioned samples were investigated to observe the presence or absence of microorganisms. Optical microscopy was used to detect microbial contamination. Two questioned samples were found to be contaminated with E. coli. E. coli and budding E. coli were found in the questioned samples (Fig. 7a & b). Figure 7a depicts the contamination of questioned samples C3 of the first brand (B1) and figure 7b represents the contamination of questioned samples C14 of the second brand (B2).

Toxicity assessment of heavy metals:

From the EDS data, the mean concentration obtained for the toxic heavy metals i.e. Pd, Cadmium, and Mercury were 100, 1000, and 2600 ppm, respectively for the case of brand B1 (mean of 10 samples), whereas for the case of brand B2, the mean concentration obtained for Pd, Cd, and Hg were 200, 700, and 1100

ppm, respectively (mean of 10 samples, Table IX). In all the confirmed counterfeit products, the maximum concentration observed was of mercury (in both kohl sets of B1 &B2). And the decreasing concentration trend found in both sets was Hg>Cd>Pd.

DISCUSSION

Packaging analysis:

It was observed from the hologram analysis that the advancement in optical technology and a combination of opaque and translucent inks has not only enhanced the appeal of holographic effects but also made it difficult to replicate and produce the exact copy of hologram by the counterfeiters.

Through the investigation of barcodes, it was found that counterfeiters did a good job of 1D linear barcode duplication on the packaging and it was concluded that counterfeit items can't be detected from a 1D barcode scanner. So a chemical analysis is needed to investigate the questioned samples.

Chemical Analysis:

The result of questioned and authentic samples was confirmed through EDX test and it was discovered that all the questioned kohl cosmetics of both B1 and B2, which were purchased from third party sellers on 70% discount, contained the very harmful heavy metals that could cause harm to the human body. Fourier transforms infrared (FTIR) spectroscopy identified organic and inorganic materials. NMR delta values for both the authentic and questioned samples B2 are different therefore it is concluded that the molecular structure and composition of both samples are dissimilar and consist of different elements. Through microbial study, two questioned samples were found to be contaminated with E. coli.

Toxicity assessment of heavy metals:

The most common route of exposure is the dermal layer as cosmetics are applied directly on the skin. The ions released from the heavy metals form complexes after getting absorbed into the skin and makes a bond with amine (-NH2), carboxylic acid (-COOH), and thiol (-SH) of proteins that lead to the distribution in the working of cells and also causes cell death. This bond formation causes various diseases, however, the information related to the exposure of metal toxins through skin is very limited. [21]

In all the confirmed counterfeit products, the maximum concentration observed was that of mercury (in both kohl sets i.e. B1 & B2). And the decreasing concentration trend found in both sets was Hg>Cd>Pd (Table IX).

The concentration obtained in the kohl samples indicated the high level of toxicity, as according to the guidelines of US food and drug administration 'standard level of mercury concentration should not exceed 1 ppm and the use should be restricted if the utilization is inevitable, FDA has restricted the use of mercury and other heavy metals in cosmetics.[22] Contradictory to the FDA regulations, the concentration attained in the kohl sample was in excess amount i.e. 2600 ppm for B1 samples and 1100 ppm for B2 samples, signifying the high toxicity level of mercury.

Many investigations reported the toxic behavior of cadmium in the biological system, according to various research studies, heavy metals such as cadmium,[23] mercury[24,25] etc release the reactive oxygen species (ROS) and also causes oxidative stress in the cells leading to cytotoxicity, genotoxicity, and carcinogenicity. These heavy metals are known to cause a high degree of toxicity and induce organ damage even at a lower level.[26] United States Environmental Protection Agency (U.S. EPA), and the International Agency for Research on Cancer (IARC), also mentioned these metals as 'known' or 'probable' carcinogen in humans.

CONCLUSION

Counterfeit cosmetics business is a hazard not only at the economic level but also at the consumer level. Consumers around the world have been victims of counterfeits. Therefore, consumers need to be informed and be vigilant. The strategy presented here is based on packaging analysis to recognize security traits such as overt and covert features to distinguish

between genuine and counterfeit cosmetics. The analysis of the chemical composition of questioned products in a dedicated lab can help law enforcement. The advantage of this combined set-up is to facilitate the screening of counterfeits in the market to speed up the investigation to determine the danger to which the consumers are being exposed.

Investigation unravel the huge nexus among the manufacturers of counterfeit products. Important information collected during the packaging examination of the greater part of the counterfeit and the chemical examination can be recorded to a database and then compared with the data acquired from former counterfeits. By promoting authentication, security features, tracking mechanisms, and investigative services, these standards will bring confidence to consumers, administration, and commerce. IJFMP

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The authors declare that there is no commercial or financial links that could be construed as conflict of interests.
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ORIGINAL ARTICLE

Evaluation of the Concentration of Heavy Metals in Kohl (Kajal) using ICP-OES

[1]Kamna Sharma, [2]Sally Lukose

ABSTRACT

CONTEXT: Kohl or *kajal* is an ancient and popular eye cosmetics. In its simplest form, it is a fine black or gray powder that contains high level of lead (usually in the form of lead sulphate). *Kohl* is a popular eye product and is prepared in ultra-fine form using galena along with some other ingredients. Presently, most of the women and adolescents use it in their daily make up regime to enhance their beauty. However, it is known to contain varying levels of toxic compounds in the form of additives making it highly unsafe for use.

AIMS: In the current study, an attempt has been made to determine the concentration of lead, nickel, and cobalt in kohl samples.

MATERIALS AND METHOD: Ten different Kohl samples of different brands were bought from online and nearby markets. The evaluation of heavy metals such as lead, nickel and cobalt were determined from the samples usingInductively Coupled Plasma-Optical Emission Spectrometry(ICP-OES).

RESULTS: Results obtained in the study have been alarming especially in the case of lead which was found to be as high as 8 ppm, nickel 5ppm and cobalt 4 ppm in different brands.

CONCLUSIONS: Regular application of kajal with excessive high content of heavy metals leads to serious health hazards.

KEY MESSAGES: Kohl containing heavy metals can have health hazards with long term usage and hence should be used judiciously.

KEYWORDS | Kohl, heavy metals, ICP-OES, toxicity

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INTRODUCTION

KAJAL (KOHL) IS ONE OF THE EASILY available and most common eye cosmetics. And it has been used since ancient times. It is prepared by using a specific powder known as "*kohl stone*" (Galena) which is later processed with other ingredients. The purpose of this powder was to keep the eyes cool and clean, improve vision and strengthen the eyes. It was believed that it could protect the eyes from direct sun light and were used to line the upper and lower lashes.[1,5] Apart from being widely used since ancient times, it was also used by other tribal communities, who used it for drawing distinct lines around the forehead, nose, and other body parts as well.[2,4] It comprises of other chemicals like galena (PbS), minium (Pb_3O_4), amorphous carbon, magnetite (Fe_3O_4), and zincite (ZnO). Due to its composition, it is considered unsafe for use and as an illegal substance to be imported or sold in the United States by FDA.[3,7,8]

The US Food Drug and Cosmetic Act[8] defines cosmetics as any articles which are intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied to the human body or any other part for

beautifying, promoting attractiveness or altering the appearance.

MATERIALS AND METHOD

OBJECTIVE

Analysis and estimation of Lead, Nickel and Cobalt in kohl samples purchased from local markets.

HYPOTHESIS

1. Kohl samples collected from the local market would contain lead, nickel & cobalt that can cause skin diseases.
2. Quantitative estimation of lead, nickel and cobalt can be done using ICP-OES.

SAMPLE TYPE AND SIZE

Kohl samples for the study were bought from the local market. Post-purchase, all the Kohl samples were photographed in their original packaging, the purchase bills secured and the composition, if any, were noted down. In all ten branded kohl, samples were taken for analysis of lead, nickel and cobalt.

SAMPLE PREPARATION

All the apparatus were thoroughly washed and rinsed using normal water followed by immersing the same in 5% solution of Nitric Acid (HNO_3) overnight, later by rinsing with deionized water before using the same.

1. 1 gm/ 1 ml of kohl was taken in a beaker.
2. The beaker was then heated in muffle furnace at 450°c.
3. After the sample was turned to ash, the digestion was done.
4. For acid digestion, hydrochloric acid and nitric acid was taken in a ratio of 1:3.
5. 25 ml of acid digestion was added to the beaker and heated on a tripod stand till the solution was clear.

INSTRUMENT USED

The Vista-MPX simultaneous ICP-OES with axially viewed plasma was used for this work. The instrument was fitted with the 3-channel peristaltic pump which further helps in easy introduction of ionization buffer to the sample via a post-pump Y-piece.

WORKING PRINCIPLE

- First of all, the sample is introduced into the chamber, in a liquid form which is sprayed using a nebuliser. Due to the high temperature inside, the chamber atomizes and ionizes the sample, creating positively charged atomic ions.
- The larger droplets are then removed from the gas chamber, and the remaining smaller droplets are transferred into the central passage of an argon plasma.
- The droplets are then dried, deteriorated, and dissociated into an individual atom in the chamber.
- These atoms are then converted into cations via interface before they enter the vacuum system.
- Electrostatic lenses keep the ions focused, as they pass to the chamber and the outcome were recorded by the detectors. It uses a higher thermal energy which discrete the cations from the photons and neutral particles.
- Analyte ions are then separated & scanned using multiplier detector. The spectrometer will measure the spectrum of each ion.
- The light intensity on the wavelength is measured and with the calibration calculated into a concentration.

RESULTS

This research was performed in triplicate analysis. The number of selected kohls were ten which were collected from the cosmetic shops in the local market and via online. The data presented in Table 1 and Graph 1, shows remarkably high concentration of lead in all the brands, the least being in Patanjali estimated at 3ppm. The maximum was observed in Coloressence at 12 ppm. The findings obtained in the present study indicate that kohl products available in the markets contain heterogeneous chemical composition and contain significant amounts of toxic elements other than lead such as nickel and cobalt which are bound to cause deleterious effects on the body.

DISUCSSION

The results obtained in the present study

BRAND	LEAD (PPM)	NICKEL (PPM)	COBALT (PPM)
Avon	8	4.5	4
Attitude	4	2	2
Colorbar	3.7	2	2
Coloressence	12	5	4
Loreal	5.5	4.5	2.8
Maybelline	6	4	3
Oriflame	4	3.7	2.4
Mac	4.4	2.8	3.7
Faces	2.8	3.2	2
Patanjali	3	2.6	2.2

Table 1: The concentration of lead, nickel, and cobalt in Kohl brands.

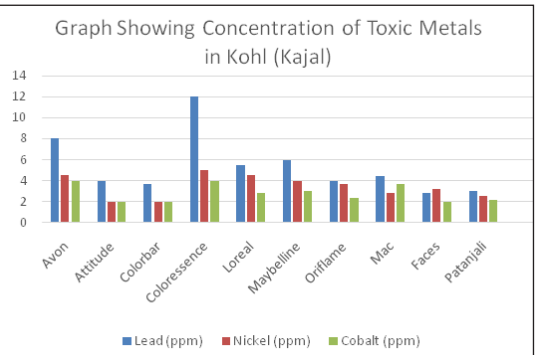

Figure 1: Graphical representation of lead, nickel, and cobalt in Kohls

confirm the findings published in previous studies that have analyzed kohl. The findings in the present study are a definite cause for concern considering that kohl is a very commonly used cosmetic product used by almost all age groups varying from infants to the adult. The easy availability of this product at retail outlets and online purchases adds to the concern. The problem however gets magnified as some of these products contain significant amount of lead and other heavy metals such as nickel and cobalt. Additionally, some of these products also do not provide the requisite information regarding the qualitative and quantitative composition.[9] The study also emphasis the need for a quality control in the product manufacture.

CONCLUSION

In earlier times, women preferred to prepare Kohl at home. Nowadays people purchase them from the market. The study has revealed that the presence of higher amount of lead may be due to the factitious elements in the samples as there are no proper awareness in the production and distribution of these products. However, the chances of mixing of sub-standard elements can't be ignored. The result clearly shows that improvements need to be conducted of these toxic metals which are used in cosmetic products. The permissible limits of potential impurities in cosmetics such as the *kajal* must be strictly enforced. The companies can take steps to minimize the impurities in their products by following good manufacturing practices. There is an urgent need for a thorough evaluation of health risks to the users from these cosmetics which are adulterated with harmful heavy metals. It was concluded from the result that most of the brands of were tainted with a high concentration of lead. It was inferred from the result that most of the brands of kajals were contaminated with high concentrations of lead. Manufacturers can help minimize impurities in cosmetics by following good manufacturing practices. This includes testing of ingredients and the finished products to make sure they meet certain manufacturing standards. Removal of toxic metals is not possible after the product has been manufactured. However, if the contents are carefully chosen while keeping in mind the toxicity of heavy metals, we can surely improve the quality of these products to protect the consumers using these products.

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REVIEW ARTICLE

Advances in Chemiluminescence based Explosive Detection

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ABSTRACT

CONTEXT: Detection of explosives is indeed crucial for the fact that it has harsh impact upon peace in the community as well as its nature as a contaminant in the environment. As of now there have been numerous additions due to clandestine synthesis of explosive materials which are not up to date with the spectral library of various spectrometric techniques. For the detection of explosives new mechanism are often required which should have advantages like sensitivity, selectivity, rapid response, low cost, remote sensing and sensors made with ecofriendly material. Techniques which are commonly utilized areIon Mobility spectrometry, Gas Chromatography – Mass Spectrometry, Fido-XT for sensing explosives like Trinitrotoluene (TNT), Trinitrophenol (TNP), and Dinitrotoluene (DNT) etc. Apart from that, military agencies, police and forensics are still having a hard time to detect the trace amount of explosives like RDX, PETN, Tetryl and HMX etc. selectively. Recent upbringing in Chemiluminescence based explosive detection showed quite a reliable way for detecting these compounds. In Chemiluminescence based detection various materials are used namely conjugated fluorescent polymers, small molecule fluorophores, supramolecular system, Bio-inspired fluorescent materials, Aggregation induced emission active materials. These techniques if combined with various techniques like electronics, imaging and sensor design then it would provide deployment over real field for explosive detection which includes buried land mines, environmental contamination by explosive material. This review article may help the readers in order to get insight about what is explosive, various types of explosive, and mechanism of explosion and explosive detection techniques.

KEYWORDS | explosive, detection, detection technique, chemiluminescence

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INTRODUCTION

The threat of improvised explosive devices (IEDs) is a major concern in the twenty-first century. Explosive devices, which are frequently used by extremists and revolutionary groups, can be created by using commercial detonators, and scrap elements from leftover army munitions. Undeployed munitions from current and historical warzones are a source of munitions. Bomb sniffing canines are commonly employed to locate hidden munitions, and even when they are incredibly successful, they have drawbacks in concerns of maintenance, instruction, expense, and temperament. An explosive has 4 fundamental properties: 1) It is a chemical substance or blend which is triggered by heat, shock, impact, friction, or a combined effect of such circumstances; 2) It decays swiftly in a detonation; 3) There is indeed a sudden discharge of thermal energy as well as massive amounts of high-pressure gaseous substances that expand speedily with considerable power to surmount constraining factors; and 4) The energy generated by detonation has four main consequences: a) stone shattering, b) stone dislocation, (c) earth tremor, and (d) atmospheric shockwave.[1] According to a theoretical

perspective of explosive, the explosion of the explosive materials generates a large blast wave as well as a massive discharge of gases. The blast wave fractures and compresses the rocks around the explosive, resulting in dozens of splits. The expanded gas subsequently fills in the fractures. The gaseous substances keep filling and extend the fractures till the compressed gases become too low to allow the fractures to enlarge any more or until they are expelled from the stone.[1]

Almost all high explosives contain at least one nitro-functional group. In general, nitroaromatic compounds including TNT, DNT and NT are one of the main explosive materials used primarily by army and as one of the main constituents present in the buried underground mines nationwide. Nitramines and nitrate esters (e.g., 3,5-trinitroperhydro-1,3,5-triazine and pentaerythritol tetranitrate, are main components of highly energetic plastic explosives, such as C-4 (91% cyclonite) and Semtex (40–76% pentaerythritol tetranitrate). Because nitrogen group containing explosives are particularly responsive to shock, friction, and impact, detecting techniques that allow for contact-free examination are preferred.[1] Furthermore, the need to identify concealed munitions in transit stations and underground bombs in conflict areas has sparked strong interest in low-cost, supersensitive explosive identification technologies. In comparison to recognition in liquid and solid phases, identification of nitro group containing explosive in vapor state is more difficult because most of them have very low volatility.1 Despite the fact that modern nitro-explosive vapor monitoring depends mainly on Ion-mobility Spectrometry and Gas Chromatography combined with Mass Spectrometry (GC-MS), their intricate methods, low accessibility, and increased price have limited their widespread applicability As a result, there is a considerable requirement for revolutionary sensor devices that are inexpensive, simple to use, hypersensitive, and discriminatory for a wide range of nitro-explosives. Some of the currently used techniques for explosive detection are discussed below.

DETECTION TECHNIQUE

Ion-Mobility Spectrometry

Explosive material recognition has become one of the primary causes for the establishment of IMS technique (together with toxic warfare weapon identification). The blend of responsiveness and toughness gave a powerful resolution to the challenging problem of explosive counter terrorism.[2] Nevertheless, ignorance about instrumentation and contextual variables with IMS has resulted in a less-than-ideal use of the approach. The machine's effectiveness has improved as the IMS's skill set of complicated gaseous phase ion-molecule dynamics has developed. The ongoing implementation of IMS technology into terminals across the U. S. highlights IMS's capabilities in the implementation of flight safety. This technique and the rules governing reaction have advanced to the point that testing strategy is becoming the primary shortcoming in deployments. One can confidently predict that IMS sensors will get compact and inexpensive in the near future, while the consequences for munition identification is still unknown.[2]

Gas Chromatography Mass Spectrometry

This study is the initial publication on the LVI-GC-MS analysis method that intends to evaluate isolated explosive materials and associated substances and allows for the convenient injection of 20 microlitre specimen quantities in a split-less configuration into a universal PTV injector.[3] This approach was proved to produce minimal identification sensitivity of 0.1 picogram/milli-litre for substances with a diverse variety of volatile substances, particularly volatile chemicals which are not normally susceptible to LVI testing.[3] Regarding air examination, sorptive pipes avoiding the need of a suitable solvent, along with TDS assessment, are often favoured for achieving low limit of detection. The technique's usefulness was proved effectively in the minute analysis of chemicals from

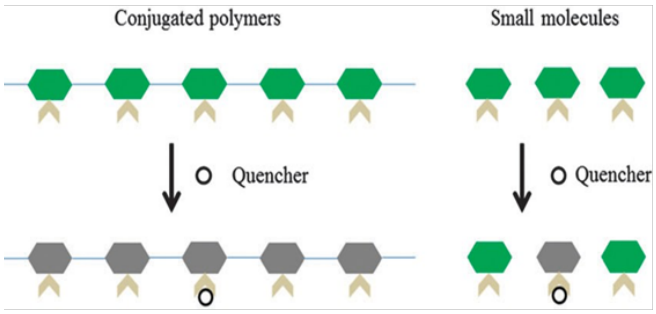

Figure 1: The schematic illustration of molecular wire theory.

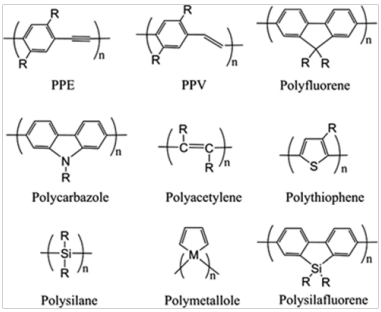

Figure 2: Basic backbone structures of the Conjugated Polymers.

specimens collected from ground in the likeness of inevitable atmospheric interventions. All investigated substances were detected reliably at a minimal concentration value of 0.5 nanogram/gram.[3]

Materials for Explosive Sensors

Conjugated Fluorescent Polymers

Fluorescent conjugated polymers (CPs) was lately been utilized efficiently in detecting nitro based explosives.[4] They feature a longer charge carrier propagation channel and excellent electronic transmission among quenchers along the polymeric matrix as opposed to ordinary small molecule fluorophores. The delocalized ϖ* exciton enhances excited state transfer and hence boosts the electro-static contact between the polymer and electron-deficient nitro-explosive solute, making CPs efficient electron donor. In instance of Conjugated Polymeric fluorescence sensory materials.

According to Swager *et al.,* engaging single receptor location resulting in an excellent dampening of all radiating subunits in a complete chained polymer molecule compared to single molecular system. This magnification is described as the "molecular wiring" phenomenon, or the "single spot touch, multiple-point action" impact, as shown in Fig. 1. In practice, luminous polymeric substances can be classified as organic or inorganic based on underlying fundamental spine architectures, as shown in Figure 2.

METHOD

Small Molecule Fluorophore

Shanmugaraju and Mukherjee demonstrated current notable instances of small molecule - electron-rich turn-off fluorescence sensing materials employed for nitro-explosive sensing.[5] Small molecule fluorophores are attractive sensor substances for detection of nitroaromatic compounds due to their synthetic simplicity, ease of functionalization, and broad array of recognition ability for chemical explosives. Moreover, the excellent solubility of small molecule-based fluorescence sensor in conventional solvents allows for simple synthesis for potential implementation. The effectual charge-transfer (donor-acceptor) complex formation of electron-rich small molecule fluorescence sensor with electron-deficient nitroaromatic explosive is responsible for their medium to considerable dampening effectiveness.[5] Although their great discrimination and effective monitoring ability small molecule fluorescence sensor are poorer to conjugated polymeric sensor. Because many charge carrier emission can be suppressed by one molecule of quencher via far range exciton diffusion all along polymer chains, linked polymeric sensor are incredibly effective. Small molecule sensor fluorescence, on the other hand, is dampened in a stoichiometric manner of one fluorophore per analyte. One proposed solution is to create supramolecular sensor

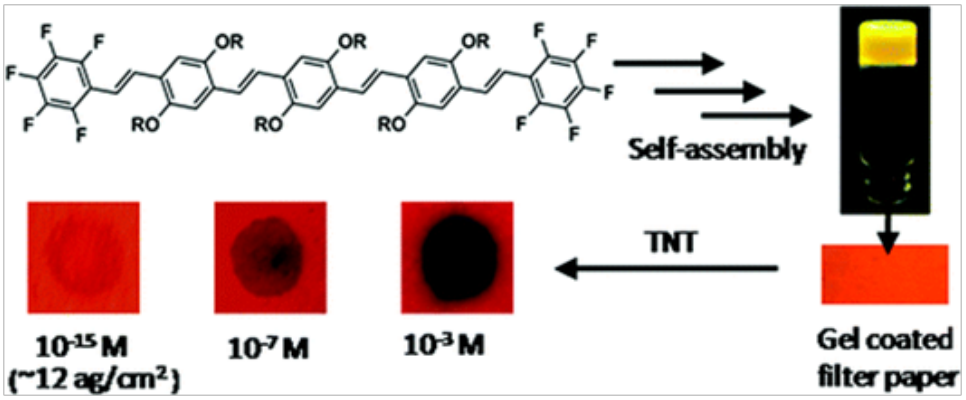

Figure 3: Molecular structure of OPVPF and its self-assembly to gel-coated filter films and the ability for attogram TNT detection in contact mode.

by connecting basic fluorophores via several supramolecular contacts.

Metal Organic Framework

Ning He *et al.,* developed a prominent fluorescent Eu-MOF and utilized it to sense Picric Acid, Trinitrotoluene, and Tetryl having a low sensing level (20-140 g/mL), excellent sensitive having stern volmer constant value of 104-105 M-1, and remarkable reusability.[7] Furthermore, utilizing UV-vis absorbance spectrum interpretation, the process of the fluorescence signal was studied, and the process may be attributed to competing absorbance among Eu-MOF and analytes. Ning He *et al.,* also created a test strip for detecting TNT and were able to obtain visual detection and fast field identification. As a conclusion, Eu-MOF is a perfect candidate to be used in explosive sensing results since it has a better selectivity for detecting Picric acid, Trinitrotoluene, and Tetryl.[7] There seems to be an ongoing necessity for explosive sensing devices that are simple to use on the site and produce accurate outcomes. Unfortunately, maintenance support would be required before such a technology could be functional and useful for forensic investigations. A development procedure is required that would have to recognize nitro aromatic, nitro amines, and nitro esters in contrast to NACs, which could have an appropriate false positive and false negative probability.[8] Furthermore, in the sector of mankind relief when field bomb identification is required, a TNT-only sensing method may discover applicability, however sensing in the gaseous state may be required. As a result, the research is mostly a technical challenge.[2] Here a visual self-explanatory (Fig. 4) is given to show how metal organic framework behaves with/without the presence of various type of molecule.

CONCLUSION

Because of the necessity for immigration enforcement and extracting operations, there is a great deal of focus in enhancing explosive identification. Almost all of the requirements necessary for an efficient recognition element, including the tailored properties, high response, compactness, mobility, and cheap price, are likely to be addressed by fluorescence-based technologies.[1] Fluorescent substances combined with complementary techniques such as electronic devices photography, and technology of experiments could play a larger role in real-world explosive identification such as underground minefields and toxic substances in land, freshwater, and saltwater. For bomb identification, the fluorescent technique was used. Nevertheless, in current history, various other approaches, like as surface Raman

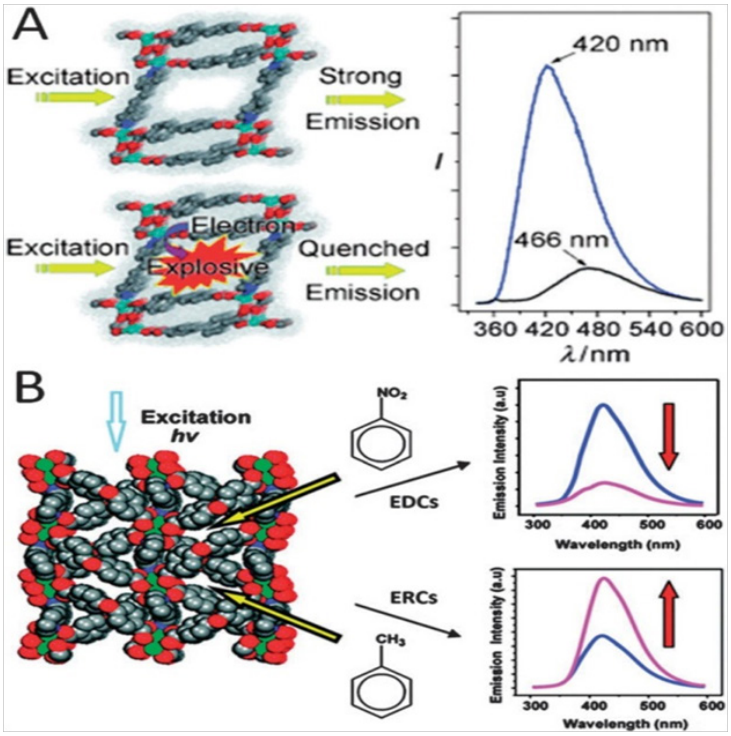

Figure 4: A highly luminescent microporous metal–organic framework, [Zn2(bpdc)2(bpee)] (bpdc = 4,40-biphenyldicarboxylate; bpee = 1,2-bipyridylethene), capable of very fast detection of the vapors of nitroexplosives and the fluorescence spectra before and after exposure to DNMB vapor for 10 s. (B) A highly luminescent 3D microporous MOF, [Zn2(oba)2(bpy)] DMA, demonstrates unique selectivity for the detection of electron-deficient explosives (EDCs) and electron-rich aromatics (ERCs) via a fluorescence quenching and enhancement mechanism.

Spectroscopy, have arisen as formidable instruments and have fiercely challenged with fluorescent techniques for explosive identification, which should not be neglected. Raman spectroscopy has spawned a new area of detection study since its emergence in the 1970s, owing to its advantages such as excellent specificity (molecule fingerprinting) and supersensitivity (enhanced signals). Several scientists found on the discriminating and precise sensing of Trinitrotolouene using the Raman approach, and Limit of detection was reached at the 100 femto-molar levels as well as at the 15 atto-molar mark, which was significantly less than that of fluorescent based explosive investigative techniques.[6] Though Surface-enhanced Raman-spectroscopy has excellent capability in detecting explosives, it has some drawbacks, including low repeatability and expensive equipment setup. I believe that in the long term, chemiluminescence-based explosive sensing and Surface-enhanced Raman-spectroscopy-based explosive identification will augment each other. Though there are many obstacles in the case of responsiveness, specificity, steadiness, and expense for chemiluminescence-based explosive sensing, I assume that with the recent advancements in structural characterization of sensing substances and advancement in control system and data processing modeling, chemiluminescence-based explosive sensor will have a hopeful and positive experience. Owing to overall higher requirements for immigration control in the face of anticipated terror activity, including the rehabilitation of situations which already pose a serious threat

as landmine clearance—the science of explosive sensing is guaranteed to continue an important and broaden the research area. Among the most significant characteristics of such techniques is scalability, and much work is currently done to miniaturize established technology Nanoparticles plays a significant part in this, as well as allowing the adoption of advance equipment. Increasing the responsiveness and selectivity of explosive sensing technologies maintain crucial components; lower limit of sensing for many compounds have grown significantly and studies in such situation continues a prominent subject of inquiry.[2] IJFMP

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Conflict of Interest:
The authors declare that there is no commercial or financial links that could be construed as conflict of interests.
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REVIEW ARTICLE

Dried Blood Spot Testing "A game changer" in Anti-doping strategies: A Review

[1]Adil Ali Ansari, [2]Aman Sachdeva

ABSTRACT

BACKGROUND: Dried blood spots (DBS) have been used for years as a supplementary matrix in sports drug testing. Dried Blood Spots are a promising technique for minimally invasive sample collection in a variety of analytical disciplines, such as therapeutic drug monitoring, preclinical drug development, and diagnostic investigation of metabolic abnormalities in new-borns. The increasing potential of DBS has been highlighted in the scientific literature, particularly when it comes to drugs prohibited in world sports. World Anti-Doping Agency (WADA) has planned to incorporate DBS as a new and much more efficient way of assessing the athletes for any prohibited ergogenic aid in upcoming Tokyo Olympics 2021. This literature seeks to evaluate the scope of effectiveness of the DBS method in identification of various banned drugs by examining previous data derived from literatures on DBS. Based on the researches analyzed, it is concluded that DBS methods is very much implementable in identification of variety of ergogenic aids along with being much more efficient way of identification.

KEY MESSAGE: DBS method of blood doping analysis can prove to be a new and time saving process of doping analysis in the upcoming Tokyo Olympics 2021 due to its vast applications & cost effectiveness and considerable safety during COVID-19.

KEYWORDS | dry blood spots, dbs technique, doping, ergogenic aids, tokyo olympics 2020

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INTRODUCTION

For many years, dried blood spots (DBS) has been used as a supplementary matrix in sports drug testing. Dried Blood Spots (DBS) are a promising technique for minimally invasive sample collection in a variety of analytical disciplines, such as therapeutic drug monitoring, preclinical drug development, and diagnostic investigation of metabolic abnormalities in new-borns. DBS sampling is distinguished by its cost-effectiveness, simplicity, resilience, and ease of storage and shipment which is a need of this hour during this pandemic.

An increasing potential of DBS has been highlighted in the scientific literature, particularly when it comes to chemicals prohibited in competition. World Anti-Doping Agency (WADA) has planned to incorporate DBS as a more efficient way of assessing the athletes for any prohibited ergogenic aid in upcoming Tokyo Olympics 2021. The WADA Prohibited List has eleven classes of prohibited substances and three classes of illegal methods, with four classes (stimulants, narcotics, cannabinoids, and glucocorticoids) regarded to be prohibited during competition.

If the analysis reveals blood concentrations of the prohibited substance below recommended or accepted levels, indicating an impact on the athlete at the time of competition, the result managing authority (RMA) obtains crucial information for a more personalized case management process that takes into account the additional evidence in

the athlete's favor. The intrusive and costly collection and transport of blood samples is a major restriction in that scenario. Dried blood spot (DBS) matrices, which are easily made, relatively inexpensive, and have stabilizing properties for the target analyte, are a possible option in this context. In conjunction with new advancements in sampling techniques, (semi-)automated DBS sample preparation alternatives, and significantly improved instrumental responsiveness in bioanalysis, collecting matched pairs of urine and DBS samples in regular doping controls would provide a valuable contribution for the overall picture of the testing. This literature seeks to evaluate the scope of effectiveness of the DBS method in identification of various banned drugs by examining previous data derived from literatures on DBS.

METHOD

A literature search was conducted across PubMed and Science Direct databases, including the reference lists of relevant papers which ranged in duration of 2015 to 2021. The specific terms used for identifying relevant literatures were 'Dry Blood Spot', 'DBS technique', 'Doping', 'Anti-Doping', 'ergogenic aids', 'athletes', 'Tokyo Olympics'. Reference lists of articles obtained from this search were also examined for additional relevant articles. The inclusion/exclusion criteria for studies were based on their potential relevance to the effectiveness of DBS methods in identification of ergogenic aids.

In a study conducted by Kim *et al.,* (2015) in 453 DBS samples, development and validation of an LC-MS/MS (Liquid Chromatography – Tandem Mass Spectrometry) method was done to calculate the reference intervals of cortisol, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, and rostanedione, corticosterone, and 11-deoxycorticosterone where the samples were taken from Korean people of various ages.[1] At three concentrations, the accuracy, precision, matrix effects, and extraction recovery were all good. The linearity range for cortisol was 1-100 ng/mL and 0.5-50 ng/mL for other hormones, indicating that the LC-MS/MS method and reference intervals validated in the Korean population may be used to assess seven drugs in DBS. In a study conducted by Tretzel *et al.,* (2014) using DBS, eight anabolic steroid esters (nandrolone phenylpropionate, trenbolone enanthate, testosterone acetate, testosteronecypionate, testosterone isocaproate, testosterone phenylpropionate, testosterone decanoate, and testosterone undecanoate) as well as nandrolone were studied.[2] It was concluded that DBS can be used to analyze anabolic steroid esters in doping controls, potentially simplifying the confirmation of exogenous testosterone administration.

Similarly, in a study conducted by Peng *et al.,* (2000) an oral 120-mg dosage of testosterone undecanoate, collection of dried blood spots and plasma from six healthy Caucasian participants was done.[3] Gas chromatography–mass spectrometry was used to assess nonconjugate testosterone, testosterone glucuronide (TG), androsterone glucuronide (AG), and etiocholanolone glucuronide (EtG). The results on dried blood spots and plasma were very similar. The testosterone glucuronide/testosterone ratio in blood or plasma was found to be a sensitive and specific marker for oral testosterone undecanoate (TU) intake (significantly increased for up to 8 hours after intake; P0.05), but not for intramuscular testosterone propionate and testosterone enanthate administration.

Apart from anabolic steroids, in a comparative study done by Kojima *et al.,* [2016] compares a quantitative laboratory urine assay to a liquid chromatography-tandem mass spectrometric approach for detecting ephedrine and methylephedrine utilizing dried blood spot testing.[4] At 4-10 hours after ephedrine administration, the urine concentration of ephedrine did not surpass the threshold in two patients. The maximum levels of ephedrine and methylephedrine in the blood were attained 2–8 hours after ingestion. The blood concentrations

had a low inter-individual variability, and the findings revealed that urine pH and/or urine volume can have a big impact on ephedrine and methylephedrine excretion.

As the banning of various growth hormones in the WADA prohibited list is concerned, in a study conducted by Reverter *et al.,* [2016] a clinical trial was done with healthy volunteers who were given a low subcutaneous dose of recombinant human growth hormone (0.027 mg-1kg-1day-1person-1) for three days.[5] A comparison was made between finger prick DBS and paired time serum samples from arm venepuncture and concluded that the DBS-based protocol's analysis revealed that positive growth hormone misuse may be detected with just a single blood spot. The detection window for DBS was confirmed in all examined samples up to 8 hours after administration, and in half of the instances it was extended to 12 hours. For 12 hours following injection, serum positivity was detected in all of the samples examined.

Apart from sensitivity to prohibited drugs, the DBS method has also shown reliable results in detection of autologous blood transfusion doping method which is used by athletes in enhancement of red blood cell counts and thereby increasing performance. A study was done by Cox *et al.,* (2017) on detection of autologous blood transfusions via novel dried blood spot method, autologous transfusion of 15 subjects who received blood and 11 subjects who received saline.[6] After transfusion, the average CD71/Band3 ratio (immature reticulocytes (IRC) and red blood cells (RBC)) in the blood group was measured from the saline group at days 5, 6, 13, and 20. (Analysis was done via cell-specific proteins digested with trypsin and measured by mass spectrometry). It was found that the average CD71/Band3 ratio decreased to a minimum of 61 ± 8% of baseline. Based on experimentally defined criteria, the CD71/Band3 ratio could detect 7 out of 10 blood transfusion subjects. Thus, concluded that the DBS method could improve detection of autologous transfusion.

CONCLUSION

DBS method of blood doping analysis can prove to be a new and time-saving process of doping analysis in the upcoming Tokyo Olympics 2021 due to its vast applications and cost effectiveness. Considering the pandemic situation of COVID-19, the DBS method can prove to be minimally invasive and least infectious method with high and significantly suitable measurements of results. One the same aspects, the rise of new doping methods and substances should be considered and more researches in DBS effectiveness are need of the hour for the same. IJFMP

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REVIEW ARTICLE

Forensic Odontology: The Periodontal Perspective: A Review

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ABSTRACT

CONTEXT: Periodontics has significant potential for application in forensic odontology as a clinical dental specialty. Forensic odontology is used in medico-legal cases in order to identify the victims and deceased. Forensic odontology plays a pivotal role in age determination of the individual. Moreover, forensic odontology plays a crucial role to maintain the criminal records. The role of periodontology to identify the deceased by gingiva, periodontal ligaments, alveolar bone, perio-asthetics, etc. has their own evidentiary value in forensic investigation. However, periodontology also has its own significant value in determining the time since death. Moreover, periodontics is also used for the gender determination. The purpose of this paper is to summarize the application of periodontology in forensic odontology, to review the role of periodontists in this field, and to analyze the future implications in this field. It inspires further "perioscopical" research in the field of forensic odontology and in forensic investigation or in the court of justice.

KEYWORDS | forensic odontology, periodontics, post-mortem

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INTRODUCTION

Forensic odontology is a branch of dentistry that assess evidences of dental origin in the aspect of justice. The identification of victims, suspects in mass disasters, such as abuses, crime scenes can be well examined thoroughly through proper evaluation and presentation of ongoing scenario's.[1]

Periodontology is a field that includes the supporting structures of teeth like gingiva, periodontal ligament, cementum and alveolar bone, its associated diseases conditions as well as treatment modalities for healthy periodontium.

Using this morphology and pathology in forensic odontology helps us to identify individual for age estimation studies, on the basis of root anatomy of tooth.[2] In medico-legal investigations the vital features of dental characteristics is considered as deciding factor, More commonly performed by comparing the antemortem (AM) dental data of the missing individual with the postmortem (PM) dental data of the deceased individual.[3]

The forensic odontologist primarily evaluate the deceased individuals in number of ways and mainly consists of 2 steps: 1) Comparative identification - it serves to establish that the decedent and person are the same at the time of inspection. 2) Non-existing identification - PM dental profile signifying the characteristics of the individual likely to limit the search for

the AM materials when AM records are not obtainable.[4]

This review summarize the use of periodontology in forensic odontology, to evaluate the role of periodontist within field and its application in perioscopic research.

Implementation of periodontal knowledge in forensic Dentistry

A postmortem examination of the periodontal structures can assist with identifying the deceased, determining the time and season of their death, deciding their gender, and estimating their age.

Identifying the Deceased

The periodontal structures along with perio-aesthetic, implant procedures & recognition helps in detection of the deceased individuals. The following categories are involved in forensic odontology in relation to periodontology.

Gingiva: Normal morphology and pathology of gingiva along with changes in the contour, color of gingiva and additional deposition of plaque and calculus will be taken as counterpart.

Periodontal ligament: The changes in the thickness & widening of periodontal ligament, subsequently the presence of any lateral periodontal cyst or periodontal abscess.[5]

Alveolar bone: bone height, contour, and density of crestal bone; thickness of interradicular bone; pattern of lamina dura; Bone loss (horizontal/vertical); trabecular bone pattern and bone ;presence of exostoses, tori & presence of any residual root fragments.

Perio Aesthetics: Aesthetic surgeries like root coverage in treatment of recession, Crown lengthening, gingival depigmentation perio-ortho procedures, periodontal microsurgery play a major role in the understanding the behaviour characterstics of person and help in relating person in the crime scene as friendly, trustworthy, intelligent, and self-confident.[6]

Implant recognition: G. Michelinakis in 2006 innovated a new implant recognition program to determine the various implant systems with aid in database.

Additionally, radiographic and clinical images of the implant systems are included in the software database. In the end, the manufacturer's details are revealed, and this aids in the case recognition and simplifies the work of a dental forensic expert.[7]

Determining the Time & Season of Death

At the time of death , the release of tissue fluids results in cell autolysis, and body continues to change hence PM changes and its examination are considered as vital part in medico - legal practice. The cellular changes are observed under microscope. It is precise and concise at the level of investigation of crime scene.

Henssge C, Madea B in 2004 identified the features of decomposition at the cellular level in PM gingival tissues at different times after death and observed cellular changes in unfixed AM gingival tissue at regular intervals and view at point of decomposition within 10 hours of death.[8]

Secondly, the histological and ultrastructural study by Pradeep *et al.,* in 2009 reviewed on the changes in the electrolytes and gingival tissue

between the three groups that included normal, 2hrs, and 4hrs since apotosis. There was no notable difference between the 2 hour and 4 hours after death samples under light microscope An ultrastructurally significant difference in gingival tissue morphology was observed between the 2 hours and 4 hours postmortem samples.[9]

Determination of season of death – Cementum can be helpful in determining the season of death. Its growth is symbolized by opaque bands representing winter or dormancy season and translucent bands representing summer or growth season. [10]

As stated by Wedel in 2007, dental cementum increment analysis (DCIA) can provide the season of death by determining the timing of changeover from winter to summer bands.[11] He further demonstrated that the teeth extracted in early October showed a shift to opaque bands whereas the teeth extracted in early April switched to emerging translucent bands.

Moreover, he found that significant correlations were found between band

thickness and the number of days in each season, suggesting bandwidth increases with the length of each season. Thus, he provided a useful tool for forensic anthropologists to determine the season of death through DCIA.[11]

Determining the gender

Using the sex-determining region Y (SRY) gene by real-time polymerase chain reaction (PCR), cells in the oral epithelium can be harvested for the assessment of minute quantities of deoxyribonucleic acid (DNA) for gender identification. It has proven to be a valuable and sensitive tool for gene amplification since DNA found in bone and teeth has a long shelf life and is not decomposed.[12]

Dental Calculus in Gender Determination – Dental calculus is used with the PCR method for detecting sex using two different primers, one for the DYZ3 region of the Y chromosome and one for the DXZ1 region of the X chromosome. Due to the fact that it does not destroy the morphology of the teeth, the calculus method is preferred for classifying sexes.[13]

Age estimation of the deceased –

Cementum: a reliable marker for age estimation – A vital component of identifying a deceased person when information about the deceased is limited is estimating their age. In the periodontium, the cementum is a connective tissue that surrounds the tooth, forms in concentric, incremental lines throughout life, each line representing one year. [14]

Comparatively to other human morphological or histological traits, tooth cementum annulations (TCAs) have been shown to be a reliable source of age estimation.[15]

Studies have shown that the tooth cementum annulation (TCA) is an accurate method of estimating age from other histopathological or morphological features.[15] Microscopically examined, the apical and middle third of the root of a tooth are assessed and counted for the alternating bands of light and dark. A light microscope, polarized microscope, or phase contrast microscope can be used for this examination.[16] The number of incremental lines (n) = X/Y In this case, X = total width of cementum from dentino-cementum junction to cementum surface. Y = width of the cementum between two consecutive lines.

Based on the eruption age of the tooth and the number of lines, we can estimate an individual's age. Nonetheless, to be able to ensure a high level of accuracy, TCAs diagnosis must be based on more than one tooth of each individual and must be supported by different methods in forensic cases.[17]

Gingival Changes: Soleheim in 1992 stated that the recession of the marginal tissue provides an indication of aging when applied to age.[18]

The method may not be as precise as a single indicator of age, however it can contribute significantly to a number of regression methods for estimating age, particularly for premolars. Alveolar bone in age estimation: The alveolar bone level of anterior monoradicular teeth was measured on the labial aspect by Lamend in 1992. He concluded that the amount of alveolar bone loss increased with aging.[19] Age can be estimated by histological evaluation of osteons in bones.[20] Periodontal ligament in age estimation: The Periodontal ligament is considered as a marker for age estimation, its visibility was visualized in orthopantomograms of mandibular third molar in paitent age range from 17 to 31 years in Portuguese population. A four-stage classification was devised based on the appearance of completely mineralized third molars lacking the periodontal ligament. Each periodontal stage (0,1,2,3) was evaluated for its median, variance, minimal and maximal age & in both sexes, age and stage were statistically significantly correlated. Stage 3 can serve as a marker in this population to indicate that a male is older than 21. It is recommended to use a different marker for females. An advantage of this technique is its ability to determine the age of males over the age of 21.[21]

Future Implications

Periodontists may use gingival epithelial cells for optimum identification of human, based on the study of Barr bodies and sex-determination.

A judicial approach to the accomplished facts should also be implemented through the evaluation of them.[22]

CONCLUSION

Maintaining the proper dental record is very important in the critical situations. Dentists should be enhanced to maintain dental records, apply distinctive marks in prosthesis and also maintain a database, which can be made available when required. The success of forensic dentistry is highly dependent on the availability of data. Literature demonstrates that forensic dentistry can have crucial contributions made by periodontists as well as an active role in possibly identifying suspected or unknown individuals before and following their death. Throughout the compilation of review articles, we have attempted to provide inputs from periodontology that could stimulate forensic research with a methodical nature. IJFMP

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REVIEW ARTICLE

Saliva: A Trump Card in Forensic Odontology

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ABSTRACT

Traditionally, bite-mark analysis has been a vital element in forensic odontology. Salivary analysis is a novel addition to this field. Recovery, detection and analysis of saliva could be crucial in a crime investigation. Hence saliva can act as a significant source of forensic evidence such as drugs, toxins, heavy metals, hormones, sex determinants, DNA and other nucleic acids, blood group antigens and oral microbiota data which can be crucial in identifying the suspect or victim. Additionally, saliva has also depicted some significant benefits over the other biological fluids such as blood. To emphasize on this aspect, the non-invasive nature for collection of saliva and the ease with which it can be collected makes it an edge over blood as a biological sample. Saliva is also comparatively safer to collect especially from some forms of acquired immunodeficiency syndrome and hepatitis. This review summarizes the use of saliva and its implications in forensic as an evidence.

KEYWORDS | forensic odontology, saliva, identification, oralfluid, forensics

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INTRODUCTION

FORENSIC ODONTOLOGY OR FORENSIC DENTISTRY, is a branch of science which applies knowledge in dentistry for the process of law reinforcement by examining dead or living body, including analysis of saliva in crime investigation.[1]

Over year saliva is gaining interest amongst the scientific community to use as a diagnostic tool as an alternative to blood or urine.[2] Saliva is a complex oral fluid which could play a critical role in forensic evidence.[3] It comprises of a mixture of secretions released from salivary glands, gingival crevicular fluid and contains exfoliated oral epithelial cells and microorganisms.[2] The gaining popularity due to its ease and noninvasive method of collection, safety in handling compared to blood which has higher potential risks transmitting diseases such as hepatitis and acquired immune deficiency syndrome from needle pricks while collecting, makes saliva a desirable fluid for forensic analysis as major source of DNA.[4]

Recovery of traces of body fluids is one of the most important types of forensic evidence. They contain valuable DNA samples which may help in identifying an accused or a victim. Saliva may get deposited unwittingly on skin during acts of biting, sucking or licking. Hence, it can act as a significant source of forensic evidence which can be crucial in law enforcement, including in crime investigation, identifying suspects & victims and also in detecting drug abuse.[5] This review summarizes the use of saliva and its implications in various aspects of forensics as an evidence.

Detection of and Collection of Saliva from

crime scene

Bite mark analysis has long been a part of forensic odontology as bite marks found in many homicides and assaults. However, elastic and distortable nature of skin compared to a good impression medium, makes it difficult to obtain relevant information. Hence, attention has been shifted to collect saliva deposited on these bite marks to get more relevant information such as DNA, biomarkers and other components that would help in identifying the suspect.[6]

As salvia is a dry colorless stain, it may not be detected by a normal visual inspection. Hence, two special methods are used to detect saliva from the crime scene. Using alternate light sources (ALS), with wavelengths from 415 to 490nm (using orange/red goggles) quartz archtube or argon ion laser, saliva appears as soft edged white spots comparatively less intense than other stains.[7] Using ultraviolet lights, saliva stains appears bluish white. However, ultraviolet is less preferred as it can degrade the DNA in the sample.[8]

In cases where the above mentioned special methods fail to detect saliva, it can be detected using chemical and immunological methods. Detection of chemicals such as nitrates and thiocyanates, amino acids like tryptophan, reducing carbohydrates, and enzymes such as alkaline phosphatase and alpha amylase, which are found in saliva, is the principle behind it. However, as these chemicals can be found in other body fluids and their presence is not always warranted in saliva, the sensitivity and specificity of these methods varies.[9]

Sheets coated Phadebas®, a reagent that consists of blue dye-linked starch which becomes soluble in the presence of ά-amylase, is widely used nowadays in forensic odontology to detect invisible salivary stains. This test is called Phadebas® Forensic Press Test, named after the reagent and the way it is performed.[10]

Rapid stain Identificationtest (RSID) is a parallel stream immune-chromatographic test similar to pregnancy testing kit is used for identifying saliva detecting the alpha amylase in it. Even though not widely used, ELISA test can also be used to detect alpha amylase11. Near Infrared (NIR) Raman Spectroscopy and Fluorescence Spectroscopy are also widely used techniques.[12]

After identifying the presence of saliva, classically it is collected from the skin using a wet cotton swab or a wet filter paper. This method is referred to as Single Swab Technique. Sweet et al in 1999 developed Double Swab Technique for better yield of saliva. It was achieved through rehydration of the dry salivary stain with a sterile swab wetted in nuclease free water. This swab is rolled over the stain for about ten seconds applying moderate pressure. Then a second swab is used to collect the saliva.[13]

The suspect's saliva is collected directly from the oral cavity suspect as well in order to analyze and compare with the saliva obtained from the crime scene. Non-stimulated saliva can be collected simple rinse with mouth wash. Paraffin or citric acid crystals are used to stimulate production of saliva. For more concentrated saliva, it can also be collected directly from the opening of Stenson's duct using commercially available kits like Oragene.[14]

Laboratory Analysis of Saliva

General laboratory tests of saliva are used to identify if the stain is saliva or something else. Those tests are parts of detection of saliva and mentioned in the above section. Apart from those tests, forensic odontology has devised various tests of saliva that would help in identifying the suspect or the victim by finding out various characteristics of a person including age appropriate and gender appropriate hormones ,presence of drugs ortoxins, gender appropriate chromosomal characteristics, and genetic materials such as RNA and DNA. Some of the important ones are mentioned as follows.

Salivary Drug Screen

Compared to the traditional urine drug screen, used to detect presence of drugs in human body,

which has the risk of getting wrong sample as patients collect the sample themselves in their privacy, salivary drug screen has an advantage of getting the right sample. It is analyzed by radio immuno assay (RIA) technique to detect various drugs such as opioids, barbiturates, benzodiazepines, cocaine, amphetamines and cannabis. Nicotine can be detected in saliva of smokers using ELISA technique to detect alkaloid nicotine also known as cotinine which is considered as a biomarker for nicotine exposure.[5,15] Recently immuno chemical strip test called Drug Wipe Test has been introduced for quick detection of commonly abused substances in saliva.[16]

This information can assist the investigative team to characterize the suspect and rule out people in their list who are less likely to abuse substances. This information can also help to know if the victim has been drugged prior to the assault or homicide.

Saliva Toxicology Screen

Presence of various poisons can be detected in saliva using appropriate techniques. Organophosphate, organochlorine and other toxic substances can be detected in saliva. For example, Atrazine, Diazinon can be detected using ELISA technique, Ethion using Liquid-Liquid Extraction (LLE) or Gas Chromatography techniques. While PVC can be identified using Light Chromatography and Mass Spectrometry, Acetone intoxication can be detected using Solid Phase Micro Extraction (SPME), Gas Chromatography and Mass Spectrometry. Paraquat is better identified using diode-array electrophoresis.[17, 18]

Detecting toxicological status can verify if the victim has been poisoned before the act of crime has occurred, which would help the investigation team to assume the modus operandi.

Salivary Drug Screen

Heavy metals such as Mercury, Nickel and Zinc which can appear in saliva as a result of leaching dental materials can also be detected in saliva using various techniques such as atomic absorption spectroscopy or mass spectroscopy. Identifying the heavy metal in saliva can help in identifying the suspect or victim by matching with the records of dental procedures involving restoration and prosthesis using various dental materials.[17, 18]

Salivary Harmone Detection

Various hormones can be detected in saliva using Radioimmuno assay (RIA). Detecting testosterone, estrogen and progesterone can identify the sex of a person whereas hormones such as beta-human chorionic gonadotropin can identify the pregnancy status of the person. Low cortisol levels and high testosterone levels were correlated to criminal behavior in a few studies. Hence, detecting such hormones can assist a forensic odontologist in identifying the suspect or the victim [15].

Sex Determination from Saliva

Apart from relative concentrations of various sex hormones, presence of barr body in exfoliated oral mucosal cells in saliva can help identifying the sex of the person. Barr body is inactive in X chromosome in cells with more than one X chromosome,[5] which are usually cells from people off male sex (XX), but it can also be seen in males with Klinefelter syndrome (XXY).

F-bodies are fluorescent stained Y chromosomes seen in sperm head. Detecting the presence of F-bodies in saliva can identify the presence of spermatozoa in saliva.[5] This information may be crucial in suspected cases of sexual abuse.

Salivary DNA Isolation and Fingerprinting

DNA of the suspect or victim can be obtained from the exfoliated oral mucosal cells found in saliva. DNA from saliva and skin-deposited saliva samples can be extracted by using phenol-chloroform method.[16] Those DNA samples are amplified by PCR. Thus amplified DNA is typed using a set of 15 STRs. This is used to establish a link between biological evidence and a suspect or victim in a criminal investigation. This is called DNA fingerprinting.[6]

Usually cells contain genomic and mitochondrial DNA. Matching genomic DNA

with available sample or a first degree relative can aid in establishing the identity of a person. Chromosomes containing genomic DNA undergoes meotic divison and recombine with paternal chromosomes during fertilization. Hence, variations occur even amongst parents and children, or between siblings except for monozygotic twins. So this method may not be very effective when a sample for comparison or a close relative is not available. Mitochondrial DNA (mtDNA) is inherited through cytoplasm of the gametocyte from mother. This does not undergo meotic division or recombination during fertilization. Hence copy of same mtDNA can be found even in distant maternal relatives across generations. Hence, matching mtDNA can identify even distant maternal relatives of the person. This can be particularly useful when close relatives are not available.[19]

Extra cellular Nucleic Acid Detection in Saliva

Apart from DNA, other nucleic acids such as mRNA and microRNAs (miRNAs) are secreted in extra cellular body fluids such as breast milk, semen, saliva, urine. RNAs has homology with DNA,which makes them useful when DNA is not available. RNA obtained from saliva is used to identify the victim or suspect by comparing it with reference sample by comparing various characteristics of RNA such as Copy number variations (CNV) or Single nucleotide polymorphisms (SNPs).[20]

Blood Group Antigens

Approximately 80% of people secretors of ABO blood group antigens in their saliva. If the suspect has bitten the victim, the suspect's ABO antigens can also be extracted from his saliva. Identifying blood group of the suspect or victim can assist in establishing one's identity in forensic investigations.[20]

Salivary Microbiota

Bacteria of oral microflora could be recovered and analyzed from saliva. Streptococcus mutans, Actinomyces, and Veillonellaare commonly found in saliva. They can be identified by methods such as bio-typing, serotyping, and bacteriocin typing. Out of the common organisms found, the uniqueness of Streptococcus mutans for every person makes it the most important species used in identification in forensic odontology. The unique Streptococcus mutans for each person is identified using genotyping it with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis.[21]

CONCLUSION

Since the human skin is not a good impression medium because of its elastic and distortable nature, analysis of bite marks, as traditionally practiced in forensic odontology poses a limitation to the advancement of the field. This led to opening of another frontier in using saliva as a forensic tool. Saliva is a biological fluid that can be easily obtained using noninvasive techniques. It constitutes various products such as drugs, toxins, heavy metals, hormones, sex determinants, genetic materials such as DNA and RNA, blood group antigens, and micro biota, which would help in identifying victim or suspect in a criminal investigation. It is a new research area still in infancy and highly underutilized. It needs to be explored further for its better utilization and raise awareness to facilitate appropriate legal policy changes. IJFMP

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REVIEW ARTICLE

Forensic Investigation of Aquatic Organisms

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ABSTRACT

Insects play an imperative role in the criminal investigations. The association of the insects with that of the corpse in the criminal investigation is forensic entomology. These insects invade the cadaver and ameliorate in decomposition process and hence aid in criminal investigation. Number of research has been conducted on terrestrial entomological insects regarding their role in criminal and forensic investigation but not enough work has been done on aquatic forensics. There are many environmental factors that may act upon the colonization of an organism on a dead or decaying matter. While this is a known factor in terrestrial settings, this aspect is also observed in the decomposition process in aquatic environment as well. The water and its physical and chemical aspects, involving parameters such as temperature and oxygen content, may not only have a significant role to play in the process of decomposition but may also impact the core process of decomposition. This review paper summarizes the studies on aquatic forensics, its importance, forensically substantial aquatic insects, and their collection, preservation and decomposition studies of human cadavers in aquatic habitats.

KEY MESSAGES: This article discusses the importance of insects as forensic evidence.

KEYWORDS | forensic entomology, aquatic forensics, metamorphosis, decomposition

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INTRODUCTION

Forensic, entomology is a promising tool used in criminalistics for determination of time since death and analysis of carrion insects and other arthropods that invade them. Biological organisms like plants, fungi, insects, mammals etc. are of great forensic importance as they are useful in establishing the linkage between the victim, suspect and the crime scene. This forensic discipline is also termed as medico-criminal forensic entomology. The insects colonize the corpse in a predictable ecological succession which relies on the environmental factors as well as body decomposition. The succession of insects plays a key role in determining the stage of decomposition along with the season in which the death took place and the movement of corpse from one location to another.[1]

Insects are most commonly the first witness to the death. Their action and sequence is determined by variety of instant and complex chemical, biological and physical reactions as the corpse degrades from fresh stage to a skeleton stage. Arthropods and invertebrates invade the corpse and the resulting community provides clues to the victim's death and post-mortem history thus provide great aid in forensic investigation. Therefore, they act as an important tool in criminal investigations. The insects act on the corpse in a specific order by forming a biological clock this helps in determining the age of the developing fly

progeny and hence, the PMI (Post mortem interval) can be analysed. The wide diversity of organisms exploit the corpse, these organisms range from microbes to vertebrates (scavengers). In case of terrestrial habitats, arthropods and insects are present in diverse amount and are constant but in marine habitats, the arthropods are replaced by crustaceans .

Researchers have claimed that the species composition as well as the insect succession differs from each other with respect to the geo-location and the season. There are several factors that play role in determining the time since death. These include:

- Temperature
- Season
- Humidity
- Insect or animal activity

The ages of insect can be inferred by analysing the immature stages found on the dead body. This could help in estimating minimum post mortem interval.[1] The study or investigation of the corpse recovered from aquatic habitat is quite different from the terrestrial one. There are no specific sacrophagous aquatic insects that are functionally evolved to feed or invade the carrion alone. A like terrestrial succession, there is no specific predictable time frame of succession in aquatic habitat. The findings in aquatic forensic may vary from one another depending upon the nature of the resting site (pool, river, ocean, etc). When the corpse is submerged in water, the worthy evidences often get demolished by the water submersion and the integrity of the evidence is lost during the recovery. Aquatic insects are the imperative tools for assembling valuable information from the corpse.[1]

The corpse become an eventual source of food for the aquatic insects like fishes and for wide variety of invertebrates. It also acts as a sheltered microhabitat for small non-scavenging species secondary predatory species also get attracted to the original scavengers. The corpse also acts as a substrate for primary producers like algae or other periphytons, there organisms colonize on the corpse and grow. At the advanced stage, which is highest stage of decomposition, there is formation for bacterial or algal biofilms on the bones, skin or the clothes and hence is attracted by invertebrate grazers. In the case of aquatic forensic investigation, it is imperative for forensic investigators and police officials have to keen knowledge of aquatic organisms that participate in colonization and decomposition process of the human corpse. Along with this, the investigators must have knowledge regarding the factors (environmental) that influence or affect the decomposition process. Time of Submergence (TOS) can be estimated by the aquatic invertebrate succession as well as the decomposition changes.

Aquatic Insects

Aquatic macro-invertebrate investigation is the first and foremost step for the basic understanding of the key role of these insects in death investigation. The variation in morphology, physiology and behavioral adaptations in the aquatic organisms allows them to inhabit in all bodies of water virtually. True aquatic organisms and semi-aquatic ones inhabit in every conceivable habitat in which human body can be found. Human corpse is tend to be found in hot and cold springs, pools, streams, ponds, saline lakes etc. In cases, when the insects are virtually absent in the open sea, it can be elucidated that other marine organisms are more important for forensic investigation in that habitat.[1]

Chironomid midges in their immature stage are considered predominantly for forensic investigations. These aquatic insects are ubiquitous in the aquatic environment and have tendency to colonize the submerged body in short duration for the sake of food and shelter. Along with this, these insects are commendable environmental indicators as they require specific conditions like pH, salinity, temperature, oxygen concentrations, pollution etc. Chironomids are good forensic indicators as they play crucial role in determination and investigation of submerged corpse therefore,

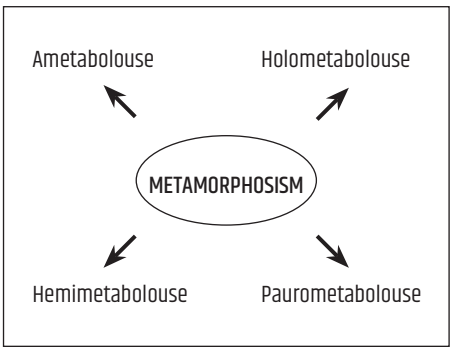

Figure 1: Types of Metamorphosism.

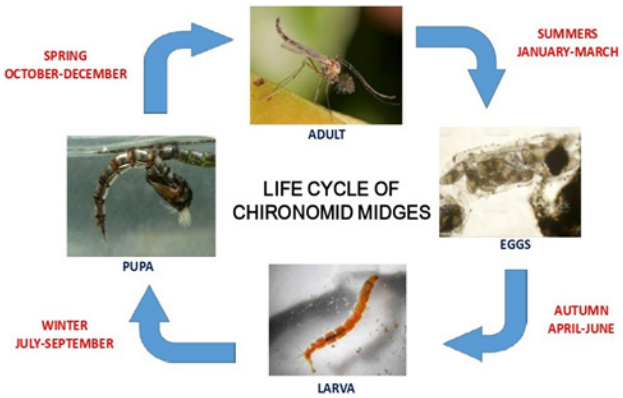

Figure 2: Lifecycle of Chronomid midge

it helps in depicting the location of the corpse. Chironomids are considered as PMSI indicators, because the larvae of these species are not at all affected by the scavengers in submerged corpse.[1]

How do insects reproduce?

Insects undergo series of stages when they develop from egg stage to an adult. The appearance and the time taken for the same varies from one species to other. Studies have revealed that the development cycle accelerates with the enhancement of temperature. The procedure of undergoing physical changes from one stage to another is called "metamorphosis".[1]

Aquatic insects undergo paurometabolous or holometabolous metamorphosis. Each stage takes specified time depending on the species type, season, clothing, temperature. These parameters helps in estimating the PMSI (Post mortem submersion interval).

Caddisflies also play an imperative role in calculating PMSI. (Post mortem submergence interval)These insects should always be kept into consideration as they can provide plenty of information for forensic investigation . Chironomid midges possess four distinctive life stages: Egg, Larvae, Pupa and Adult. The duration of the entire life span may vary from 2 weeks to several years. Parameters like species and environmental conditions play a vital role in the life cycle. With the separation of larva from the substrate that is pupal integument, there is beginning of the pupal stage. Chironomid adults genuinely survive for few days, except for some species which remain alive for several weeks. Adults deny feeding in this particular stage and they only reproduce and disperse. Copulation occurs in aerial swarms, especially on the surface of water where they sink towards the bottom or they release some gelatinous egg masses either on the open water or emergent vegetation.

Aquatic and Semiaquatic Insects associated with Corpses:

Mayflies (Order: Ephemeroptera)
Stoneflies (Order: Plecoptera)
Caddisflies (Order: Trichoptera)
Trueflies (Order: Diptera)

METHOD

Collection and Rearing of Aquatic Evidences

Collection, Rearing and preservation of

INSECTS	ORDER	METAMORPHOSIS	CHARACTERISTIC FEATURES
Mayflies	Ephemeroptera	Hemimetabolous	• Occur in freshwater exclusively. • Duration of adult stage is 2 to 3 days. That's why these insects possess ephemeral nature. • Species of these flies are found feeding on and around the pig carcass. • Proved to be an important entomological evidence that aids in forensic investigation.
Stoneflies	Plecoptera	Hemimetabolous	• These species are found in clean and cool running water. • Eggs hatch as soon as the decomposition process initiates. • Some species of this order were found colonizing and feeding on the carcass of pig and carrions. • Presence of such species on the corpse indicates that the body remained in the riffle zone of the lotic environment.
Caddisflies	Trichoptera	Holometabolous	• Occurs in freshwater, brackish water or sometimes in marine habitats. • Presence of caddisflies on the corpse might indicate whether it had moved from one specific lotic habitat to another or it is transported to different location via water currents.
True flies	Diptera	Holometabolous	• Largest family of freshwater insects therefore are forensically important. • Most successful colonizers and can survive in extreme environmental conditions. • One of the first colonizers that invade the corpse. • These flies aid in determining PMSI, linking corpse with specific aquatic habitat, ascertaining the time of the year as well as the duration of submersion.

Table 1: A list of aquatic and semi aquatic insects associated with corpse and their salient features. [2]

aquatic insects is different from terrestrial insects. Aquatic insects have varied survival adaptation depending upon their species and the environment in which they are residing. Specific insects aid in forensic investigation by providing information regarding the geographical location of the time of the year. Aquatic arthropods like crawfish, crabs will feed extensively on the human tissue that leads to post-mortem artefacts. In some aquatic insects, entire life span as well as the stages are in water, others grow and feed in water but reproduce in terrestrial habitat that is why, and only larvae, pupa and other immature stages are found on submerged bodies. During summer, there is probability to encounter newly emerged adult stages having wings to be found on corpse either on the surface or along the shorelines or riverbanks. If there is presence of some terrestrial insects on the corpse, one can elucidate that the body was on land and then it was sunk to the bottom. Proper protocols are followed by police and investigators while recovering the bodies from the water. Some type or shroud or sheet in placed underneath the body if it is floating or if it is partially in water. This is placed before moving the corpse. The key function of this shroud is to maintain the integrity of the evidence on the body that might get disturbed. Care must be taken while dealing is this evidence as some insects residing on aquatic substrates would detach themselves even with slight disturbance. For this purpose, sheet or large, finely woven mesh net can be employed so that all the insects can be captured. Most of the insects make use of corpse for shelter as well as for food. The samples or evidences are collected with proper care, they are either preserved if dead or are reared if they are alive. Specific methodology is followed for preserving and rearing of insects.

- Live immature samples should be collected and transported in the jars containing water collected from the environment in which they were residing.
- Some of the immature insects are usually heat sensitive and hence, temperature of the water must not be elevated and one must keep the jars containing the insect samples in shade, covered in wet cloth or make use of chemical ice as such things will help in reducing excess heat during transportation.
- The specimen jars are placed in a Styrofoam or an ice chest.
- All the specimens should be labeled and data labels should also be placed on the vials

as soon as collected and with same label format.
- The wings of the insects play imperative role in the identification of the species hence, they should be collected with immense care.
- Forceps or fingers must be used for picking off the larvae, eggs and pupa from the corpse and the collection of these evidences should be done prior to movement of the body as some aquatic insects crawl off or might get disturbed during the movement and may not be found.
- Aquatic insects possess small size and are inconspicuous, therefore, the investigating officer must notice the insects carefully on and around the corpse. A hand lens is preferred for the same.
- The investigator and his team must have keen knowledge regarding the entomological evidences for species identification and to know the behaviour of such insects.
- It is mandatory to put some aquatic plant material in the preserving jar. This feeding material should be collected from the scene itself as it will aid in rearing and in supplementing with fish food.
- Emerged adult aquatic insects are placed in 80-90% alcohol for preservation.
- The rearing containers work well for both terrestrial as well as aquatic insects and are requisite part of rearing equipment.[2,3]

Preservation Solutions for Aquatic Evidences:

Kahle's Solution

This solution is used for preserving adult insects and larvae.

95% ethanol – 30ml
Formaldehyde – 12ml
Glacial Acetic Acid – 4ml
Water – 60ml

Carnoy Fluid

It acts as a killing agent and preservative for most soft-bodied aquatic insects. It is not used much because of restriction for the use of chloroform.

Chloroform (30%) - 30ml
Ethyl alcohol (95%) – 60ml
Glacial acetic acid – 10ml

Pampel's Solution:

Formalin – 10ml
Ethyl alcohol – 30ml
Glacial acetic acid – 7ml
Water – 53ml

Ethyl alcohol (90-95%):

Ethyl alcohol is the most favoured preservative for eggs, larvae and pupae of aquatic insects. The adult form of these insects can be preserved in 75-80% ethanol.[2]

Decomposition of Corpse

Decomposition study in aquatic habitat is not well understood because there has not been much research on the same. Most of the research conducted in past have been conducted on the individual cases. As soon as the corpse submerge in any aquatic habitat, the decomposition process initiates. The decomposition rate and procedure completely depends upon the environmental factors and the condition of the corpse. The key role of decomposition study is to determine time since death for analyzing decomposition in aquatic habitats, one must have knowledge regarding environmental factors and their effect on the decomposition process. Decomposition characteristics that corresponds with aquatic habitat includes:[2]

- Bloating
- Lividity
- Skin sloughing
- Flesh decaying
- Exposure of internal organs
- Algae accumulation
- Silting discoloration of bones

Stage of Decomposition:

Decomposition of corpse in aquatic environment usually takes place at approximately half the rate as that of in terrestrial habitat because of the cooler temperature and less insect activity.[2,3] The stages of decomposition in aquatic habitat are described by several authors and are

discussed below:

Submerged Fresh

It is time period when the corpse is initially submerged and when it starts to bloat and rise to the substrate. This observation is based on the study of pig carcass. The process depends on the geographic location of stagnant or flowing water, microhabitat inside the water body and the season. During this stage, truly aquatic insects like chironomid midges, mayflies and caddis flies were observed by the researchers and investigators.

Early Floating

After death, the humans and animal body releases certain gases which are produced by anaerobic bacterial respiration. This leads to the movement of the carcass or the corpse on the water surface. When the body moves toward the surface. When the body moves toward the surface, the terrestrial insects get attracted towards it. These insects lay their eggs on the carcass where exposure is there. If the carcass is totally submerged, then aquatic insects like aquatic isopods, caddisflies, mayflies, chironomid midges invade the carcass. Researchers have found that there is release of decay odor in this stage along with this, the tissues turned from pinkish to blue-green color, yellow fluids and gases are released from the anus, algal or periphyton growth enhances significantly more on the carcass or corpse. The season when the process initiates plays an imperative role in the determination of duration of each stage of decomposition.

Floating Decay:

Calliphorid maggots feed intensively on the carcass of pig which was floating on the surface and they create many apertures on the exposed skin. Certain beetle species invade on carcass in abundance during this duration, as they find predators and copulate studies have revealed that aquatic micro-invertebrate colonization on the pig carcass varied in both temporarily and spatially between riffle and pool microhabitats.

Bloated determination:

In this stage, the exposed portions of the carcass that float above the surface of water usually disappear due to constant feeding activity of blow fly maggots. On the flip side, completely colonized by aquatic insects like chironomid, black fly, larvae etc. Researchers have found that the hind limbs get disarticulated, body fluids including the blood leak from the orificies of the carcass, the flesh gets slaughed off in large sections.

Floating remains:

Little maggots were found on the limbs and tissues that were floating on the surface of the water. This happens because of the migration of insects from the carcass, drowning death or the predation from aquatic and other terrestrial insects. Sloughed off flesh, disarticulation of phalagial as well as limb bones were all the factors recorded in completely submerged carcass. Black fly larvae and chironomid midge are the dominant organisms for invasion in this stage. Other vertebrate predators like dace, sunfish etc. were also found feeding on the carcass flesh as well as in the micro-vertebrates that were present on the carcass. Studies have revealed that some fishes and other arthropods also feed on the maggots that invaded the corpse in the pond during floating remains stage.

Sunken remains:

This stage has vast variability in its duration. This stage primarily focuses on the bones and skin pieces that remain on the substrate. Researchers have claimed that the decomposition process is completed by microorganisms like bacteria and fungi during this stage. Benthic organisms like fauna might be recovered within the carcass remain along with other organisms like snails, mayfly, amphipods, annelids.[3]

Factors Influencing Aquatic Decomposition

Corpse immersed in water would likely to be exposed to variable and changing environmental conditions. The factors affecting the decomposition process are:

Flora and Fauna:

Microbes like bacteria, fungi, algae play an imperative role in the decomposition process of human remains or corpse. These organisms are somewhat present in the body or are injected along with the water during drowning. Their

occurrence inside the body aids in estimation of time since death and in analyzing the cause of death. Many studies have been conducted in past which focused on the use of these microbes but there is no such reliable method for analyzing them.[4]

Forensic limnology is the scientific discipline which focuses on the examination of diatoms recovered from the crime scene or the body of the victim. Diatoms can help in detection of type of drowning whether it is ante-mortem or post-mortem. If a person is alive and falls into the water, these diatoms enter the lungs through the water inhaled by an individual. Further, they are circulated to different organs of the body like the brain, bone marrow, kidneys, cavity fluids etc. If any individual is dead and is thrown into the water body, in that case the diatoms do not get entry inside the body. It is an undeniable fact that absence of diatoms inside the corpse does not exclude drowning as the cause of death. Ante-mortem injuries during the drowning process, post-mortem injuries due to flow of dead body or invasion by aquatic organisms can be a barrier for detecting the cause and manner of death in drowning. [4,5]

The use of insects in determination of time since death in the aquatic habitat is deficient as compared to those in terrestrial habit. This is due to inadequacy of sarcophagus benthic organisms. Therefore, it is merely impossible to develop an entomological succession for benthic creatures. The interaction the aquatic organisms especially insects with the corpse is an accidental encounter as they do not colonize on the corpse deliberately. If the maggots are present on the submerged body, it indicates that the corpse was exposed to air for longer period of time. Thus, resulting in post mortem drowning. Such maggots are predominantly used for toxicological analysis as compared to the time since death analysis.[6]

Fauna activity on the corpse enhances the decomposition process because these organisms consume the soft tissues and play major role in dismembering the tissues. This action proves to be a complication for identification of the victim as well as for identification of the wounds. Scavenging activity by fishes and other arthropods and molluscs expedite the process of decomposition and can significantly cause skeletonization within week.[7] Microbes also play an eminent role in natural decomposition of the body. The microbes invading the cadaver are either endogenic or those which are already present in the environment. Various aquatic communities have remarkable forensic value. Marine bacteria have been proved as a commendable indicator for drowning cases in the seawater. Moreover, they also play crucial role in the decomposition process. These marine bacteria and their succession in the aquatic habitat act as an innovative indicator for estimating the PMSI.[8] Algae is also considered as a reliable evidence for forensic studies. Algae is also utilised as an aquatic evidence because of its ubiquitous nature and the community composition also varies according to the seasons. The study of algae in forensic scenario is known as forensic phycology.[9]

Applications of Forensic Phycology includes:

- Confirmation of drowning death
- Crime scene identification
- Novel indicator of PMSI

Temperature

Temperature is the paramount environmental factor that persuade the decomposition rate through the colonization time, insect growth and therefore, is the most imperative factor in determining PMI specifically in terrestrial habitat. Aquatic insects also respond to absolute ambient temperature as well as to the summation of the thermal units. Temperature decreases with the increase in depth of water. One needs to have accurate information regarding the surface water temperature as well as the temperature at the depth from which the remains were recovered. These parameters are important for observation of post-mortem changes. Water temperature suppresses the gas formation and the time of reappearance of the body at the water surface.

Some researchers claimed that temperature ranging from 21-45C is suitable for adipocere formation as this much temperature is also suitable for microbial growth and survival. Others have claimed that this adipocere is formed in cooler temperatures. Coldwater temperature decelerates the process of decomposition, specifically the microbial breakdown. Higher temperature enhances the larval growth rate of benthic organisms by altering the quality and quantity of the organic matter which is related to the corpse.[10]

Water Chemistry and Water Flow

Salinity and bacterial content of the aquatic habitat both affect the decomposition process. Freshwater is taken into lungs by the process of osmosis and hence lead to haemolysis due to blood dilution due to high saline content, osmosis will allow to move water from blood to other organs. Specifically lungs and hence, leads to thickening of blood. Because of the modification in bacterial activity, the decomposition process is slower in saline environment as compared to fresh environment. For example; body submerged in water comprising of high organic content will decompose at higher rate as compared to that in which body is submerged in freshwater.

The rate of flow of water is also a determining factor during the investigation of any corpse. The motion of the body has notable effect on the soft tissue and its decay process. Different parameters like interaction with sea bed or river and other environment factors can destroy the tissues and also enhance the decomposition.[13,14]

Body Coverings

The decomposition process is affected by the presence of clothes or other coverings of the body. The putrefaction gets delayed if there are presence of bodily coverings as they acts as a barrier. In some cases, these clothes accelerate the decomposition by keeping intact the temperature of the body as well as the conditions favorable for all the microorganisms that play role in decomposition. Moreover, it also depends on the clothes or the covering that are worn by the victim. For example; heavier clothes have more insulating effect, synthetic clothing materials absorb less water than natural textiles. Bacterial effect gets dwindled when the corpse is wrapped tightly in plastic, there is no source of oxygen in that case leading to the disruption of microbes. Unclothed area of the corpse was subjected to feeding activity of carrion at comparatively higher rate.[14]

CONCLUSION

From the literature survey being conducted in past, it can be inferred that plenty of study has been conducted on forensic entomology but there is scarce research work in the field of aquatic forensics. Both terrestrial and aquatic forensics play crucial role in estimating time since death or Post mortem interval (PMI). In many cases, the body is discharged into aquatic habitats after the commission of crime in order to mislead the investigators regarding the cause of death, as the investigators may infer that the death is caused by drowning. In such cases, forensic aquatic entomology comes into play. The study on this discipline is quite challenging because of different environmental and allied factors that affect the rate and nature of decomposition that goes on in the aquatic environment on the human corpse. Decomposition is a complex phenomenon involving various variables that influence each other. These aquatic organisms have immense significance in criminal investigations, therefore a reference data comprising of all the forensically imperative aquatic species should be made all over the world which can assist various law enforcement agencies to deal with different cases and investigate the cases with great aid.

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REVIEW ARTICLE

Charred Documents and the Techniques used for their Forensic Examination: An Update

[1]Ankita, [2]Gaurav Kumar Singh

ABSTRACT

A number of publications have been published pertaining to the forensic analysis of the questioned documents. Along with that new methods and techniques have been used nowadays to solve the question document cases. Questioned documents are those documents whose legitimacy or authenticity is disputed. Often, the documents are demolished by burning and different intends to cover crimes termed as charred documents. Charred documents are a sort of questioned documents that are probably to contain crucial data. These types of documents are principally connected to ransoms, arson case, accidental fires, financial and insurance issues, extortion, suicide, white collar crimes etc. The present article reviews the introduction to charred documents, the cases in which charred documents are supposed to found, their handling and all the related information up to their forensic examination.

KEY MESSAGES: Charred documents are found in arson cases, sudden fire accidents, financial and insurance disputes, etc. Documents may be willfully burnt or destroyed with the intention to hide crime or to destroy evidence. Charred documents at the crime scene should be handled with utmost care as they are too fragile to be handled casually.

KEYWORDS | charred document, fragile, arson, questioned document, handwriting enhancement

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INTRODUCTION

A DOCUMENT IS A PIECE OF PRINTED, WRITTEN, or electronic matter i.e. generally consisting of signatures, handwritings or some modified data providing information that makes a record. Questioned documents are those documents whose legitimacy or authenticity is disputed.[1] Papers are made up of cellulose fibers, derived from the sources including the wood, rags, grass etc. which are further chemically treated to discard the undesired components such as lignin, resin etc. This is further followed by the draining of water leaving the soft moist mat of interlaced fibers. After this, the moist mat is dried by the heat and pressure and resulting in the paper.[2] According to the Indian Penal Code (IPC), document is defined in the section 29, states that, 'The word document denotes any matter expressed or described upon any substance by means of letters, figures or marks or by more than one of these means, intended to be used or which may be used, as evidence of that matter'.[3] Charred documents are defined as, ''A document or a record that has gotten darkened and fragile by burning or subjecting to extreme heat is named as charred or burnt document.'' Charred documents are mostly found in the arson case, sudden fire, deliberate fire, revealing of examination papers, financial and insurance issues etc.[4] Burning of documents is one of the techniques utilized by the culprits to demolish the evidences.[5]

The present article gives a brief introduction to charred documents, the cases in which

Figure 1: Showing a sample of some of the charred documents

charred documents are supposed to be found, at crime scene, their handling and all the related information up to their forensic examination & the tools and techniques have been reviewed in this paper.

Principles of Forensic Document Examination

The forensic document experts deal with the questioned documents or can say with the questions of those documents whose authenticity is disputed. To determine whether a document is genuine or not, an examiner may attempt to confirm who created the document, determine the timeframe in which it was created, identify the materials used in its preparation or uncover modifications to the original text. Documents can be examined for evidence of alterations, obliterations, erasures and page substitutions. The ink, paper, writing tools, ribbons, stamps and seals used in production of the document may all reveal important clues. The examiner may even discover valuable evidence in a document's invisible impressions.

The forensic examination of the documents consisting of handwriting and signatures based of the following three principles:

1. No two persons can write the same way.
2. There is always a range of natural variations in handwriting.
3. No writer can go beyond his skill level.[4]

METHOD

Search Action & Study Selection: A systematic search was done thoroughly on various papers related to the charred documents, their examination & instrumentation on 'PubMed', 'Google scholar', 'Web of sciences' and 'Research gate', along with many news articles, internet sources also taken into consideration. A comprehensive search methodology was put into action to include all the points in the review paper. The preliminary investigation was done on Google Scholar, which gave promising results and updates. Among the results found, authentic and published papers were used as the first basis of gathering information were studied and analyzed independently.

Methods for the examination of charred documents:

There are several tools and techniques used for the examination of questioned documents. In this section, those tools and techniques have been represented followed by the crime scene investigation of charred documents, their handling & transportation and the decipherment methods.

Tools and techniques used in questioned documents

Figure 2: Showing the charred documents found at the scene of crime

Basic Measurement Tools	Magnification & Light Sources	Chromatography
Scale Protractor	Magnifiers: Magnifying Glass Microscopes: Steromicrosope, Light Microscope	TLC, HPLC GC, MS
Basic Measurement Tools	**Magnification & Light Sources**	**Chromatography**
Infra-red Spectroscopy FTIR, Raman Spectroscopy UV-Visible Spectroscopy XRF	Photography tools Computers, Visual Spectral Comparator, ESDA (Electro-static Detection Apparatus) Electrophoresis	TLC, HPLC GC, MS

Figure 3: Showing all the tools and techniques used in the forensic examination of documents.

The tools and techniques used for the examination of questioned documents are represented in the following figures shown above (Fig. 3).

METHOD

Crime Scene Investigation of charred documents

During the field investigation, the charred documents at the crime scene should be handled with utmost care due to the fragile nature of documents. So, to prevent pointless breakage, proper care should be taken and proper procedure should be followed. The crime scene investigation should be carried out as follows:

1. Protect and secure the crime scene.
2. The crime scene should be preserved with the least possible disturbance of any physical evidence and contamination.
3. Proper maintenance of the documents and all the related details at the crime scene.
4. Firstly, separate the unburnt, evidential documents at the crime scene and keep them under the custody.
5. The documents should be photographed at the crime scene on arrival as a proof further in the legal proceedings to prove that these documents were actually recovered from the scene of crime.

Handling and Transportation of the Charred Documents

Proper handling and transportation of charred

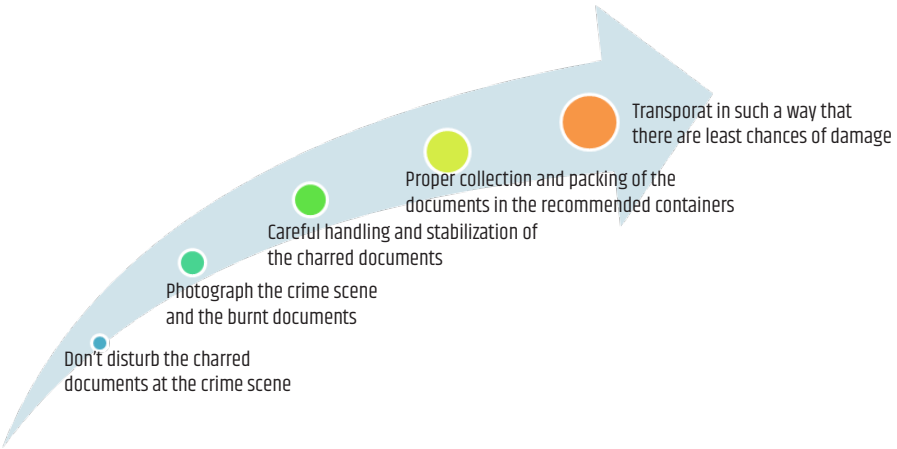

Figure 4: showing the methodology being followed at the crime scene for charred documents

documents should be carried out as follows:

1. The crime scene should be secured.
2. Restrict the flow of wind by closing the windows and turning off the fans. This means burning of documents or other materials is also restricted.
3. The container that holds the burnt documents should not be bothered unless transported to the laboratory.
4. When there is a stack of charred documents, make an effort to secure partially burnt documents from the central and middle part of the stack as unburnt documents may be obtainable due to incomplete burning.
5. Do not disturb the heap of papers. Let them be in their original position.
6. The scattered papers should be lifted carefully by using a spatula and then transfer to the sheet of glass. Place a cotton over that and transfer to the cardboard box one after the other.
7. Use plastic sheets for preservation.
8. The transportation of the exhibits should be done in a way so that there are least chances of damage.
9. Handling of charred documents requires a great patience and care.

Collection & packing of charred documents

1. Close up photography with a high resolution camera at the scene of crime should be done of the burnt or charred documents prior.
2. Wet and burnt documents should be sent to the chilled storage to avoid the cast formation.
3. Place the documents under the controlled air condition to eradicate the moisture.
4. To provide strength to the charred documents, the solution of polyvinyl acetate (3%; 3gm polyvinyl acetate in 100 ml acetone) or methyl methacrylate should be sprayed out on the burnt documents. By doing this the charred documents would gain some weight and can be placed on glass for further examination.
5. The charred documents are usually found twisted or curled. So there is a need to carefully remove or segregate those curled sheets.
6. No forceful attempts should be made.
7. All the documents should be dried, restored and refilled.

METHOD

Decipherment of charred documents-
Deciphering of charred documents can be done in the ways shown above in Fig. 5.

The methods for the decipherment of Charred documents are explained below.[7,8]

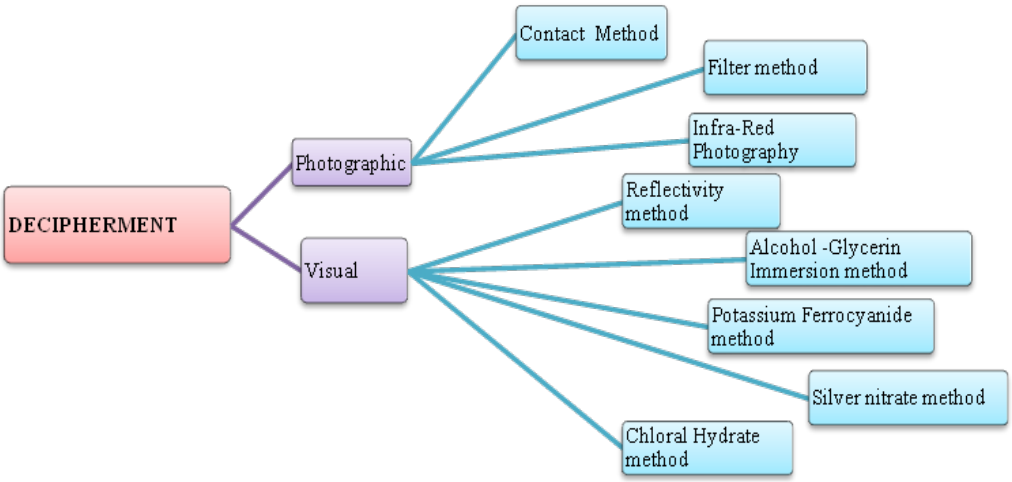

Figure 5: showing the decipherment methods for the charred documents

Photographic Methods:

a. Contact method - In this method, the fragment brought in contact with the certain gases and vapors without exposure to light.
b. Filter photography - This method requires the Wratten # 48 deep blue filter paper along with a commercial film. This method helps to differentiate between the charred documents background with the printing ink containing papers.
c. Infra Red photography – It is one of or highly broadcast method for the decipherment. This method involves the Wratten 87, deep red filter in combination of the Eastman infra plates.

Visual methods

a. Reflectivity method - Among all the decipherment procedures, the reflectivity method is one of the simplest and versatile methods. This method involves the examination of charred documents directed at various angles relative to the paper surface under a controlled light source.
b. Alcohol - glycerine Immersion method- In this method, the charred documents are immersed in a solution of alcohol-glycerine dissolved in a ratio of 5:3:2 i.e. alcohol, glycerine and water respectively for a varying intervals of time.
c. Potassium – ferrocyanide method- Iron is found in all variety of writing inks which are available and in use nowadays. Some chemical reagents results in the formation of a color compound when they get combined with the iron. So, this kind of phenomenon is also used to decipher the writings on the charred documents under some ideal conditions the traces of iron base inks present on paper will give positive reactions.
d. Silver nitrate method - 5% silver nitrate (aqueous) solution is poured on the piece and then second glass plate will be placed over that piece. Direct sunlight should be avoided and after three hours the writings will be deciphered against the gray background of the paper as a black image.
e. Chloral hydrate method - In this method the fragments of charred documents immersed in the 25% of the chloral hydrate solution dissolved in the alcohol and then drying at a temperature of about 600 C. After the fourth or fifth immersion, ten percent of the glycerine will be added to the same solution and dry it as same as before. The crystals of chloral hydrate will be deposited on the charred paper surface which can be further deciphered by the reflectivity method making it likely to read the characters at

certain angles of reflected light.

f. Visual spectral comparator (VSC) - The VSC provides a good, reliable and alternative approach to enhance the writings on the charred documents. For the same, the white spot light and flood light settings can be used to decipher the writing.[1,6,7]

CONCLUSION

The documents are often getting burnt intentionally or accidentally. Such burnt documents are often recovered in the arson and fire investigation cases which may be containing significant evidentiary importance and crucial information which may be linked to the case, crime and investigation and demands the decipherment. Such documents may be related to the ransoms, extortion, suicide and any other offenses. Charred documents are extremely delicate, brittle, blackened and become fragile due to the heat exposure and may get shattered into the smaller sections or ashes. Charred documents are not containing any actual shape and often twisted around the borders. So, there is a need to handle the charred documents very carefully and with a great patience. Handling and decipherment of charred documents is one of the challenging tasks in the field of questioned documents. Therefore, the handling of the charred documents should be done carefully and separately. Tweezers should be used to lift the pieces and those pieces should be placed in the boxes and tightly shut. The moisture contents should be minimized. The transportation of the charred documents to the laboratory is another challenging task which might be taken seriously. Therefore, the charred documents needs careful handing while collection, packaging and transportation to the laboratory. With the help of suitable techniques, possible matters on the charred documents can be easily deciphered which might be helpful in the crime investigation. IJFMP

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REVIEW ARTICLE

A Systematic Review on Restoration of Obliterated Serial Codes from Iron and Steel Surface by Chemical Etching Process

[1]Abi KS, [2]Ankita, [3]Gaurav Kumar Singh

ABSTRACT

Serial numbers are mostly observed on the surfaces of iron, steel and metallic-alloys. Such kind of serial numbers in the form of alpha-numerical codes are obliterated to conceal the identity of the materials by various methods. The decoding or restoration of erased numbers from the surfaces is one of the main challenges in criminal investigation and quite difficult to restore on tougher obliterations. However, deciphering the obliterated unique codes using chemical reagents (chemical etching) has been proved of its ability to deciphering the mechanical erasures.

Here in this study we discuss about the ability and effect of particular chemical reagent on the iron, steel and aluminum-alloy surfaces. Such types of metallic surfaces have been proved of various applications in chassis of motor vehicles, railway iron rods, engine parts etc. The chemical reagent's properties on obliterated metal surfaces were studied using different types of Fry's reagent (Table1-4). Hence this study may have application relevance in criminal investigations involving erasures of identity serial number. **KEY MESSAGES:** The author(s) tried to include scientific explanation restoring serial numbers on iron and steel surface using chemical etching process and some challenges.

KEYWORDS | obliterations, chemical etching, erasures, alpha-numerical codes

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INTRODUCTION

SERIAL NUMBERS ARE ALPHA-NUMERICS with or without logo or unique codes which are cast, engraved or stamped on the metal surfaces which are used for the purpose of identification.[1] These alpha-numerical numbers are sometimes erased from the surface to conceal its identity. Restoration of these codes plays a vital role in providing crucial information as evidence in criminal investigation. Unfortunately, erased marks are impossible to restore. Meanwhile, the other two types of markings may be restored using appropriate chemical etching and other procedures.[1,2,5] The restoration process will also be affected by the nature or method of obliteration i.e. obliteration using drilling[2] and welding.[3] There are different techniques that are used by Forensic experts to restore erased serial numbers. The adoption of particular technique is determined by the type of surface where serial numbers are obliterated.[5] Nowadays, forensic experts encounter different challenges involving different metal-alloys in motor vehicle serial number on the chassis and varies from vehicle to vehicle and also in the breech end of firearms. Automobile's engine and chassis have been constructed or preferred with metallic alloys rather than steel to reduce mass and increase metallic strength.[14] Nowadays improvement in technologies made

serial numbering more advanced and engraved by pin stamping and laser etching methods. Innovation of Fry's reagent for the deciphering the obliterated number from steel surface made a huge impact in investigation of crime involving erasures of serial number.[6,8] Some years back Graham and Jennifer[1] have reported successful restoration of erased identification number on steel surface by Fry's chemical reagent and also study stated that obliteration caused due to over-stamping can be regained by polishing and chemical etching. Better results have been showed by Fry's chemical reagent made by 90g crystalline cupric chloride, Hydrochloric acid (Concentrated HCl) 120 ml, 100 ml of water and also alternate solution of 80 ml hydrochloric acid, 60 ml water, 12.9g of copper chloride and 50 ml of alcohol successfully restored marks erased, it presents itself as the responsive reagent for steel surface. Zaili *et al.,* studied the effect of Fry's reagent to restore obliterated marks on steel surface.[2]

METHOD

Search Action

An organized search was conducted for articles related to the particular topic includes Restoration of erased number, chemical etching process in *PubMed*, *Google Scholar*, *Web of Sciences* and *Research Gate*. A comprehensive search methodology was used to put together all the criteria included in the review and beside the point are excluded. Preliminary investigation in *Google Scholar* gave auxiliary unique results with respect to particular topic; therefore, the investigation for journal was restricted to *PubMed, Web of Science* and *Research Gate*. Detailed strategy is mentioned in (Appendix 1)

Study Selection

The search results from the database gave back 547 articles from *PubMed*. A supplementary 36 journal reports and articles were received from Web of science and 18 from *Research Gate*, relevant to the study criteria. These contents were analyzed independently. 601 articles were included for the study, and 550 were removed due to their insignificance to the pertained topic. The complete texts of 51 articles were scrutinized for inclusion, and 34 were rejected in final scrutiny, by reason of reiteration of information. 16 journal reports from the initial search action were used in the study selection.

SL. NO	METHOD	EXPLANATION
1	Filing or grinding	The surface is removed by means of grinder
2	Peening	The superficial layer is hammered to conceal number
3	Over stamping	New serial number is stamped or engraved over old serial number
4	Centre punching	Obliteration using pointed punch like nailing
5	Substitution	The serial number is substituted with another by means of pasting
6	Drilling	Removing content and surface with welding machine
7	Welding	Heating with oxy-acetylene welder
8	Sometimes an original appearance would be given to a previous erased number surface	

Table 1: Describing the method of obliteration

Protocols of Caring and Analysis of Evidence

Preliminary examination

The method of obliteration may affect the restoration but the degree of destruction caused affects the most. Before restoration using chemical etching process preliminary examination is mandatory using hang magnifier to see whether physical removal has been found place at all. The surface has to be clean with methanol or acetone to remove debris and dirt to avail better visualization and to avoid chemical hindrance during chemical etching process.[1,2,4] The disturbance in the pattern of serial number is suspected of erasure. Identification of erasure and appearance of serial number left is important as well as identifying whether it has been repaired after erasure. The surface may also examine using alternate light source to obtain vital information.[7]

Preparation of surface

The preparation of surface for chemical itching process in much crucial for restoration of obliterated number. Cleaning of surfaces using benzene or acetone is significant to remove the dirt solvents such as chloroform also used. This procedure is commonly preceded with emery cloth or cotton. Sometimes polishing is avoided as the surface is smoothened with sand paper.[4,7]

Chemical etching

SOLUTION 1	
Crystalline Cupric Chloride	90gms.
Concentrated HCl	120ml
Distilled Water	100ml
Solution 2	
Nitric Acid	15%

Table 1: Sol A (Fry's Regent 1)

SOLUTION 1	
Crystalline Cupric Chloride	45gms.
Concentrated HCl	180ml
Distilled Water	100ml
Solution 2	
Nitric Acid	15%

Table 2: Sol B (Fry's Regent 2)

SOLUTION 1	
Crystalline cupric chloride	12.9gms.
Concentrated HCl	80ml
Distilled Water	60ml
Ethanol	50ml
Solution 2	
Nitric acid	15%

Table 3: Sol C (Fry's Regent 3)

SOLUTION	
Copper Sulphate	5gms.
Concentrated HCl	60ml
Ammonium Hydroxide	30ml
Water	60ml

Table 4: Sol D (Fry's Regent 1)

SOLUTION	
Nitric acid (65-68 %)	10ml
Hydrogen Fluoride (40%)	-
Acetic acid (99%)	15ml
Acetone	-

Table 5: Sol E (Fry's Regent 1)

SOLUTION	
Nitric acid (65-68 %)	10ml
Hydrogen Fluoride (40%)	0.5ml
Acetic acid (99%)	10ml
Acetone	-

Table 6: Sol F (Fry's Regent 1)

SOLUTION	
Nitric acid (65-68 %)	10ml
Hydrogen Fluoride (40%)	0.5ml
Acetic acid (99%)	-
Acetone	10

Table 7: Sol G (Fry's Regent 1)

SURFACE UNDER CONSIDERATION

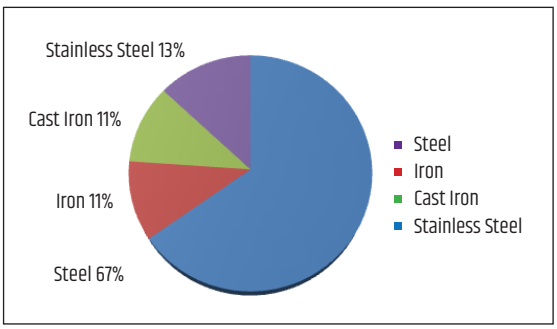

Figure 2: The surfaces considered for restoration in the study under consideration

Chemical is selected according to the surface identified. Fry's reagent is known to be very effective on steel surfaces. The methodology includes cotton swabs or emery cloth dipped in the chemical solution. The chemical reagents are very specific for the surface material.[9] The focused chemical reagents are mentioned below in table format (Table 1- 4).

RESULTS AND DISCUSSION

The study shows effective reagents on different surfaces and conducted. The most commonly used restoration technique is Chemical etching process and found very triumphant. The chemical reagent selected was Fry's reagent of three types (Table 1- 3) for steel and cast-iron surface where obliterations were observed. Use of reagent 5g copper sulphate CuSO4, 60 ml hydrochloric acid, 30 ml ammonium hydroxide (NH3OH), 60 ml H2O (Table 4) found successful on steel surface but the same time failed to decipher the serial number on cast iron engine block of car engine. The chemical reagents solution E, solution F, solution G (Table 5-7) found very successful to re-establish the identity by means of recovering serial number within 6 minutes on motorcycle engine shell and fraction of minutes on car frame.[9]All the Fry's reagents were given moderate contrast on cast iron surface whereas faint and transient on steel surface.[1,2] The solutions considered in restoration is defined in Table 1-7 (Sol A-G) for the surfaces described in the study (fig 2).

Automobile theft investigators often deals

with obliterated serial numbers and without finding the serial number the investigation may not be able to lead. Chemical etching process have been proved the capability of restoration in such cases. The development must be observed closely and may not be available for long. Hence, the development is also known as magical development in midst of forensic professional.[14,15]

Preparation of chemical etching solution is described in MA Zalli *et al.,* study. The preparation scheme has distinct impact on restoring erased number. At the same time factors such as type of obliteration, depth of obliteration, choosing appropriate chemical influence in restoration and time. The restoration is not an instant process, the author recorded the minimum time for deciphering on various depth of obliteration is found to be varying from 10-20 minutes to 35-60 minutes.[16] The surfaces of chassis are developing nowadays. Light weight and more strength alloys are developed for the vehicles. Automobiles work in CNG, hydrogen and electricity may use different alloys for its chassis and engine. The developed chemical agents may not be working for restoration of numbers on such alloys. Thus, it is recommended for research in newly arrived vehicle's alloys for restoration.

CONCLUSION

From the studies, it is clear that chemical etching process especially Fry's reagent as well as reagents mentioned in table 5-7 on steel surface made significant impact on restoration of obliterated number. Here we conclude that the study on different metallic surfaces using chemical reagent etching's having a greater forensic significance in the restoration of serial number on chassis, automobile engine, metallic pipes etc that might lead to solve the crime. In spite of destructive nature of the chemical etching process it gives lead to decipher the concealed sequence number. IJFMP

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REVIEW ARTICLE

Microbiology as Forensic Tool in Investigation of Bioterrorism

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ABSTRACT

Bio weapons were always a known part of wars and very much acknowledged form of terrorism even from Ancient times. Microbes can be used as good arms as their mass production does not requires much skills, quantity and time, along with that they can grow well in numbers from a single cell. Highly pathogenic micro-organisms and their toxins are always in concern as they were used as Microbial-terrorism, one of the much known examples of bioterror is spores of Anthrax attacks at United State in 2001. The word 'microbial-terrorism', itself Indicates, the use of pathogenic microbes, to be dispersed among the group of people/ in certain areas/ or certain countries as a fatal disease, which act as epidemic or some time pandemic. In view of these Forensics investigation, microbiology plays an important role such as sample handling, configuring, tackling, investigation, and validating of bio crimes. The main purposes of this review on Microbiology as forensics tool, is to explain how microbiology helps in investigation of bio-terrorism, and also to suggest the systematic scientific diagnoses of bioweapon. With this knowledge, people and nations can be secured from emergent bio-threat of future.

KEY MESSAGES: Microbial forensics is one of the most essential and significant technologies for detecting bioterrorism and bioweapons. As microbes are associated with decomposition that can impact the investigation results of autopsy, toxicological studies, and histological views. As a resutlt, microbial-forensics can always be of tremendous assistance in criminal investigations.

KEYWORDS | Microbiology, bio-weapon, bio-terrorism, microbial- forensic

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INTRODUCTION

MIRCOBIAL FORENSIC SCIENCE GAINED LIMELIGHT after the attack of Anthrax spores via postal service in 2001 at USA.[1] Microbial forensics, is combination of two field which deals with cause of crime but with use of microbiological tools. This field mainly comprises of all the techniques used in microbiology, such as biochemical reactions, normal flora identification, culturing, molecular assays, genes, phylogenetics etc. in addition to that, basic principles of forensics, such as collection of evidence, chain of custody presentation in court etc., are also a part of that.[2] Few of the similar methods used in DNA studies of humans are also been explored to microbial analysis such as single nucleotide polymorphism (SNP) analyses, microsatellite and minisatellite loci typing, RT-PCR etc. Bacterial DNA fingerprinting is much more difficult than the humans DNA fingerprinting, because bacteria are haploid, and can reproduce much more rapidly and often undergo recombination gene transfer.[2] The diversity of bacteria can be studied by use of phylogenetic tree, which give knowledge for relationship of different genus and their spp.[2] In other words,

we can say Microbial forensics is the branch of science using scientific methods for evaluation of evidence from hoax, bioterrorism outbreak, bio-crime such as assertion of a bio-products or toxins, etc. Provenance of microbial evidence comprises exploring an allied source or culprit or set of entities to the highest degree of possibility. The arena of microbial forensics is based on a various fortes of biological sciences (e.g., forensic sciences, microbiology, biotechnology, genetics, biochemistry, immunology, bioinformatics, molecular biology, population genetics, epidemiology, etc.) and along with that law, public health, the law enforcement, policy and intelligence communities are also part of that.[3]

After the outbreak of Anthrax bacillus spores in the US, Microbial Forensics and Epidemiology are collectively working for determination of any type of outbreaks (some are summarized in Table 1), whether it is accidental, natural, or intentional. In addition to that, predefined forensic approaches such as analysis of DNA, fingerprinting, trace elements or materials, and handwriting examination, are also used for the investigation of crime.[3]

Microbial forensics is a noble field of research, and still in initial stages of expansion; because of this it is still going through lots of ethical and scientific trials to find a strong base for its existence. This fact is well known that the Illicit use of microbial or biological products, having potential hazard to environment, humans, economy, global peace and world-harmony.[4] As we know, the "microbial Forensics" principally deals with crime study and legal proceedings, maybe It may not crack the entire case, but definitely may lead the investigator in a correct direction.[4]

Bioterrorism is a word used for microbial agents and their toxic products which utilized for causing harm to the humans or other living bodies. The use of microbial agents in wars is being rehearsed from prehistoric eras. For example, usage of toxin coted arrow, putting poison in water and food supplies and knowingly dispersing fatal microbes (*Clostridium tetani*) to injured soldiers in clothings such as blankets. These infectious agents are proficient to lead or cause pandemics, epidemics or large outbreaks in a short span of time and the best example we have seen is outbreak of Corona virus.[5] The bio-weapons are not only a threat to humans, but also to plants, animals, economy, agriculture, country, and to the whole ecosystem.[5] Bio-attacks can be classified as either obvious or secret and the main difference among the two is obvious attacks are normally identified immediately, whereas covert attacks take a long time. On the other hand, covert biological attacks are much difficult to determine than the obvious attacks.[6]

S.NO	CASE STUDY	YEAR	REFERENCES
1.	Specially characterized strain of Staphylococcus aureus from paraphernalia to track drug networks	2000-01	25
2.	Reports, origin of Bacillus anthracis from heroin users in Scotland	2009	12
3.	The Origin of the Haitian Cholera Outbreak Strain	2010	26
4.	Phylogenetic analysis to provide evidence against Richard Schmidt that he intentionally injected HIV/HCV-contaminated blood to his girlfriend.	1994	18
5.	Investigation compared different Ames strain morphotypes to determine the origin of the source material released in the 2001 anthrax attacks	2001-02	1,13
6.	Escherichia coli 0104:H4 outbreak in Germany in 2011	2011	27
7.	Initial report of molecular tracking of HIV infection from dentist to patients after invasive health care procedure		
8.	Phylogenetic and molecular clock analysis to provide evidence that a Spanish anesthetist infected 275 patients with hepatitis C virus	1998	29
9.	Ebola outbreak	2014	11
10.	Parvovirus B19 characterization from skeletal remains revealed the likely origin of World War II casualties	2015	30

Table 1: Cases of microbial forensic along with epidemiological (in interest to forensics) investigations.

Standard Protocol of Investigation

It is very much required to identify natural outbreaks and intentional attacks. And for these identifications, microbial forensics comes into the picture. Separating intentional from non-intention biological outbreaks needs an in-depth understanding of normal pathogens and their epidemiology. Therefore, a substantial method for detection, awareness, and surveillance measures are required. The microbial-forensics collects the evidences and provides the information about such cases (intentional attack).[7] Once the biological samples of evidences are collected and preserved, then it is sent to a specific laboratory (depends on the type of biological samples) for further analysis. In the lab, a set of analytical apparatuses are present to provide required assistance in characterization and classification of the evidentiary material. As microbial forensics mainly focuses on chasing microbiomes with location and individuals, different approaches may be applied, and it is totally liable on the type of specimen and the attack. In an obvious attack, for example, weapon, package, and allied materials, along with forensic evidence such as nail, hairs, fingerprints, fibers, etc.) may be evaluated. On the other hand, in a secret attack, the evidences are restricted to medical antiquities, isolates from victims and diagnoses. In 2001 anthrax attacks, a blend of both methods was applied in the investigation and these shows that an investigative analytical plan for biological attacks may requires several and diverse approaches or strategies. After identifying the cause of bio-crime, microbial-forensic expert proceeds for a more investigation for example, detail study of bioweapon (pathogen) by custody of evidence. The protocol for investigation or evidence collection comprises, collection and preservation of samples, and then investigation with respective techniques at National forensic laboratories.[8]

Tools of Microbiology

Numerous of methodologies already exist, but molecular biological tools are conspicuously absent in the selection criteria of the microbial forensic scientist. Relative genetic categorization of pathogens (bioweapon) is a key segment of their provenance process. There are a number of genetic markers and approaches that permit accurate and high potencies of specificity in classification of microbial diversity. For forensic determination, rapid assaying markers of high quality are in use for providing better lineage-based evolutionary analysis, defining strain and species of microbes. As viruses, bacteria, and some fungi reproduce by asexual means, their genomes are much constant and can be clonal too. Therefore, it may be uninformative for differentiating samples by genetic analysis as it is proficient in human DNA identification testing. Uncertainty in microbial genome is more than the humans due to limited database, unknown diversity, huge manipulations and limited genetic analysis. [9,10,11]

Genetic analysis methods used by epidemiologists to track infectious disease outbreaks employ interpretation guidelines to evaluate the results and to determine case relationships in epidemics. Forensic analyses can often use the same or similar methods but may require additional criteria, such as identification of individualizing characteristics for higher resolution source attribution. [12,13]

Hence, genotype variation analysis, for example, polymorphism and DNA fingerprints need to be stressed. By these techniques, genomes from specimen (evidentiary sample) need to be compared to a standard reference specimen for analyzing if they both are belonging from the same source and also shares the recent lineage. Few techniques which are used for human DNA studies have been also explored for studying microbial investigation, such examples are single nucleotide, minisatellite and microsatellite loci typing, polymorphism analyses, and RT-polymerase chain reaction (PCR). For imputation of bioweapon (causative agent), DNA typing method is to be considered as enormously significant technique and the first step in the analysis is the extraction of DNA, for that various biological tools and biomarkers are

present to assist the analysis, some examples are, sequencing, microarray analysis, pathogenicity array analysis, single nucleotide polymorphisms (SNP) characterization,16S rRNA sequencing, variable number of tandem repeats, antibiotic resistance gene characterization etc.[14]

For the evaluation of the microbial pathogenicity and for improved determination of microbial strain or sub-strain various methods are used, such as immunoassays, bio-functional assay, peptide or protein-based assay, mass-spectrometry.[15]

The traditional physical evidence that described microbial forensic specimens are: physical morphological characteristic of microbes which were used as bio-weapon; isotope analysis mainly used for determining origin and age of microbe; further microbial Identification at species and subspecies level can be done by usual physiologic methods such as serotyping, fatty acid composition and phage typing; the sign to origin to the weapon or technique used to prepare material from the residue of growth media attach to the microorganisms; addition of stabilizer to the samples for preparation: time, year and location of the sample need to be recorded and labeled; along with all geological normal flora need to be studied and IgG and IgM antibody reactions need to be analyze for determination of recent exposer to microbes.[15]

DNA fingerprinting performed for bacterial genome is much more tough than the humans one, because bacterial species numerous in number and all need to be considered. In addition, bacteria they reproduce very fast and are haploid too. Most of them reproduce asexually, and endure recombination and horizontal gene transfer.[16]

DNA microarrays, also called biochips and are among the popular selections because it can precisely detect quantity of causative agents in a short span of time. Microarrays comprehends numerous of DNA sequences which may help in forming complementary sequences with suspected bioterror samples and evaluate it for its specificity. [17]

Matrix aided laser desorption ionization assay has been effectively explored for RNA, DNA characterization and protein studies at gene level. These approaches help in analyzing of subtyping of microbes.[17]

Comparative genomic sequencing: this is another valued means for identification of microbes. Though, it's not that fast as other methods but at the same time has the potential to deliver more specific and detailed information about the microbial strain.[17]

2D Spectroscopy: this is mainly working on the principle of optical and infrared spectral regions emphases on the technically scientific challenges faced by microbial forensic investigations. This technique helps in the visualization of time dependent morphological variations occurs in biological specimens, which may range from liquid to solid changes, new findings, and vitalphysio-chemical changes etc.[18]

Determination of ratio of stable isotope: this technique mainly helps in identification of molecular markers carried out by bacterial species. Stable isotopes of nitrogen, carbon, oxygen and hydrogen, can possibly provide the geo-location or medium in which bacterial species were grown in.[19]

The culture media provided for growing the bacteria has unique association with these stable isotopes atoms as, these isotopes are part of their molecular synthesis. The variation of Isotopes in the bacterial cell can be determined by the constituents, used in culture media used for their growth.[20,21]

Applications of Microbial Analysis in Forensic

- Help in distinguishing natural, deliberated and accidental outbreaks with high level efficiency and short span of time.
- Aids in identification and characterization of microbial strains at subspecies level along with their normal habitat and pathogenicity.
- Help in rapid evaluation and development of novel microbial based forensic diagnostic approaches with capability of detecting numerous of parameters associated with

crime.

- Sampling of bio-weapon based on microbial stains.
- Forensic aspects of microbial crime need to be characterized by microbial based basic procedures.
- Help in understanding microbial diversity and flora endemism for assessing the information about the purpose of an attack along with effort may have been developed or perpetrated, or how perpetrators may have exploited the microbial background.
- Help in determination of causative agent and tries to give the answers of questions such as how the microbe and crime was associated; how should scientific and legal significance be determined and supported when the causative agent is a minority constituent in a "probative sample; how much of the threat agent of interest must be contained in a sample to be considered significant.
- Help in determination and characterization of causative agents other than genomic methods such as omics (e.g. proteomics) and approaches such as multitarget examination of culture media.
- Help in forensic based characterization and evaluation of biological toxins. In addition to those credentials, it can also evaluate the development of aid rule-in/rule-out determinations.
- Outstandingly dropping the "discovery-to-decision" timeframe, across all bio-threat agents, with highest probative value and assurance. All the answers in forensics are integrated with the decision process.
- It helps in validating even very low-level analytics (very small quantities of a target analyte) in an operative scenery. Capable of evaluating precise level analyte (e.g., DNA) might be made up of canonical single-nucleotide polymorphism (canSNP) or other isotope signature or at the few- or even single-molecule(s) level.
- It provides international data-sharing forums along with quality and nomenclature standards; these are vital, and help as reference for microbial-forensic analysis.
- Help in determining, how to evaluate and quantify with certainty and report whole-genome–sequencing contrasts achieved throughout the forensic analysis for example, associating an evidence "profile" with a reference specimen that may have a direct link or common ancestor. Sequencing errors along with other factors will probably inflate variation between specimens, which may create a degree of uncertainty.
- Ensures the eminence of sequence data and the outcomes of bioinformatics analyses as high as possible. Factors that affect data genomic data representation is sequencing errors; uncertainty about databases (e.g., interpretations based on available data, including metadata), criteria for comparisons (similarities, different, inconclusive etc.), and the consistency of proficient perceptive (which comprises formulation of well-defined hypotheses, and evaluating techniques), for calculating the mass of microbial forensics evidences.
- Help in standardization of bioinformatics software, which help by ensuring the proper analysis, and all based on previously assessments and comparisons of technologies can be made effectively?
- Avoiding the filtering of data on the basis of individual preferences and bias.
- Instituting processes to inform decision makers in a way that ensures that the science is properly understood. Many nonscientists who make decisions based on forensic science
- Microbial forensics results that are very informative, have high confidence, and are rapidly obtained—and perhaps better leveraged with other capabilities—could enable investigators to manage risks so that energy is dedicated to anticipating and preparing for an event rather than reacting to a surprise, scrambling to mitigate consequences, and seeking attribution.
- It also helps in studying medical and public health issues.[17,22,23]

Legal Steps In Microbial Forensics Investigations

Microbial forensics are well known field for tackle the bio-crimes. For better understanding, example (hypothetical case), one microbial agent sustained or detected from the crime scene, is isolated and characterized and have some reference to be associated with the crime. Via a well-established scientific assay, the origin of the agent can be traced; either it is belonging from any laboratory or a person. When the lab or person's profile is matched, they will be summoned by investigatory bodies. Then judicial system has to prove their involvement in bio-crime act, and their motive of crime too. Then it is also required to provide the details about the origin of microbial agent. Some time, it might be impossible to verify the fact that same genotype of microbes exists in nature. In this hypothetical case, all efforts to prove the criminal activity may be unproductive unless strong statistical evidence is are gathered with unique signatures from the microbial agent and then only the entire purpose of doing it may be jeopardized.

In comparison, there are lots of similarities between human forensic and microbial forensic DNA analysis, such as use of qualitative conclusions of test results, population databases, and application of quality assurance/quality control practices, but differences do exist, for example, database size, protocols, contents and the analysis techniques. Contrasting an epidemiological survey, in microbial crime the evidence and samples should be preserved till the approval of the reports, that need to clearly show, whether the sample belongs to natural outbreak or holds as an evidence for judiciary purpose.[5,6]

Scope of Microbial Forensics in India

As the United States is well known for technically advanced technologies for investigation of bio-weapons by using microbial forensics, many other countries are also investing and pursuing R&D for the development on microbial forensics. In the same context, India is also exploring their potential in microbial forensics and it is incredibly vast. In India, government bodies such as National Microbial Forensic Laboratory act as a knowledge center composed of genomic databases of microbial flora, methodology based on microbiology, advances in forensics methods, SOPs, evidence assays such as bioinformatics, fingerprinting, and genomic standardized tools. These bodies also maintain the strong partnerships between the existing government, the laboratory scientists and investigating officials. Along with that they also monitor the quality control and validation of novel techniques and assays.[24]

CONCLUSION

For the prediction of bioterrorism, microbial forensics has great value because it uses numerous of biological techniques for identification of natural and manmade bioterrorists by means of both molecular genetics and non-genetic technologies. RT-PCR, whole genome sequencing and sequencing of 16S ribosomal RNA, are molecular genetics based technologies used for causative microbial agent's characterizations and on other hand, non-genetic techniques are from the physical sciences, such as electron beam-based methods, mass spectrometry and, etc. can be used for evaluation of physical properties of causative microbial forensic evidence, for example, the existence of additives for enhancing the dispersibility of agent, or physical signatures from the location where the agent is originated from. By using these technologies, it will be easy for the investigator to analyze the threats more easily, and in short span of time. As Microbial world have good potential to be applied in forensic investigation, because they are ubiquitous. But at the same time is very important to develop consistent and reliable methods for evaluating microbes and their ecological habitat, because they can provide both temporal and spatial evidences applicable to investigation. Recent advances in microbiology have substantial inferences for illicit investigation and medico-legal death studies. For the microbial based evidences

studies, a development of reliable protocols is must and should also have properties like high quality, reproducibility etc. The advances in microbiology, directly or indirectly helps in forensic studies. As micro-organisms are involved in decomposition, they can always influence the investigation results of autopsy, toxicological studies, and histological views. Similarly, the investigation of postmortem bacterial translocation using biological-sciences based modern techniques is of countless importance. This is always recommended that forensic-microbiologists need to work in collaboration with forensic entomologists and anthropologists. IJFMP

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REVIEW ARTICLE

Digitopalmar Dermatoglyphic Traits in Medical and Genetic Conditions: A Potential Indicator

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ABSTRACT

Dermatoglyphicsis the investigation of ridge patterns on hands and feet. Dermatoglyphs are created in the fetus by the 12th week and are under solid yet not restrictive hereditary influence. There have been applications of dermatoglyphics in various fields but its relevance to diseases has not been explored in the Indian context. The present review focuses on the history, embryogenesis, theories, and topology of dermatoglyphics with emphasis on the various studies in India. The databases that were searched for the keywords 'dermatoglyphics' and 'medical diseases' were EBSCO host, Academia and Google scholar. Out of the obtained results, 40 studies were selected as per the relevance to the topic. This review highlights the utility of dermatoglyphics in different areas of science. Dermatoglyphics can be essentially used as a potent tool for diagnosis of medical conditions. Dermatoglyphics also offers the advantage of being a simple, cost - effective, and non-invasive screening tool for the prediction of disorders having a genetic predisposition.

KEYWORDS | dermatoglyphics, digitopalmar, genetic disorders

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INTRODUCTION

Human skin on the palm and sole is marked by various grooves of different configurations. Dermatoglyphics is the science, which investigates the ridge designs on palms, fingertips, toes and soles.[1]

The investigation of patterns on fingers and its application for individual identification proof was started in 1892 by Francis Galton. Joannes Evangelista Purkinje is credited with the logical investigation of these in 1823. The broadest depiction and characterization of skin patterns were made in 1926 by Harold Cummins, who coined the term 'dermatoglyphics'.[1]

Dermatoglyphic designs are created by the 12th week of fetal life and are influenced by hereditary. The pattern setups on the palm are shaped by raised equal columns of sweat gland openings. Environmental fetal impacts are clear if the distinctions existing between both the hands of monozygotic twins are considered.[1]

In 1924, Bonnevie found that the epidermal cells at the basal layer were under mechanical pressure forces due to their fast growth. As a result, the cells get directed towards the delicate underlying dermis, bringing about the arrangement of the essential ridges.[2]

Kücken put forward a numerical model to show the effect of pressure influences on the formation of these dermal patterns. The authors expected that the ridge course may be controlled by zones of increased stress during the association of Merkel cells with each other and with the dermal areas. These cells may be moved towards the main forces, which characterize the position of the palmarridges.[2]

Numerous investigations have upheld a connection between dermatoglyphics and the cerebrospinal axis, particularly with a setting to the type of pattern and the division of the nerves and blood flow pathway. Such

examinations propose that these designs match the pathway of the nervous and venous network beneath. Despite the fact that there is clearly a relationship between the advancement of the nervous system and the skin patterns, adapted by their basic ectodermal source, palmar distribution cannot be clarified distinctly with the nervous division, particularly with respect to the intricate dermatoglyphic patterns, for example, loops and whorls.[2]

The fibroblast theory on the arrangement of dermatoglyphic designs suggests the association of fibroblasts which can create critical stretch forces, influencing the extracellular framework and the development of these ridges. However, there is no support for the affiliation occurring among finger ridge designs and fibroblasts.[2]

The morphogenesis of the dermal ridges happens all the while along with the development of the axial brain structures of the same origin from ectoderm. Ridge designs show up on the pad of the fingers, palms and soles. The process of the development begins in 6.5 gestational weeks, starting in the palms, followed by the fingertips, and the soles. Nearing 10.5 weeks, palmarpads display fast development and start differentiating in location and shape. At the same time, there happens a fast development of the cerebral hemispheres. Toward the termination of the fourth embryonic month, the fetus obtains epidermal ridge arrangement similar in a grown-up, whereas the primary divisions of the central nervous system are fully formed.[2]

A basic time during this differentiation is the third embryonic month when diverse external variables may influence the normal differentiation of the ridges bringing about structural brain anomalies.[2]

When created, the ridge designs stay unaltered for the duration of entire life, even unchanging after superficial skin wounds. Based on the normal ectodermal origin of papillary ridges and brain structures, characterized times of development, dermatoglyphics may act as possible biomarkers in deciding the hour of effect of the genetic factors.[2]

The digito-palmar dermatoglyphic studies include the observation and analysis of the following parameters:

1. **Fingertip pattern:** The ridge patterns on the fingertips were divided into three main forms by Galton (1892): Whorls, Arches and Loops.

(i) Arches (*A*): "It is the simplest pattern formed by almost parallel ridges, which form a proximally concave curve".

(ii) Loops (*L*): "It is the most common pattern on the fingertip where aridge series enter at one side of the digit, recurve, and leave on the same side".

(iii) Whorls (*W*): "It is any ridge configuration with two or more triradii".

2. **Ridge Counting:** The pattern size is determined by it. The ridges are counted along a line joining the point of core to the triradius. Counting of ridges can be done between two digital triradii; a and b, b and c, & c and d.
3. **'ATD' Angle:** "It is formed by lines drawn from the digitaltriradius (a) to the axial triradius (t) and from this triradius to the digital triradius (d)".

Uses of dermatoglyphics as an indicator of various diseases:

Cardiopulmonary Conditions:

Bronchial Asthma: It is a syndrome where patient can experience a variety of symptoms such as wheezing, airflow obstruction,chest tightness,coughing and shortness of breath.[3] It is one of the most extensively studied condition with a well-established genetic basis.[4] On assessing the correlation of fingerprint pattern with bronchial asthma, it was found that there was a decreased mean value of arches and high frequency of increased whorls & radial loops in females and ulnar loops in males, with a higher Absolute Finger Ridge Count (AFRC). It was concluded that the fingerprints can serve as a biomarker for bronchial asthma and help in early recognition and thus effectively managing the disease.[3,4]

Pulmonary Tuberculosis: The genetics play a major role in the cause of pulmonary tuberculosis as "Mannose Binding Protein Gene" has been associated with the vulnerability to pulmonary

tuberculosis in India.[5] Palmar dermatoglyphic studied in pulmonary tuberculosis showed that there was a dominance of whorl patterns with a decrease in loop pattern. A narrowed atd angle was also observed along with a significant difference in Total Finger Ridge Count (TFRC) and AFRC.[5,6] These findings can act as supports in the early diagnosis of pulmonary tuberculosis.

Hypertension: The existing literature shows that genetic factors have significant role in the genesis of essential hypertension. Analysis of dermatoglyphics in essential hypertensives showed a greater number of arches, radial loops, and ulnar loops than controls. The chances of an individual acquiring essential hypertension can be determined by the use of study of dermatoglyphs as genetics is involved in the cause of essential hypertension.[7]

Neurological conditions:

Mental Retardation: "It is characterized by a sub average intellectual function in combination with deficit in adaptive behaviour". When the dermal patterns were studied in the mentally retarded group, it was found that the number of ulnar loops was more than any other pattern in the mentally retarded group. There was a statistically significant lower mean TFRC value in mentally retarded males as opposed to the females. A significant increase in the palmar patterns in hypothenar & fourth and third interdigital areas in affected males was found. In case of females, an increase in the palmar pattern in hypothenar areas and fourth interdigital was seen when compared with controls. A higher value of atd angle with an increased inclination towards Sydney line and Simian crease could be seen in affected children.[8]

Epilepsy: "Epilepsy is a neurological disorder characterized by abnormal brain activity resulting in seizures or periods of unusual behavior, sensations, and sometimes loss of awareness". On studying the dermatoglyphic patterns in epileptics a significant variation in the c-line, a-b ridge count, finger-tip and palmar pattern was found. When analyzing the mean values of a-b ridge count, they were higher in epileptics. C-line patterns had a decreased count of ulnar and proximal type patterns. A significant difference in arch type of palmar patterns was found. In the affected population, the count of loops was higher and vestiges were not seen. These results can help in correlating antenatal factors may to the cause of epilepsy.[9] A statistically significant increased ATD angle in both hands of male and female epileptics and only in left hand of female epileptics was also noted.[10]

Schizophrenia: It may be caused by an interaction of genetics and environment with modified brain chemistry and structure. In catatonic schizophrenics, it was found that patients had characteristic qualitative and quantitative dermatoglyphic features like more arches, loops and fewer whorls; less frequency of patterns in thenar area; smaller ATD angle. These differences might be genetic marker for catatonic schizophrenia.[11]

Medical conditions:

Diabetes: Diabetes Mellitus is a condition that may result either due to insufficient production of glucose or failure to utilize insulin properly resulting in high blood glucose levels.[12] Numerous studies have found following dermatoglyphic features in the diabetics: increased frequency of whorls[13] and arches,[14] higher Total Finger Ridge Count (TFRC)[13, 14] and Absolute Finger Ridge Count (AFRC)[15]. The findings of the research suggest that dermatoglyphic characteristics may be used for diagnosis or prediction of chances of developing diabetes later in life.[15]

Thalassemia: Thalassemia is a complex series of genetic disorders, which involve underproduction of hemoglobin. People whose hemoglobin does not produce enough β protein, are said to have β thalassemia. Studies on association of fingerprint patterns and β thalassemia revealed an increasing number of whorls, lesser number of loops, lesser atd angle mean in patients than in controls. A significant difference in the dermatoglyphic patterns in thalassemic patients can be observed.[16]

Congenital Deafness: Researches suggest that about half of the cases of childhood hearing impairment are genetically influenced. The risk for hearing impairment increases in a

child with an affected parent. Among affected males, the pattern distribution on right hand fingertips, number of whorls, various patterns frequencies in III interdigital area of right hand and in IV interdigital area in the left hand showed statistically significant differences. Also, frequency of arches was found more in the deaf in the hypothenar area. The III interdigital area pattern frequencies in left hand of affected females were higher than controls. It was noted that the simian crease was of higher incidence in the left hands of the affected individuals, and the mean ridge count was decreased in them in contrast to the control group. These characteristic findings may prove to be helpful in screening the population.[17]

Carcinoma:

Carcinoma Breast: Various genes (BRCA1, BRCA2, p-53 etc.) have been linked to the causation of cancer.[18] The arch pattern was seen to be increased and a lower count of the radial loops in both the thumbs, the left index and middle finger was seen in individuals with breast cancer. Highest frequency of arch pattern in all five fingers, lower values of Total Finger Ridge Count (TFRC) & Absolute Finger Ridge Count (AFRC) were also found.[19] In another study, it was also observed that six or more whorls were characteristic to the cancer patients. Such findings may indicate the use of dermatoglyphs as a screening tool for carcinoma breast.[18]

Head and Neck Cancer: "Head and neck cancer is the group that affects the mouth, nose, throat, larynx, sinuses, or salivary glands". On examination of dermatoglyphic features in cancer patients, the finger tip ridge pattern of thumb showed lower percentage of loops, arches and higher percentage of whorls, the finger tip ridge pattern of index finger showed a higher percentage of whorl in cancer patients. Finger tip ridge pattern of ring finger showed lower percentage of ulnar loops in control and lower percentage of whorls in cancer patients. Finger tip ridge pattern little finger showed higher percentage of ulnar loops and lower percentage of whorls in cancer patients. The finger prints do not necessarily establish a diagnosis but should prompt the physician to look deeply for hidden diseases.[20]

Musculoskeletal conditions:

Rheumatoid Arthritis: "It is a multifactorial condition and the dermatoglyphic patterns can be affected by factors determining rheumatoid arthritis in utero".[21] In males increased arches and decreased loops / whorls were found. An associated increased partial simian crease was also noted. An increase in whorls in both hands, arches on third finger; both arches, whorls on 4th finger of left hand;decrease in loop in first finger in both the hand was seen in the females.[21, 22] A study also reported an increased total finger ridge count in patients. A statistically significant increase in the pattern intensity was observed among female patients. It can be said based on these findings that there is some relationship between the qualitative as well as quantitative traits of dermatoglyphics and rheumatoid arthritis.[22]

Dermatological conditions:

Vitiligo: It is a condition where there is a loss of pigment cells of the skin leading to areas of skin becoming discolored. On analyzing the dermatoglyphic traits in patients of vitiligo, it was observed that there were increase in total ulnar loops, total loops, and finger ridge counts. Also, the true palmar patterns (TPP) in right thenar, right hypothenar, and the interdigital area of both the hands was seen to be raised in the male patients. A lower number of arches, radial loops, whorls, absolute finger ridge count, and true palmar patterns (TPP) in right ID (Interdigital) 3&ID (Interdigital) 4 was also recorded in them. Whereas, in the female patients, a higher number of true palmar pattern, arches, in right ID, right hypo-thenar, and a-b ridge count and a lower number of total ulnar loops, total loops, whorls, total radial loops,absolute finger ridge count, total finger ridge count, TPP in right ID2 and left ID3 was seen. A higher value of ATD angle was observed in all the patients.[23]

Eczema: "It is an inflammatory condition of the skin, characterized by spongiosis with varying degrees of acanthosis and a superficial perivascular lymphocytic infiltrate". It can occur due to a complex interaction between

genetic susceptibility and environmental risk factors. Pulmonary function tests are valuable investigations in the management of patients with respiratory symptoms in eczema. Studies established that there is a random relation between dermatoglyphic pattern, eczema and lung functions. Population with decreased arches in both hands may develop eczema.[24]

Psoriasis: "Psoriasis is a common familial chronic papulo-squamous inflammatory skin disorder of unknown cause". A significant increase in total ridge count, decrease in a-b ridge count has been noted in this population. The frequency of palmar patterns has been found to be increased in all areas in psoriatic males; but in female psoriatics it is increased in thenar, 3rd and 4th interdigital areas. A significant decrease in ATD angle in female psoriatics has been associated. There are characteristic dermatoglyphic patterns in psoriasis affected individuals as opposed to healthy population.[25]

Leprosy: Leprosy, an infectious disease caused by Mycobacterium leprae has been one of the causes of high morbidity and deformity in India. A screening method helps in early diagnosis and thus prevention of these.[26] In these patients, in multibacillary: a decreased number of whorls and increased number of the loops, in paucibacillary: increased whorls and decreased loops, was seen.[27] Also, a reduced atd angle was associated with the leprosy patients.[26] Dermatoglyphic analysis can be useful diagnostically to differentiate multibacillary, paucibacillary leprosy and control.

Dental Conditions:

Malocclusion: It is a widely occurring condition seen influenced by diet patterns, genetics and environment.[28] On studying the relationship between fingerprints and malocclusion, a statistical association was revealed between increased frequency of whorl patterns and class 1 and increased frequency of loops with class 2 malocclusion.[28,29] Class 3 malocclusion had predominance of arches and whorls as compared to other classes of malocclusion.[28] Based on these studies, it can be said that dermatoglyphics and types of malocclusion are related to one another.

Oral submucous fibrosis (OSMF): "OSMF is a precancerous condition characterized by the accumulation of collagen in the lamina propria of the oral mucosa".[30] There was predominance of ulnar loops and arches, decreased frequency of fingertip whorl patterns, decreased ATD angle, frequency of palmar accessory triradii on right hand and total a-b ridge count, presence of hypothenar pattern in OSMF.[30,31] However, a decrease in arches, radial loop and whorl in these patients was also found in one of the studies.[32] Thus, it can be said that dermatoglyphics can predict the probable occurrence of OSMF in smokeless tobacco users.

Oral Squamous Cell Carcinoma (OSCC): Increased frequency of ulnar loop and arch, decreased number of simple whorls, palmar accessory triradii on both hands, mean ATD angle and total a-b ridge count, presence of hypothenar pattern, was noted in the studies.[30,31] The results of these studies emphasize the role of dermatoglyphics for establishing the genetic tendency of a person to develop OSCC.

Impacted Teeth: "Impacted teeth are those which fail to erupt in dental arch within expected time or classically defined as teeth retained in the jaw beyond their normal date of eruption, encircled by their coronary bag and without contacting the oral cavity". In the study of the dermatoglyphics of the impacted teeth group, a lower frequency of whorl,arch regions and a higher frequency of loop region was seen. The findings highlight a relationship between the tented whorl and arch patterns with the chances of impacted teeth. This indicates that the formation of ridges is influenced by genetic factors.[33]

Dental Caries: Dental caries is one of the major conditions of dentistry and genetics plays a determining factor for an individual's resistance against them. A statistically significant increased number of whorls in the 2nd finger, a decreased total ridge count in children with a dental caries were found. It can be concluded that dermatoglyphic patterns and the relationship with total ridge count could emerge as a new method to establish the risk towards dental caries.

Periodontal Diseases: These diseases are unique in themselves owing to a varied aetiology and symptoms. Dermatoglyphic studies have shown an increased tendency of loops in subjects with healthy gums and an increased tendency of whorls in subjects with calculus. It was concluded that a possible relation between dermatoglyphics and periodontal disease stages exists.[35]

Dental Archform: In dentistry, the arch form is important for stability, occlusion, & esthetics and is the typical expression of a person's development. In persons with square or ovoid arch form, an increased frequency of whorls; with ovoid or tapered archform, increased ulnar loop pattern and with tapered arch form, increased radial loop pattern were observed. The treatment of malocclusion can be benefited by the dermatoglyphic analysis as a pointer for formation of the dental arch form at an earliest time.[36]

Non medical uses of dermatoglyphics:

Handedness: Handedness is the preference for the one of the hands over the other for daily activities. The presentation of hand is developed in fetus and is based on the hand which is held close to the mouth. The leucine rich repeat transmembrane neuronal (LRRTM1) gene is associated with left handedness which brings about the fact that handedness has a genetic basis. The left- handed people were found to show particular fingerprint patterns, like peacock and the radial loop modified. Also, characteristic patterns on the thenar and hypothenar regions of the left- handed people were observed, along with changes in the crease patterns. Thus, these typical findings can help us in determining the handedness of an individual.[37]

Blood Groups: The ability of fingerprints as a method of identification (as in determination of blood groups and sex) has led to its increased use in the detection of crime and criminals. An increased number of loops were found in persons with blood group 'O' followed by blood group 'A' and 'B'. Incidence of whorls was found to be predominant in 'O' followed 'A' and 'B' blood groups. in Among all the blood groups,arches were least commonly found.[38] Also, loops were found to be dominating in blood group A, B, AB and O (Rh positive and Rh-negative) individuals, except O negative where frequency of whorls were increased. There is a relationship between fingerprint patterns, blood group and gender and thus predicting gender and blood group of a person can be done on the basis of fingerprint pattern.[39]

Sports Performance: One of the motives of sport competition is to detect sports ability at its earliest and direct it effectively.[40] In a study involving national level boxers, it was found that loop patterns were predominant and composite loop patterns were very few in number in the fingertips.[41] Another study demonstrated an increased frequency of loops on thenar area which might act as a genetic marker for detection of sports talent.[42] ATD angle was found to be low in students involved in sports as compared to controls, in a study suggesting that measuring the ATD angle before selection of athletes is preferable for a better performance in sport.[40]

Ethnic Affiliation: As the formation of dermatoglyphs is influenced by genetic factors, this science can be used to establish the eccentricities of human populations. One of the researchers studying the bilateral differences in the finger and palmar dermatoglyphics of the Limboo population of Sikkim noted that the Limboo showed similarities with the East Asian populations of Assam and North-East India.[43]

Academics: Dermatoglyphic Multiple Intelligence Test (DMIT) can be used as a screening tool for determining the career choices according to the prominent innate intelligence.[44] It may also be used by the academic institutions to help in determining the growth and development of the student's brilliance, perspective and competence on the educational front. Similarly, the results may be used by the facilitators to guide the students according to their natural learning manner and skills.[45] IJFMP

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REVIEW ARTICLE

Forensic Optometry: A New Tool for Forensic Identification

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ABSTRACT

Optometry is an eye care profession that involves examining the eye and visual system. It also helps in prescribing the correction of refractive error with glasses, contact lenses, and the treatment of various eyes diseases. Likewise, the use of optometrical studies for the criminal justice system is a new paradigm in forensic sciences. Additionally, individual identification of a person may be done by examining certain characteristics of eye. Hence, eye shape, iris color and contact lenses are prevailing vital roles in forensic investigation. This review provides examining iris color for personal identification of the individual in different ethnic population as a whole.

KEY MESSAGES: This review enumerates the role of personal identification with the help of iris color.

KEYWORDS | optometry, iris color, personal identification, ethnic population

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INTRODUCTION

The human eye is the gateway to one of our five senses. Reaction of light to the eyes leads to light perception and color vision. The most fragile organ, eye contain eyelashes, eyelids for protection from injuries, cornea for making eye to focus light, sclera, iris the colorful part of the eye, pupil controling the excessive light rays, lens for focusing light rays to retina and retina that connect light rays into nerve signals.[1] As per laws of individuality, eyes are unique. Every person's eyes are different, with aspect to shape and color. Hence these characteristics of difference among different population relates to the personal identification of the individual.

Eye Color

The color of eye of an individual is determined by iris pigmentation known as melanin. The iris pigmentation has been classified into six colors such as amber, blue, brown, gray, green, hazel, or red. The color of eye is directly proportionate to the quality of melanin in the front layer of the iris. It has been reported that the large amount of melanin is found in brown eyes, whereas less amount of melanin is found in blue eyes.[1,2] These differences in the color of eyes is the most impressive feature which makes person unique in the ethnic population. On the basis of melanin pigmentation of iris, it was found that Negroid population shows the distinct color of iris. The following pie graph depicts the percentage distribution of eye color where in 69% was dark brown found to be common and 16.30% was light brown, 12.30% was dark and 2.30% was blue or gray.

Similarly, percentage distribution of eye color in Mongolian population is found to be 50% hazel, 21% green or gray, 20% brown and 9% blue.[4]

Likewise, the Caucasian population shows 45% brown, 27% blue, 18% hazel and 10% others.[5]

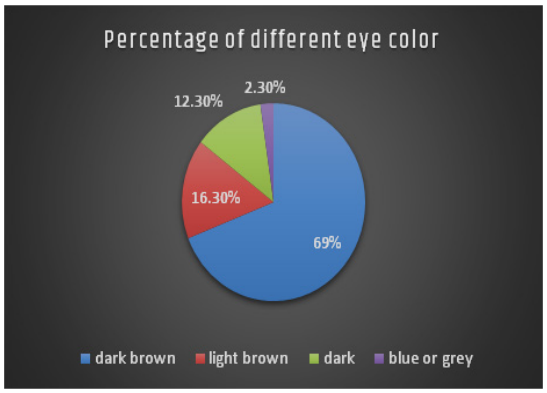

Figure 1: The distribution of eye color among the nigroids population. "dark brown" was the most common color in nigroids, whereas blue or gray color was the least prevalent.[3]

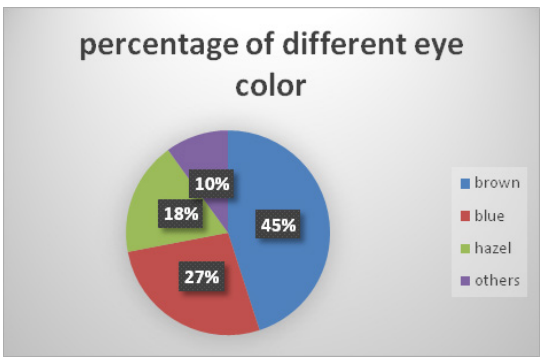

Figure 3: The distribution of different eye colors among the caucasian population

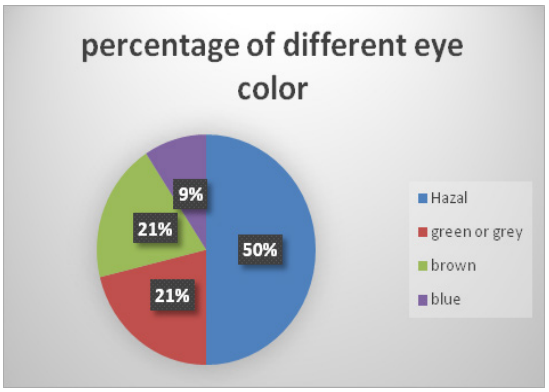

Figure 2: Distribution of different eye colors in a Mongolian population.

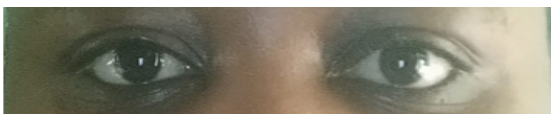

Figure 4: Downturned' eye shapes is mostly seen in Negroid population

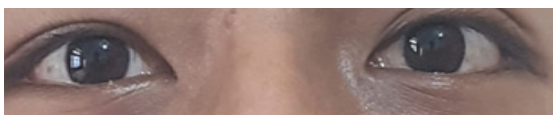

Figure 5: Hooded' shape eye is mostly seen in Mongloid population. In this slant which is going from the outer corner of the eye to the inner corner known as the palpebral slant

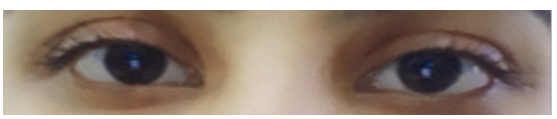

Figure 6: Almond-shaped eyes are mostly seen in the Caucassion population. Here, the iris properly touches the upper and lower eyelid margin

Eye shape

The shape of eye mainly depends on the position of the upper and lower lid, palpebral height, and crease. Eye shape can be categorized into six types: round, monolid, hooded, downturned, upturned, and almond. Visibility of colored part and creases indicates round shape eye.[6] Downturned type of eye defines drooping of outer corner of eye. Whereas, hooded eye represents the nonvisibility of creases due to skin flap. Likewise, when iris touches the bottom and top of eyelids margin it indicates almond shape eye and when flick is in outward direction of the outer corner of eye it shows upturned eyes.

CONCLUSION

In a nutshell, it is reported that iris color and shape of the eye plays pivotal role in personal identification of the individual. These findings and in forensic investigation as an substantial tool for criminal justice system. IJFMP

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REVIEW ARTICLE

Death due to Asphyxia: A Forensic Prognosis

Abhinav Singh

ABSTRACT

From ancient times, hanging, strangulation, and ligature marks have been the topic of interest in forensic science, psychology, forensic medicine, and other branches of science. Hanging is a courageous act for committing suicide; people kill themselves by hanging with the means of ligature. Nowadays, the cases of committing suicide by the standards of hanging are tremendously rising; adults perform this act, but teenagers find it easy to commit suicide and hang themselves. In comparison, strangulation causes death by strangulating the surface of the throat or neck. Hence the essential responsibility of the forensic investigator and the forensic medicine practitioner is to determine the mode of death. Significant and minor findings are located at the time of death, differentiating whether the end is due to hanging or strangulation. Hence, the study aims to detail the hanging, strangulation, the ligature marks, and the post-mortem findings of the major and minor findings during investigation in the cases of hanging and strangulation, which would help the attentive soliloquist to differentiate among these two modes of death and attempts to discuss the scientific grounds, techniques, and methods used to study the cases of hanging and strangulation, along with the type of ligature used and the methods used for the strangulation with their post-mortem findings while performing an autopsy.

KEY MESSAGE: Role of Death due to Asphyxia: A Forensic Prognosis in Forensic investigation

KEYWORDS | Hanging, Strangulation, Bansdola, Ligature marks, Hyoid bones, Mugging, Neck compressions

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INTRODUCTION

Suicides, homicides, or murders the acts which are pernicious in our society. Mechanical asphyxia causes death in case of hanging and strangulation. The pressure is exerted upon the neck, or the body is placed in the position, which creates a ferocious condition in which it becomes knotty to breathe or respire. These acts are performed to kill oneself or to kill someone else. People perform these acts ensuring the ultimate fatal of own-self in the case of suicide.[2]

In contrast, in homicides or murders, the offender kills the victim for personal reasons, greed, retribution, marital reasons, or other reasons. Nowadays, the cases of suicides are rising vigorously, which is not only a fraught but also this act perpetrated by the teenager and adults of our country has some dark number. The death through hanging is serene and painless compared to the other suicidal deaths like deliberately putting fire oneself, poisoning, or any other form of an act that results in the ultimate painful fatal. Suicidal death in India is defined under the three sections of the Indian penal code (IPC, 1860), section 305, section 306,

and section 309.[12] Hanging also plays a pivotal role in our judicial system, as in India, the verdict taken by the judiciary for some criminal cases is prone to the death penalty. The judicial Hanging in India is also known as justifiable homicide as the death penalty is given to the offender to do justice for the victim, to maintain discipline along with rules and regulations of the country, and to keep fear of the death penalty among people of the country so that they would think about the judicial system before committing the crime and know the consequences of their wrongdoing. The death-sentenced in India is legally known as capital punishment, and for hanging the offender, the jail rules and regulations are followed.[9]

In contrast, strangulation is a violent crime in which the offender strangulates the neck of the victim, which leads to blunt trauma on the neck leading to asphyxia death. Strangulation by ligature: the act of killing someone by strangulating the neck through a ligature. It is not necessary that in the case of strangulation, the victim's death occurs with any intention of the offender. Sometimes accidental strangulation also leads to the death of the victim. Hence it set off a task for the forensic investigator and forensic medicine practitioner to differentiate between intentional strangulation and accidental strangulation. Moreover, the ligature mark and post mortem findings play a crucial role in leading the investigation. It assists the investigation in differentiating the mode of the death, whether it is suicidal, homicidal, or accidental death due to hanging or strangulation.

Suicidal Hanging

Suicidal hanging is expounded as an asphyxia death in which the person tries to self-suspense themself through ligature from the fan, tree, poles, or any possible object or thing that supports the heavy object's suspension. It is a frequent type of hanging that is comprehensively used to commit suicide in India and worldwide. The majority of the death in the case of suicidal hanging transpire death due to asphyxia5. The ligature used for the suspension are ropes, scarfs, bedsheets, wires, leather straps, belts, sarees, turban, sacred thread, or any other means that suspend the body. In a forensic investigation, the hanging cases are investigated at the crime scene based on two aspects, which also classify the hanging. The first aspect to look for the hanging is the degree of suspension. In the degree of suspension, the investigator looks for the type of suspension. There are two ways of ligature suspension in which the body is recovered from a crime scene.

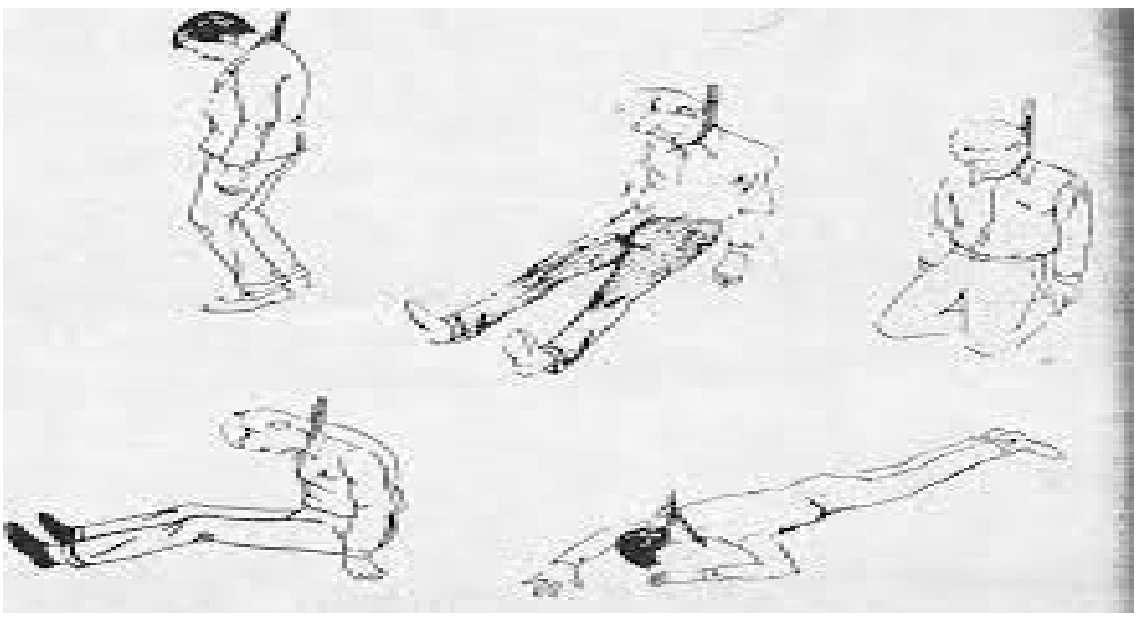

Figure 1: Partial hanging (**Souce:** Image Courtesy Internet Source Jaypee Digital| eBook Reader)

- ***Complete Hanging:*** When the person hangs themself at a height more than their height so that the victim's body or any part of the body does not touch the ground is known as complete hanging.[11]
- ***Incomplete Hanging:*** This type of hanging is also known as partial hanging. In this type of hanging, when the body is suspended, the body or its part is in contact with the ground or any object. In incomplete hanging, the head, chest, and hands apply the pressure on the neck, and these body organs work as the constricting force, which ultimately leads to the fatal[7] as shown in figure 1.

Another facet that plays a pivotal role in forensic investigation is the tying of ligature or any suspending mean, hence based on the position of the knot, hanging is classified further into two types:

- Typical Hanging: In this, the investigator looks for the running of the ligature and the knot's location, if the knot is tied at the center back of the neck and the ligature passes from the side of the neck to the occipital region.
- Atypical Hanging: In this, the knot and ligature are irregular, and the ligature does not run from the occipital region. In the atypical hanging, it is noted that a knot is present either on the right side or left side or in front. The binding of the knot depends upon the feasibility of the person[11] as shown in figure 2.

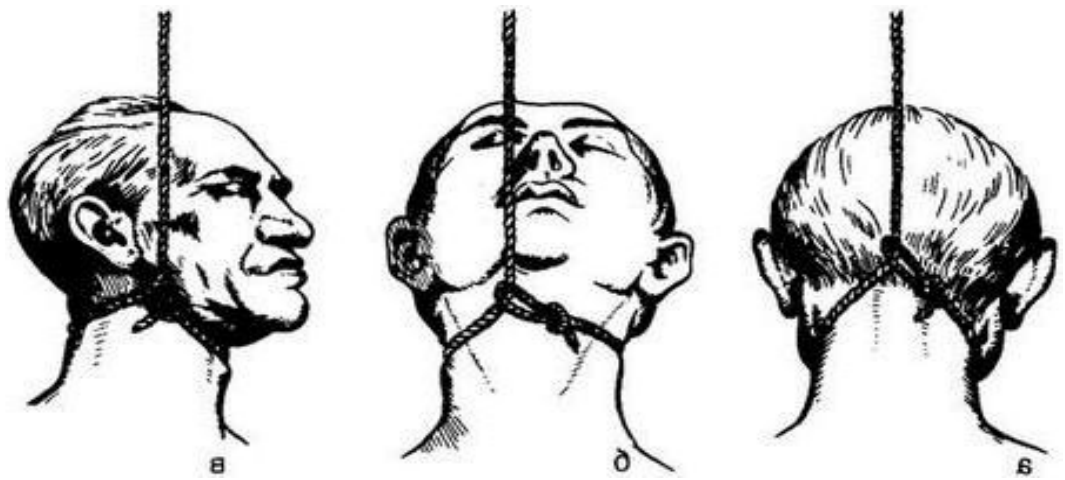

Figure 2: Ligature position around the neck [**Source:** Image Courtesy "Mechanic Asphyxia Prof. Giyasov Z.A.]

Furthermore, homicidal hanging is a rare type of hanging. It is generally spotted in the cases where the person is under the influence of drugs, alcohol, or heavily injured, which leads to numbness of the body. The person is in deep sleep so that in those cases, the offender hangs the victim with the criminal intent of causing fatality to the victim.[13] The deaths in homicidal hangings are caused due to Asphyxia.[3] The significant symptoms of homicidal hanging and suicidal hanging are similar, and the offender tries to imitate the appearance of suicidal death. The word "Lynching" came into existence from the United States of America and was given the name of "William Lynch," the one who used to pass the order of hanging instantly. He used to hang people based on racism, where he targets the black people who were offenders of rape or rapes. The white mob used to play a significant role in hanging the offender. Hence this type of homicidal hanging is also known as mob lynching because of the indulgence of the mob causing fatal.[11]

Accidental hanging is expounded as the hanging in which a person or kid fortuitously hangs themselves, leading to their death. This type of hanging is usually seen in the cases of children where they suspend themselves while

playing, climbing trees, mimicking the action performed in the wrestling, or following their action superstar. In adults, the cases of accidental hanging are more miniature than almost negligible.[12]

Autoerotic Hanging: This type of hanging is bizarre and is shared among the groups of gays or in abnormal sexual behavior when a person obtains sexual pleasure by choking the neck through ligature of the partner for a wild sexual activity, which results in the diminished supply of blood and oxygen to the brain. The person becomes unconscious, and the death is unanticipated; the participation of the males is more in autoerotic hanging which are younger than 30 years of age and more than 11 years of age.[14] In India, the incident of mob lynching raised vigorously from the year 2010 to 2017.[6] As per the study of the web portal, it asserts that among 60 incidents of cow beef exportation cases, 25 people were mob lynched.[1]

Strangulation

In strangulation, death occurs due to constriction of the neck employing any substance or usually ligature without suspending the body. It is generally classified based on the act followed by the offender against the victim; it includes mugging, garrotting, bansdola, and throttling.

- Mugging is expounded as compressing the victim's neck by applying the pressure with a forearm; it is generally known as the choke-hold.
- Garrotting is performed from the victim's back, and the offender strangulates employing ligature followed by tightening it swiftly (Line Jr et al., 1985).
- Bansdola the neck is compressed using complex objects, usually sticks of bamboo; the cases of death by bansdola are usually recorded in north India.
- Throttling causes asphyxia by compression of the neck by applying pressure through the hands. Palm strangulation is one of the most common acts used strangulation. In this, using the palm of one hand offender closes the nostrils and mouth, and by using the heel of another palm, the offender applies pressure to the front neck of the victim.

It is noted that the cause of death in strangulation is not only asphyxia but also cerebral anoxia, congestion of venous, combined venous congestion and asphyxia, vagal inhibition, and cerebral vertebrae dislocation causes death.

External and Internal Sign of Post Mortem Findings

The external appearance and internal sign during post-mortem differentiate the cause of death, in case of hanging externally, the neck descent, and saliva dribbling opposite to the ligature knot due to gravitational pull. The facial features include paleness, congested swollen with profuse petechiae at the portion of the head and neck. Cyanosis is observed at the nail and hands. Moreover, the tongue is turgid and protrude due to the pressure of the ligature. Internally in hanging, the hyoid bone is rarely injured; exceptional cases include the aging factor, disease, osteoporosis, brittleness of the bones.[10] The congestion and hemorrhage above and below of the lymph node near the region of ligature, along with the frictional tear of the subintimal carotid arteries, the tissues under the ligature marks get dry and compressed with the formation of a white band of subcutaneous tissues. Hyperaemia of the tracheal and epiglottis region is more common. While in dealing with asphyxia death due to strangulation, external injury is most common, including injury on the face, chest, tongue, specifying the struggle, bleeding from ear, nose, mouth, and raised body temperature along with the clenching of hands. The bruises occur at the front and sides close to the larynx, and the pattern of injury signifies the way of strangulation. In comparison, internally, the laryngeal fracture of hyoid bone fracture is more common. The hyoid bone fractures are based on the force applied and its direction: inward compression fracture, anteroposterior, and avulsions[17] as shown in figure 3 and in table (1).

Ligature marks

Ligature marks are generally expounded as the marks surrounding the neck due to the pressure of the ligature at the site of its contact. The ligature marks change periodically from

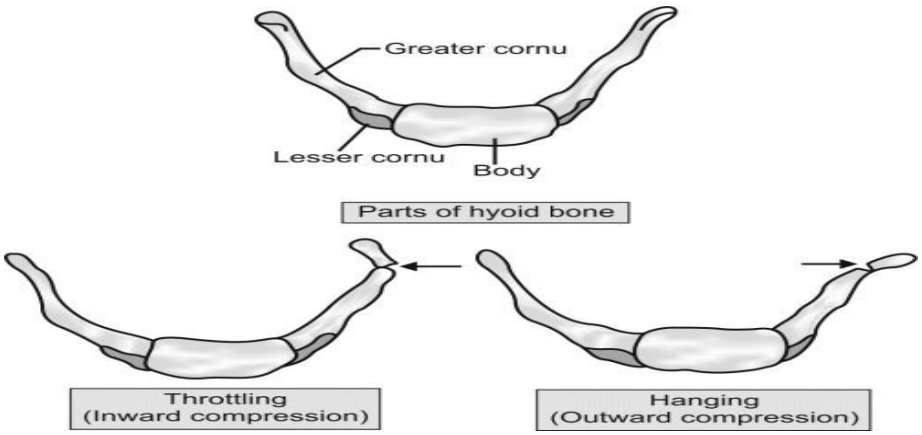

Figure 4: Compression Fractures in Hyoid bone (Image Courtesy)[17]

pale to yellowish-brown, dry hard, and parchment-like. The ligature mark is characterized by the nature of the ligature, bodyweight of the victim, time of suspension, and the number of turns of ligature surrounding the neck site. The ligature and the marks produced by it play the role of distinguisher to justify whether the death was suicidal or homicidal.

Suicidal rate

The act of committing suicide by hanging is prevalent in our society, as fatality is higher than the survival rate. In suicidal deaths due to hanging, the location and surrounding plays a pivotal role for the forensic investigation and helps build up the scene of the crime or reconstruct the scene of the crime. Bhosle et al., 2015 conducted a study for the cause of death in adolescents and how adolescents choose to kill themselves or commit suicide. Their studies found that suicidal deaths employing Hanging are most common among the youth, and about 96.08% opts for this technique to execute the suicidal deaths. The researchers provided the data figures of committing suicidal death employing hanging among the children and teenagers is a matter of concern, as per their study 81% of the teenager within the age of 15-19 years commits suicide employing hanging whereas 19% are of the age group of 11-14 years of the age.[4]

Furthermore, Vijayakumari in 2011 voiced the factors that lead adolescents to perish: a high expectation of the parents, aversion from the parents, scarcity situations, failing to achieve academic goals, being bullied, and failing to survive within the competition. In the case of adults, depression, inveighing from colleagues, medical illness, failure in love life, penitent, the influence of the drugs or alcohol, and penurious factors create execrable conditions in which they falter and decides to end their life and reported that most of the cases of suicidal hanging occur within the victim's home, whereas 95.50% of the teenagers try to locate the secluded place to execute their set out.[16] Line Jr et al., in 1985, conducted a study on the deaths due to strangulation and discussed deaths that occur in strangulation due to the pressure applied on the neck through rope or ligature any other object8. Furthermore, Arya et al., 2019 bring to light National Crime Record Bureau data of suicidal hanging from (2001-2014) based on the sex, age group, and geographical region. The results obtained from their study have some dark numbers; there is a 56% rise in suicidal cases among males and 24% in cases of females, whereas the suicidal death by poisoning decreased by 44% among males and 52% among females. Moreover, considering the suicidal rate by the age of the victims, 30-59 years of age usually opt for suicidal hanging to kill oneself.[2]

CONCLUSION

In our country, there are numerous reasons or cases in which people are hanged, whether to

do justice, criminal intent, suicidal intent, or abnormality in sexual behavior, and cases are differentiated as per the doer's intention. The post mortem findings in the autopsy and the evidence collected from the crime scene help link the scene of a crime with the offender or with the victim. Nowadays, offenders try to manipulate homicidal death with the means of strangulation as a suicidal death by killing someone in a fierce, or intentionally by constricting the victim's neck through ligature, hard object which cause the person's death due to the mechanical asphyxia. In most cases, the relatives, beauties, or the police personal are unaware of dealing with the hanged dead body.

Furthermore, certain cases are reported where friends, relatives, laypeople, or area police don the body down, which causes the disturbance of the scene; hence, in these situations, post mortem fwindings play a significant role in differentiating between the mode of death; hence there is a need to impart knowledge about the cases of hanging and their dealing methods.

In the occurrence of death in the case of strangulation, the victim's body is not suspended; instead, immense pressure is applied over the neck, which causes the cessation of the passage of oxygen through the windpipe. The victim starts losing their sensibility, which ultimately leads to the victim's results in the fatal.

Whereas death in the judicial hanging occurs, the second-third, or third- fourth cervical vertebrae get dislocated or fractured. If a zygomorphic fracture occurs within the pedicle or laminae on the arch of the cerebral vertebrae, then it causes the hangmen's fracture. Hence, as forensic scientists and medical practitioners are hanging, strangulation cases are the mildest and require extra attention and particular measures to follow the investigation. There is a need to know about the particular characteristics of the death occurring to differentiate the type of death.

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Conflict of Interest:
The authors declare that there is no commercial or financial links that could be construed as conflict of interests.
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ORIGINAL ARTICLE

Impact of Degradation of Blood Samples on RNA, DNA and HB: A Review

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ABSTRACT

CONTEXT: Collection of biological evidence from various crime scenes for identification purpose is an important task to achieve. Where different biological evidence serve as an important source of information, whole blood samples and bloodstains act as prime source of identification. Blood samples are mostly found to be exposed to various environmental conditions, which results in the degradation of samples. It is quite inaccessible for forensic experts to re-evaluate the evidences and get the same outcomes as earlier because of storage conditions of the biological samples. Hence, the stability of nucleic acids and hemoglobin is very crucial. This study was conducted to present a review on the available facts of different storage conditions and temperature on the stability of ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and hemoglobin (Hb). The study also provides an outlook for ideal storage temperature and conditions for blood samples and appropriate preservation of same immediately after the collection from the crime scene.

KEYWORDS | RNA, DNA, haemoglobin, extraction yield, degradation, storage, forensic analysis

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INTRODUCTION

Various biological evidence redeemed from the crime scene are useful evidences for the purpose of identification and individualization. It is also useful in creating a linkage between crime scene, suspect and victim. Over the last few decades, among all the biological evidences, blood appears to be the prime source for providing abundant source of identification of individuals. Moreover, before the process of collection, the samples are exposed to varied environmental conditions, and whenever the re-evaluation of results is required, the storage conditions have been found to have affected forensic analysis. The purpose of this study is to present a review on the available facts of different conditions and temperature on stability of RNA, DNA and Hb.

Properties of Blood

A circulating fluid that fulfills the nutrition and oxygen requirement of the body is blood. It bears various physical, chemical and biological properties that is to be kept in mind while examining whole blood and bloodstain evidence for forensic examination. Blood falls under the category of liquid connective tissue, which comprises of various blood corpuscles and plasma. Around 7% of total body weight comes from blood in an average human being.[1] Three major types of blood cells and cell fragments are white blood cells (WBCs), red blood cells (RBCs) and platelets. Deoxyribonucleic acid extraction is not possible from red blood cells as nucleus is absent, yet the major element of red blood cell is a protein called hemoglobin which is responsible for oxygen transportation and bears iron.

Blood as Evidence

Blood plays a vital role in identification of a person. It is quite important to ascertain the basic properties and characteristics of blood retrieved from the crime scene. Numerous preliminary examinations are conducted to

endorse if the red droplet encountered at the crime scene is actually blood or not. Kastle-Meyer phenolphthalein test or benzidine test is used to detect and confirm that the sample is blood. Precipitin test is conducted to confirm that the blood of human origin.[1,2] Following this, the individualization of blood is to be done. Having various techniques in hand for RNA and DNA analysis it is quite evident to determine to whom the blood at crime scene belongs. Results of deoxyribonucleic acid analysis authenticate that the blood belongs to the accused or to the victim. Tissue type of body fluid and determination of cell could be identified through the RNA analysis.[3]

Sample Collection

The biological samples need to be carefully collected and stored in a way so that all useful information can be acquired from the analysis. Evidence that can be collected for the purpose of DNA isolation and analysis are of biological nature as mentioned below:

- *Blood and Bloodstains*
- *Semen and Seminal stains*
- *Saliva*
- *Urine*
- *Hair*
- *Bones and teeth*
- *Cells and tissues*

The transfer of biological samples either through direct contact or through the secondary transfer will always be present on the target surface. The collection process must be followed based on the physical properties of the evidence, the liquid state evidence will be present as the absorbent and the solid state evidence will adhere at the surface. For the purpose of acquiring the sufficient amount of DNA from the biological evidences, each sample must be collected and should be stored in dry and cold environment until it reaches the forensic laboratories.

Blood and Bloodstains

Blood and bloodstains appear to be the prime source for providing the high degree of information from hemoglobin, RNA and DNA analysis. The collection process of blood samples from the crime scene is dependent on the surface from where it is to be collected.[4,5]

- *Collection from a Person* This is of great importance to collect a sample as control or for reference from either suspects or victims. While collecting the sample, every tube bearing the sample must be labeled with time, person's name, date, collector's name, case number, location of collection and exhibit number. Blood should be collected by a qualified medical person and in vacutainers with a suitable anticoagulant. Samples must be refrigerated and forwarded to the laboratory. Rahilla *et al.,* (2017) has collected the samples using 5ml disposable syringe and transferred into 6ml K2 EDTA vacutainers and stored in various temperatures in order to assess the influence of storage methods of whole blood samples on integrity of DNA.[6] Di Pietro *et al.,* in 2011 collected the whole blood samples in 1.5 ml of eppendorf tubes of heparin.[7] Schröder and Steimer (2018) also one of the researcher who conducted the study on long term impact of storing samples on DNA methylation and extraction yield by collecting EDTA blood from different individuals and stored at varied temperatures for different durations.[8]
- *Blood in liquid state* Liquid blood must be collected using clean syringe or using spatula in a clean test tube. With proper labeling as mentioned above should be refrigerated and submitted to the laboratory within limited time. Permenter *et al.,* (2015) collected samples and stored using two different preservatives viz. heparin and EDTA at different temperatures and the impact on DNA degradation was assessed for this study.[9]
- *Wet Bloodstains* Small articles like cloth, weapons etc. containing wet bloodstains must be first air dried and then collected and transported, where the large articles like cupboards bears the wet bloodstains, there the wet bloodstains must be transferred to the clean cotton cloth and kept to air dry.

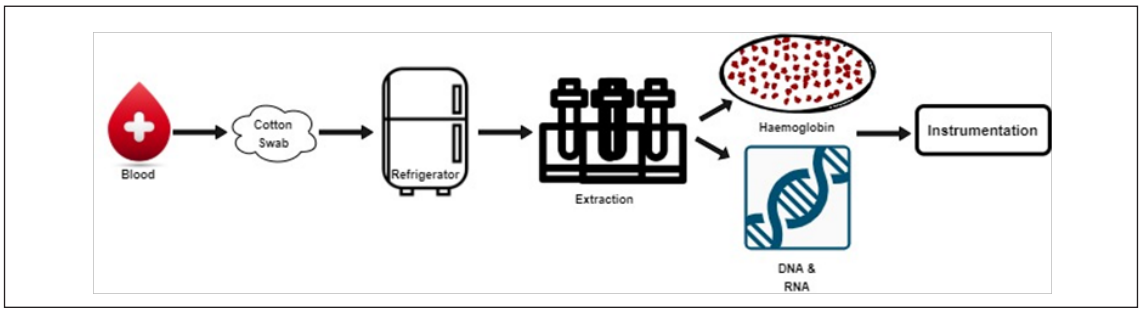

Figure 1 : Schematic representation of blood collection, storage, extraction and instrumental analysis

After packaging with a paper container all the objects should be labeled and transported to the forensic lab. Kaur *et al.,* (2020) utilized the blood samples that were soaked using cotton gauze from different crime scenes for the purpose of quantification of DNA and Hb using UV-Visible spectroscopy.[10]

Dry Stains: Bloodstains on small articles like weapons, cloth etc. must be collected independently, packed in paper and transferred to forensic lab. Whereas the articles that are not possible to be collected like from wall, floor or any other non-movable object the samples scrapings should be collected in a paper or tape lift method. If the scrapings lifting is not possible than the dried bloodstains should be collected using saline cotton swab. Then the cotton swab should be air dried and packed in paper envelopes. For the purpose of collection of control an unstained scraping should also be collected from the article.[11,12,13] Stored bloodstains at varied temperatures by absorbing the blood on cotton gauze to analyze the effects of different storage conditions on forensic examinations of blood. Zupanic *et al.,* (2019) stored the samples in varied forms where one of the form used were FTA cards for the blood samples collected at autopsy.[14]

Storage Conditions and Temperature

For the purpose of transportation and storage of evidence which is important for forensic analysis, samples must be secured in paper envelops with proper sealing and labeling. Samples that may bear nucleic acid must never be placed that have warmer conditions or in contact to direct sunlight. All the wet bloodstains collected from the crime scene should be air dried and heat should not be artificially provided to facilitate the dry state of evidence. All the evidence should be kept separated to one another so as to decrease the possibilities of contamination. Various studies were conducted where the samples were stored at specific temperatures like room temperature, 4°C, -20° C and -80°C.[6,7,9,11,12,13,14,15,19] Another study conducted by Howlett *et al.,*. (2013) storage temperature for the blood samples were kept at -20°C, +37°C, +50° C and room temperature. He also used bio stabilizer viz. DNAstable™ for storing the blood samples at varied temperature.[18] Kaur *et al.,* (2020) has considered the dry bloodstains and stored the samples in the form of cotton gauze.[10] Several storage conditions could also be considered for the purpose of analyzing the effect of storage temperature and conditions on degradation of DNA, RNA and forensic analysis of hemoglobin. Bulla *et al.,* (2016) classified the samples in three major categories on the basis of EDTA coupled with DNAgard Blood solution.[15] Sirker *et al.,* considered the dry and humid conditions for the storage of temperature.[3]

Extraction

DNA Extraction

Deoxyribonucleic acid extraction methods play a major role in the quantity, quality, purity and integrity of the isolated DNA from varied biological samples. The selection of the method to be used for the purpose of DNA extraction is based on various factors like, type of evidence retrieved, reduction of contamination risk, cost-effectiveness, simplicity and throughput potential. A study has been conducted where the comparison of three in general used extraction methods (phenol-chloroform, proteinase K and silica based extraction) were used.[7] Where the efficiency of Phenol-chloroform was indicated through various findings. Various other studies were conducted for the same where authors also used commercial kits.[3,15,16]

Cell Disruption In various protocols of DNA extraction different process could be followed for the disruption purpose. It could be achieved by using boiling, alkali treatment, enzymatic digestion and mechanical disruption based on the sample through which the DNA needs to be extracted. Cell disruption plays a major role in the good yielding of extracted DNA, as the decalcification process is required to remove the calcium ions from the sample matrix. Most commonly used decalcifying agent is EDTA which is generally used for the bones and teeth samples, where the calcium ions could interrupt in the process of extraction.[29]

Cell Lysis: Cell lysis is required in order to release the DNA from the membranes, which can be conducted using SDS, sarkosyl and guanidinium salts. Where these substances facilitates to destruct the membranes, denature and dissociate the proteins from the strand.[29]

Removal of protein and Cytoplasmic Contents: After the process of cell lysis there is the need to remove the protein and other cytoplasmic contents from the sample matrix, for this purpose proteins and lipids are to be removed by extraction rounds with organic solvents and cytoplasmic contents could be removed by the reversible binding of DNA with any solid substance. Al-Griw *et al.,* (2017) utilized the Chelex - 100 method for the purpose of genomic DNA extraction.[17,29]

Storage of DNA Solutions: Purified DNA sample is mostly stored in TE buffer (pH 8.0), where EDTA is used as the additive as chelating agent. Isolated DNA could be stored at 4°C or at -20°C for short duration and at -80°C for long durations. Where the cycles of freezing or thawing should be avoided to prevent the breaks of single and double stranded DNA. Udtha *et al.,* in 2014 stored the blood samples at room temperature by adding DNAgard blood, which shows notable extraction yield at room temperature.[19] Howlett *et al.,* evaluated the results utilizing the DNAstable™ medium for storing the isolated DNA samples at room temperature with an approach of low budget and effective storage method for extracted DNA.[18,29]

RNA Extraction

RNA has come up as a favorable tool in recent years for identification of body fluids retrieved from crime scene in forensics. Examination for stability of various mRNA markers specific for human blood in diversified environmental conditions and contaminants has been conducted.[21,28] Moreover RNA is also useful in determining the age of bloodstain through the rate of degradation.[5] For the purpose of both RNA and DNA extraction from the same processing RNA-DNA co-extraction is applied[3] where the procedure of extraction is conducted with an approach of extraction of total RNA, as total RNA bears the considerable amount of mRNA. RNA- DNA co-extraction is recognized for providing the simultaneous extraction of good and effective quality of both RNA and DNA for the purpose of forensic identification of body fluids.[29] Various studies have been conducted for extraction of miRNA for the purpose of understanding the impact of storage methods. Because of less molecular weight and length of miRNA it is required to first extract

the total RNA using organic-solvent method and followed by solid-phase extraction method to improve the small amount of RNA.

Hemoglobin Examination

For the purpose of identification of blood various tests viz. Benzidine test, Kastle-Meyer test, luminol test and leucomalachite green test are being conducted for various years.[22]A proteomic approach for identifying various body fluids has been proposed by Kamanna *et al.,* where the biological samples including blood were firstly mixed with ammonium bicarbonate, reduced with 1, 4-Dithiothreitol, than alkylated with iodoacetamide and in the end digested with trypsin at 37°C overnight. All the digested samples were added with uniform volume of saturated α-Cyano-4-hydroxycinnamic acid matrix solution and deposited on MALDI target plate MTP 384. [23]

Instrumentation

Blood and bloodstain evidences encountered at crime scene are of great importance for identification and individualization purpose. Various preliminary and confirmatory tests are being applied that are destructive in nature. [24,25] Different analytical techniques are developed in recent years where to measure the concentration and purity of the sample can be identified using UV-V is spectrophotometer[15,17,7,10,18,19] and RT-PCR.[3,6,13,16,12] Where on the other hand for the purpose of identify the integrity of extracted sample specially for nucleic acids 1% agarose gel electrophoresis[7,9] is conducted. ATR FTR was applied by orphanou et al. for the differentiation between different biological fluids, where albumin and hemoglobin were tested for blood.[26,27]

DISCUSSIONS

Hb, RNA and DNA are three major factors for the forensic identification and individualization. As the chances of encounter the blood as evidence at a crime scene are very high, it is the area of concern that how the blood undergo changes due to various environmental factors, storage temperature and conditions. The recovery of important information with the help of Hb, RNA and DNA is based on the method of collection and storage as well as on the process followed for the extraction of nucleic acids and proteins from the cell. Di Pietro et al.,. when calculated A260/ 280 nm and A260/ 230 ratio, it was found that silicagel spin column and revisited phenol-chloroform gave the purest genomic DNA respectively.[7] Moreover, while conducting gel electrophoresis to assess the integrity of extracted genomic DNA it was observed that revisited and proteinase-k gave more amount of yielded DNA. Although it was noted that the former method of extraction showed lower level of degradation when used. Various studies were conducted where the blood was kept at room temperature by adding bio stabilizers.[18,19,15,17] Hara *et al.,* in year 2016 has stored the blood samples for more than 20 years showing the stability of bloodstains and whole blood samples can be achieved for long term storage by keeping the whole blood samples at -20°C and -80°C whereas bloodstain samples at room temperature, 4°C, -20° C and -80° C, where -20° C and -80° C are suitable for both.[12,13] The samples received at the laboratories are mostly the piece of cotton gauze soaked in blood [10] Kaur et al., in year 2020 proposed a study with appropriate procedure to be followed for collection and preservation of blood samples from the crime scene and its impact on retrieval of information using UV-Vis spectrophotometer technique.

CONCLUSION

Biological evidences are more likely to be found at a crime scene wherein, blood out of all biological evidences acts as more valuable and corroborative evidence. For the purpose of identification and individualization of victim and accused, blood plays a vital role. In India diversified temperature and climatic conditions at crime scene may lead to degradation of the blood samples before the laboratory analysis. Also whenever the re-analysis of the sample is to be done, it is impossible to provide the same

findings as earlier due to the impact of storage temperature and conditions of the sample even in laboratories. To overcome this factor there is a need to identify the actual impact of different collection, storage conditions and temperature on the degradation of three major components of blood, viz. hemoglobin for the purpose of identifying the presence of human origin blood, RNA to analyze the age of bloodstain as well as the type of body fluid and DNA for the purpose of individualization.

Various studies has been reviewed and it can be concluded that the whole blood samples and bloodstains gave best results when stored at -20°C and -80°C. Although the temperature to be maintained is not possible always, for this purpose the blood soaked cotton gauze after air drying can also be used. The instrument and extraction methods, used in mostly cases is UV-VIS spectrophotometer, other techniques like Raman spectrophotometer and FTIR can also be applied for low yielding samples. The most common extraction method being used currently is phenol-chloroform method, which is quite time consuming and various expensive commercial kits are also available for the extraction from degraded samples.

On the basis of various studies it has been observed that there is no specific study available about the impact of various climatic conditions on the rate of degradation of blood evidences.

There is a need to identify an efficient extraction method for extracting the related information from the degraded blood evidences and to formulate the method which is comparatively less time consuming as well as less expensive than the methods being utilized presently. IJFMP

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REVIEW ARTICLE

Establishing Individuality using Palatal Rugae

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ABSTRACT

Palatal rugoscopy is a method to analyze and categorize palatal rugae patterns. These patterns can be analyzed on the basis of its size, shape and quantity in an individual. These patterns turn out to be extremely helpful in the cases when it is difficult to identify an individual with the help of fingerprints or dental records. The review article is based on the study of rugae pattern of male and female on the shapes and to do a comparative analysis of previous data. The present review article highlights the importance of accuracy in using digital software for recording the rugae pattern from photograph of dental casts also it showed that the shape of rugae remained consistent, showing the stability of the rugae patterns which are used for the individual identification.

KEYWORDS | palatal rugae, rugoscopy, biometrics, pre & post orthodontic treatment dental casts, individual identification

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INTRODUCTION

IDENTIFICATION OF AN INDIVIDUAL IS based on the definition given by Prosthodontic Terms – 8, the muscular folding or wrinkles present anatomically is known as Rugae. These are the folds of connective tissues which are on the preceding third of the palate. The other name for it is "Plica palatine" or "Rugae palatine".

Identification of an individual is of utmost importance to our culture, all the persons have their identity, whether living or dead and the primary motive in Forensic is to establish the identity of an unknown person. The identification of a human individual is based on the scientific facts of DNA analysis, fingerprints and dental records. In an effort to bring about newer methods of personal identification, scientists have highlighted the role of palatal rugae. This study is known as palatoscopy, which helps in the identification of an individual with the help of palatal rugae. Rugae are irregular in shape, with asymmetric ridges of mucous membrane. It is said that the palatal rugae are stable throughout our lifetime and never undergoes growth, ageing, or any treatment. These patterns occur in the third month of a fetus.[1]

Classifications of Palatal Rugae

Gloria First one to classify these patterns, considered all complex patterns as same. Classification was elementary and the patterns were said to be categorized in two ways: by specifying the extent of zone of rugae and also by specifying the number of rugae.

López De Léon Classification: It determined a link between our personality and these patterns.

Trobo Classification: Divided these patterns as simple, classified and composed rugae.

Carrea Classification: Does not apply any formula and divides the patterns

depending on its type.

Da Silva Classification: Simple and composed rugae types were given in this classification.

Martins Dos Santos Classification: Position and type of the patterns is the base of this classification.

Lysell's Classification: This is the most used classification in research due to its comprehensiveness.

Basauri Classification: It is the easiest classification, similar to Trobo and depicts the variations between innermost and outermost rugae patterns.

Lima: Composite, straight, curved and punctuate are the four types of patterns categorized under this classification system.

Tzatscheva and Jordanov: Branching, direction, radiality and symmetry are the base of this classification.

Cormoy System Classification: The patterns were differentiated based on the size.

Thomas and Kotze Classification: Lysell Classification was its base, but was modified depending on new findings.

Advantages of Rugoscopy

- Rugae patterns are used for identification because they are unique and stable throughout lifetime
- Low utilization cost
- Easy to establish post mortem data with the help of dental records
- Rugoscopy is a simple technique which does not include any complex instruments[2]

METHOD

The dental casts of the palatal rugae patterns were collected. After marking the patterns, they were analyzed using the Thomas and Kotze classification (1983).[3,7,9,12] This particular classification was also used to analyze the patterns on the basis of number, type and unification of the patterns while the shapes were recorded according to Kapali *et. al.* (1997)[16] Dental casts of the patients were collected before and after the orthodontic treatment. Followed by the marking of the patterns which was then checked for similarity and unification in a family.[4] Dental casts of pre and post-treatment were divided into a group of three random arrangements for which 13 evaluators were selected to find the closest match to known patterns after the patterns were marked with a sharp graphite pencil.[6] Collection of dental casts of pre and post-orthodontic treatment which was later 3D scanned to record the number, strength, characteristics and area of the palate to develop a statistical model and the probability of correspondence.[11] Selection of evaluators to compare the patterns was also used after the digital images of the patterns were marked with a newly designed software Palatal Rugae Comparison Software (PRCS Version 2.0).[5] In the comparison of fingerprints and rugae patterns, the fingerprints were collected on an A4 size sheets and photography of rugae was used. Vucetich's method (1891) was used to analyze fingerprints while the Carrea's classification (1937) was followed for rugae patterns. They were compared using the Chi square test.[8] Fingerprints collected on paper was also analyzed using the Galton's criteria while the alginate impression of the rugae patterns were analyzed following the Kapali's classification. The unpaired t test was used as a statistical tool in the analysis.[17] A laser system was used to scan the maxillary dental casts to record angular, transverse and anteroposterior measures of the palate. The differences in sides and sexual dimorphism were devised using the independent and paired sample t test. The patterns on the collected dental casts were marked and was statistically analyzed using the SPSS 16 software. The Man-Whitney test (1945) was done for pair analysis while the Man-Whitney two tailed test was done on separate gender.[10] SPSS was also used for the statistical analysis of the shape and distance between median and lateral points on first and last patterns on the dental casts.[14] The classification and statistical analysis of the lip prints, rugae and tongue patterns were done. The classification was done using Tsuchihasi

(1970), Lysell (1955) and Stefanescu *et. al.* (1990) and the method of Chi square test was followed for the statistical analysis.[15]

RESULTS

Each research showcased a different result according to the methods adopted and samples taken for the study. It was found that converging type of rugae was more common in females while the circular type of rugae were common in males. The sex prediction by logistic regression analysis (LRA) was found to be 99.2%.[3] When the fingerprints and rugae patterns were analyzed and evaluated, it was found that the external clip was more common on right hand while the internal clip was on the left hand. The Type IV (Carrea classification) rugae which is extended in all direction was found to be more common. While there was significant correlation in fingerprints of and individual, there were no correlation in rugae.[8] A comparison ofrugae in two different genders showcased a greater number of rugae in males and less in females. It was also found that wavy patterns were common in males and straight was common among females.[8,13] It was found that the probability of correspondence of palatal rugae patterns is very low for a palate of six rugae with average palatal area of 1453.9mm2.[11] The medial and lateral points of the first and last two rugae was found to be statistically different.[14] In the analysis of lip prints, rugae and tongue patterns, the type 3 in male and type 1 in female lip prints were more common. In rugae, wave patterns in male and straight patterns in female was more common. The U-shaped tongue in male and V-shaped in female was found to be common.[15] According to the dermatoglyphic study, arch pattern in male and loop in female is more common while according to the rugoscopic analysis straight patterns were frequent in male and circular in female.[17] Rugae established asymmetry laterally in the maximum bilateral measures. In the t-test, males presented the larger value of parameters being 9 out of the complete 28 parameters.[18]

DISCUSSION

All the samples of rugae patterns were different and was stable consistently after orthodontic treatment.[4] The accuracy of the software used for the identification of the individual was found to be 99%.[5] It is well known that the rugae patterns are unique and have negligible differences after any form of treatment. The analysis of the rugae patterns by dental examiners is more efficient and accurate as compared to that of the non-dental examiners.[6] The treatment of extraction decreased the pattern and expansion increased the patterns which was negligible. But the shape remained consistent in all the samples.[7] Based on the geographically different location, even after having statistical difference between genders there were a greater number of total rugae patterns in females as compared to males (It is inclusive of all different types of rugae patterns).[12] In a similar study on two different communities of Kodavas and Tibetans, wave pattern was highest while circular pattern was totally absent. But there was difference in unification of rugae in both communities.[10] Talking about the communities, rugae patterns were significant in western and northern Indian communities. The patterns were significantly different in both the genders.[16]

CONCLUSION

The palatal rugae patterns was found to be unique and can be used as a mode of personal identification with the use of LRA technique.[3] It concludes that no two rugae patterns are similar and possesses unique characteristics.[4,9,10] The computer software was found to be efficient in the rugae analysis. It is proved that the rugae patterns are unique and can be used for individual identification.[5] Dental examiners should be preferred for the evaluation of the rugae patterns as they are more accurate with the analysis. The patterns are unique in every individual.[6] The study showed that the shape of rugae remained consistent, showing the

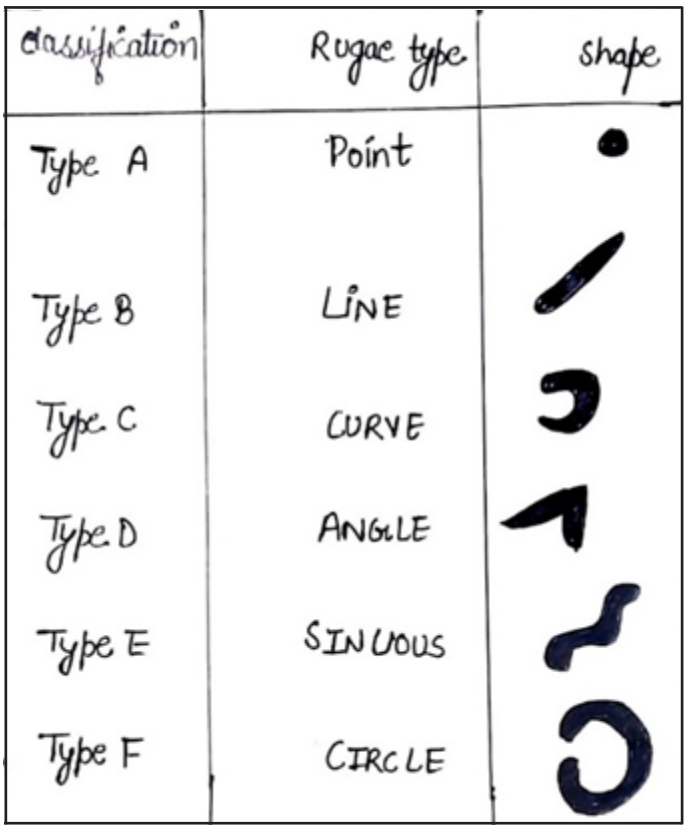

Figure 1:
Trobo classification

CLASSIFICATION	Rugae type
Type I	Posterior-anterior directed rugae
Type II	Rugae perpendicular to the raphae
Type III	Anterior – posterior directed rugae
Type IV	Rugae directed in several directions

Figure 2:
Carrea classification

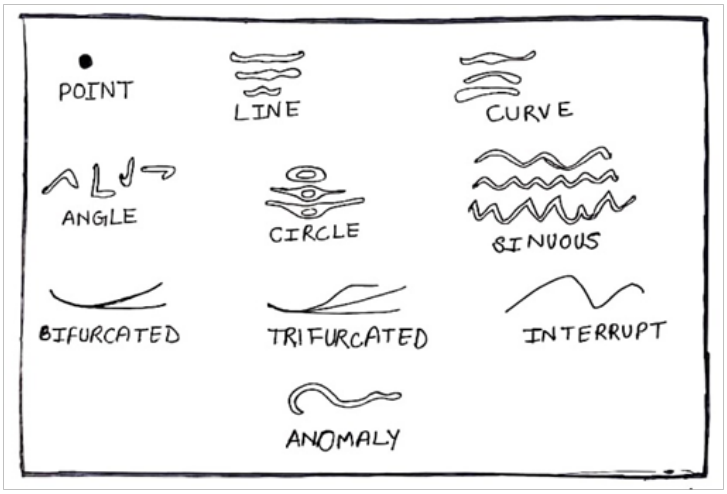

Figure 3:
Martin Dos Santos
Classification

stability of the rugae patterns which are used for the individual identification.[7,12] While genetic intervention is the major factor for the correlation in hands, further study will open up more information.[8] The information gathered from rugae patterns can be used for automated biometrics in forensic identification.[11] Palatoscopy can be used as a method of personal identification since it is effective, easy and a stable method.[13] During the maxillary expansion of palate, rugae is stable on the basis of number and shape but is not stable on the basis of position.[14] Lip prints, rugae and tongue patterns are the modes of individual identification which can be further processed with more number of samples.[15] We can also combine the uniqueness of both fingerprints and rugae patterns for the individual identification.[16] Rugoscopy and dermatoglyphics also shows significant differences in males and females and hence can be used for gender identification.[17] The morphometric measurements is also really helpful in the gender identification and sex prediction. Hence, it can also be used with the most common classification.[18] IJFMP

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REVIEW ARTICLE

Occupational Stress: A Descriptive Study among Forensic Professionals

Ritika Indoria[1], Amarjyoti Nayak[2], Rita Sharma[3]

ABSTRACT

Stress is not an illness, but it can significantly contribute to an individual's health and safety at work if it is not addressed. For decades, forensic science has gone on without devoting sufficient attention to the critical function of human cognition in forensic work. However, forensic scientists face a variety of industry specific pressures, including method criticism, exposure to crime scenes or gruesome case facts on a regular basis, financial resources, working in an adversarial court system, and a zero-tolerance policy for "errors". Thus, stress is an important human factor to mitigate for overall error management, productivity and decision quality (not to mention the well-being of the examiners themselves). According to the researches it has been concluded that forensic experts are facing high level of stress in and during their working hours, which in response hampers the decision making. The present review is undertaken to discuss the level of stress experienced by forensic experts during the course of their field work/Laboratory work.

KEY MESSAGES: This review discusses the stress among forensic professionals. Also, enumerates the on job working compatibilities of decision making and solving the cases in a more effective manner.

KEYWORDS | Forensic experts, Stress, Work place stress, Crime experts, crime scene investigators, forensic decisions, mindfulness, Occupational Stress.

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INTRODUCTION

In the scientific literature, the term stress is employed in an ambiguous and ambiguous and contradictory manner, and it is seldom defined. A stimulus, a reaction to a stimulus, or the physiological repercussions of that response are all examples of the word.[1] There are multiple definitions of stress, especially work-related stress. Job-related stress is defined by the UK Health and Safety Executive (HSE) as "the process that occurs when work demands of various sorts and combinations exceed a person's capacity and competence to cope."[2] Stress is not an illness, but it can significantly contribute to an individual's health and safety at work if it is not addressed. As a result, businesses in the United Kingdom are required by law to protect the safety and well-being of all employees.[2]

Stress is defined as an external force that causes tension or strain in a person. Stress is generally viewed as a bad occurrence or scenario that a person is subjected to and resulting in negative repercussions when it occurs in the job.[3]

Studies have consistently shown that physiological effects of stress on human body plays important role in the functioning. It has significant effect on cardiovascular system,

respiratory system, musculoskeletal system, blood, immune system, gastrointestinal system, metabolism, endocrine system, reproductive system and nervous system.[4,5] Stress causes cognitive disorders, especially in memory and judgment.[5]

Both job-related and individual factors have a role in the development of work burnout. These variables frequently lead to job burnout as a result of persistent job stress. The Job Demands-Resources (JD-R) paradigm states that an employee's well-being is determined by the balance between job demands and job resources, and that high demands/low resources are linked to physiological and/or psychological costs (exhaustion).[6]

Burnout is common in jobs where employees are exposed to stressful and upsetting events and circumstances on a regular basis. Forensic physicians/medical examiners/coroners, like other first responders and law enforcement officers, are frequently exposed to several traumatic occurrences, and their work tasks might be classified as very stressful. Autopsies, dealing with disfigured or decayed remains, and the notion of death are all stressful experiences.[6]

A substantial number of studies have shown the evidence of post traumatic stress disorder or work related stress in forensic experts during their tenure in the field or in the laboratories. Work stress and job satisfaction in criminal justice jobs are associated with physical ailment, turnover, poor job performance, and absenteeism, according to many research.[7] Understanding the levels and sources of job satisfaction and work stress can help legislators design policies that decrease negative work responses for the benefit of employees, their agency, and the broader public. [8]

METHODOLOGY

A literature search was conducted across PubMed and Science Direct databases, including the reference lists of relevant articles which ranged in duration of 2010 to 2021. The specific terms used for identifying relevant literatures were "Occupational stress in forensic experts", "Stress level of crime scene investigators", "Work place stress", "forensic decisions", "Judgement factors", "mindfulness", "crime investigators". Reference lists of articles obtained from this search were also examined for additional relevant articles. The inclusion/exclusion criteria for studies were based on their potential relevance to the high stress level in the forensic experts. Articles published before 2010 were excluded.

DISCUSSION

In 2011 a study done by Hold et al, included 54 certified forensic examiner. This study was to explore levels and sources of job satisfaction and work stress among a sample of digital forensic examiners to improve our knowledge of the complexities and challenges they face in there course of their jobs. There is increasing evidence that digital forensic examiners experience high levels of stress and burnout, particularly due to the investigation of child pornography case. It is not surprising that respondents who experience a large amount of role conflict also report higher work stress. Forensic examiners who have worked for their present agency for a longer period of time face higher job stress since they may be assigned more duties and obligations.

According to Adderley et al (2012) as might have been anticipated for the participants who completed non administrative activities, the results showed that sedentary activity categories produced the lowest mean above resting heart rate, whereas the physical activity category produced the highest mean above resting heart that suggested stress reactivity during routine scene activity such as a crime scene or vehicle examination. The psychosocial stress being felt by crime scene investigator and the stressors are unlikely to be same as those recorded by Anderson et al. (2002)where police officers displayed stress reactivity during , for example having a hand on a holstered gun or

during interaction with suspects. This may well apply not only to police officers but also to crime scene investigator engaged in examining what might be perceived to be routine crime scene.

Other variables might contribute to a scene inspection being a source of stress for a crime scene investigator. These include dealing with a hostile victim, working alone, the crime scene investigator's concern that their vehicle may have been vandalised or that they are working in an untidy house, and difficulty determining what might have been touched by the offender, all of which can cause blood pressure to rise during clinical visits or normal daily activities.

Elliott et al, 2012, Concluded that a substantial proportion of Forensic health care professional (FHCP) experienced elevated levels of occupational stress and psychological distress, while moderate levels of burnout were demonstrated in terms of emotional exhaustion, depersonalization, and personal accomplishment. FHCP employed a variety of problem-focused, emotion-focused, and palliative coping methods, according to the findings. Overall, the findings tended to confirm the widely held belief that forensic services is an inherently demanding and hazardous workplace, which can lead to high levels of psychological distress, burnout, and stress among FHCPs.

In 2016, another study was done on crime scene investigators by Leone et al. The research examined the relationship between exposure to critical incidents and the investigators' perceived stress. They concluded crime scene investigators were high in three of the four areas of job related stress. Stress caused by factors internal to the organization included training and access to stress mitigation, stress resulting from factors external to the organization included CSI gender, and many stressors resulted from the job of being a CSI.

Jeanguenat et at (2017) states that human aspects in forensic examinations, job quality, and error control are all developing areas of forensic science. Understanding and controlling human variables may improve a laboratory's quality and technical procedures, as well as its decision-making capacity. Workplace wellness, particularly stress, has been extensively researched across a variety of sectors in order to better understand employee retention, work satisfaction, health, and absenteeism.

Salina et al, (2018), staes that there were no differences in stress and coping mechanisms between sworn officers and civilian crime scene personnel. Male crime scene personnel reported slightly higher levels of stress and anxiety than females, with males also reporting significantly lower use of emotional support, instrumental support, and positive reframing.

According to Kriakaus et al (2019), FHCPs experienced moderate degrees of emotional fatigue and depersonalization, they maintained a good feeling of personal accomplishment and so felt capable and competent in carrying out their responsibilities. Because higher levels of dispositional mindfulness were linked to reduced levels of maladaptive coping, stress, and burnout, MBIs may show to be effective techniques for assisting FHCPs.

Ellickson-Larew et al (2020) concluded that forensic experts seem to be well in the current state of the literature on the dissociative subtype of PTSD, including how different analytic approaches can inform understanding of the diagnostic criteria and how best to assess the subtype in order to assess the potential for malingering and to inform triers of fact about both the presentation of the subtype in the courtroom and the availability of healthcare.

Sehsan et al (2021), did a study on Egyptian forensic physicians using a self-administered questionnaire. It included personal and occupational data, Maslach Burnout Inventory, and the Brief COPE Inventory. Multivariable logistic regression was performed to identify significant independent predictors of burnout. The correlation between burnout and coping was examined. They concluded that burnout is quite common among Egyptian forensic physicians. Female forensic examiners who

work in high-stress environments are more prone to experience burnout. As a result, psychoeducation and psychological support services should be implemented and made available to them.

CONCLUSION

Forensic experts have different fields to work on like field based, crime scene, laboratories, and all the back hand work. Their work load and job requirements can cause stress which can further affect the judgement, decision making and mindfullness in solving cases. Stress management in these individual is very much required to improve the quality of forensic judgements and more importantly health of these experts. Looking at the high level of crime in India, further research on Indian forensic expert population is needed to be done to understand their stress level and job satisfaction.

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REVIEW ARTICLE

Effect of Nicotine on Haematological Parameters in Healthy Population: A Review Literature

[1]Sumedha Rabra, [2]Meenakshi Verma

ABSTRACT

Tobacco smoking is one of the causes of the incidence and mortality of cancer in the world. The prevalence of tobacco consumption is a hallmark menace of today's era. Lot of countries are planning to curb such menace but uncertainly, the consumption is still at par. The usage of tobacco not only harm the individual but it also effects the society. Irrespective of any morbidity the tobacco consumption is causing deleterious effects on healthy population. The counter effects of this consumption on hematological variables are significantly high. The attempt has been made to portray the baneful impact of tobacco among healthy population on the basis of Hematological parameters like white blood cells, red blood cells, platelets, concentration of Hemoglobin, Hematocrit, mean corpuscular volume, packed cell volume, mean corpuscular hemoglobin concentration.

KEY MESSAGES: Nicotine is a highly addictive and hazardous substance. The deleterious effects of nicotine are not only harnessing the individual but society too. Hence, this review culminates the effect of nicotine on important hematological variables among healthy individuals.

KEYWORDS | smoking, tobacco, Complete blood count

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INTRODUCTION

SMOKING IS A MAJOR HEALTH RISK THAT IS associated with a number of co-morbidities. Tobacco smoke contains more than 4,000 distinctive poisonous and cancer-causing synthetics. These synthetic compounds, like nicotine, tar, carbon monoxide, and others, have various adverse consequences on the body's different functionalities. It is particularly related to the pathogenesis of aspiratory and cardiovascular diseases.[1]

Smoking is the most common way of consuming different techniques like cigarette, stogie, Biri, and hookah pipe. It gives the smoker a feeling of joy and satisfaction It is a perplexing outer and inner improvement with visual, material, mechanical (mouth development), gustatory, olfactory, and bothering parts. Tobacco use is one of the main reasons for death among ladies. Various examinations have been directed all around the world to explore the danger factors for cigarette smoking. Hereditary and segment factors, accepted practices, peer impacts, and parental mentalities and conduct are only a couple of models.[2]

Impact of Smoking on White Blood Cells

Smokers are more likely to develop coronary artery disease (CAD), atherosclerosis, severe myocardial localized necrosis, hypertension (HTN), thickening problems, aggravation, respiratory illnesses, cancers, and other diseases. Smokers have a 20-25 percent greater complete white cell count in their peripheral blood than non-smokers. Smoking is undoubtedly responsible for the increase in white cell count as shown in Table-1. Smoking causes increased blood leukocytes, neutrophils, lymphocytes, and monocytes, The goal of this study was to see if the concentration of nicotine in plasma

HAEMATOLOGICAL PARAMETER	SMOKERS	NON-SMOKERS
Blood Leukocytes	+ + + + +	Normal
Neutrophils	+ + + +	Normal
Lymphocytes	+ +	Normal
Monocytes	+ + + +	Normal

Table 1: Impact of smoking on White Blood cell

HAEMATOLOGICAL PARAMETER	SMOKERS	NON-SMOKERS
RBC Haemolysis	+ + + + + + +	Normal
Haemoglobin	+ + + + + + +	Normal
Macrocytic RBC	+ + + + + +	Normal
Red Cell Distribution Width*	-------	Normal

Table 2: Impact of smoking on Red Blood cell

HAEMATOLOGICAL PARAMETER	SMOKERS	NON-SMOKERS
Mean Corpuscular Volume	+ + + + +	Normal
Mean Corpuscular Haemoglobin*	+ + + +	Normal

Table 3: Impact of Smoking on Mean Corpuscular Volume and Mean Corpuscular Haemoglobin

or carbon monoxide in end-terminated air correlates with the number of white blood cells in smokers.[3]

Impact of Smoking on Red Blood Cell

Cigarette smoking has adverse effects on red blood cells (RBC), several RBC-related parameters, and hemoglobin (Hb) in previous studies. It was found that RBC haemolysis in smokers was 20-25 % higher than in non-smokers. Cigarette smoke increases 2,2′-azo-bis-(2-amidino-propane) dihydrochloride-induced RBC haemolysis. Several studies have shown the fact that there is an increase in the percentage of macrocytic RBCs and a decrease in the red cell distribution width (RDW) in smokers compared with non-smokers as depicted in Table 2.[3]

Impact of Smoking on other Hematological Parameters

Lymphocytes have been found to have specific morphine receptors. Opioid receptors are thought to function in both an autocrine and paracrine manner. Although it has been proposed that morphine affects some immune cells indirectly, it can also directly affect the functions of macrophages and polymorphonuclear (PMN) leukocytes, as well as regulate the expression of some T-cells. Some researcher reported that the endogenous opioid peptides, including α-endorphin and the dynorphin peptides, and exogenous alkaloids such as morphine plays an important role in the lymphocytes and other immune cells function.[4,5]

Constantly raised carboxyhemoglobin levels, like those found in cigarette smokers, invigorate erythropoietin creation. Smokers have higher hematocrits than non-smokers in examinations and different investigations propose that smokers have a more prominent expansion in hematocrit than non-smokers when presented to high elevation. The effect of adjusted aspiratory work in cigarette smokers comparable to HA transformation has not been tended to. Persistent hypoxemia from the low oxygen immersion of hemoglobin actuates polycythaemia[9], an expanded mean corpuscular volume (MCV)[10] and decrease in Mean Corpuscular Hemoglobin than normal levels in smokers as discussed in Table-3.

Nicotine makes a coagulation structure in the coronary supply routes, decreases vascular movement, and increments endothelial break. An increment in carboxy-hemoglobin levels might cause hypoxia, and it is additionally liable for sub-endothelia odema since it adjusts vascular penetrability and lipid gathering. Tobacco smoke unmistakably contains free extremists and peroxides. They are connected with physiological marvel like union of prostaglandins and thromboxane, and they are likewise engaged with the pathogenesis of different infections including atherosclerosis, carcinoma, and provocative processes.[11]

CONCLUSION

The review concludes with an attempt of various contraindications which are reported among smokers and non-Smokers. Numerous Hematological parameters like Blood Leukocytes, Neutrophils, Lymphocytes and monocytes level shows significant increase in

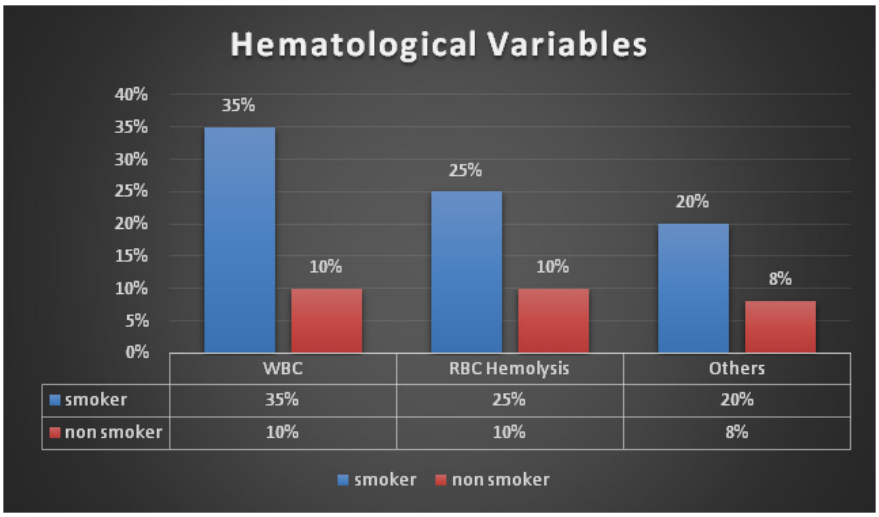

Figure 1:
A Comprehensive Description of Hematological Variables among Smoker and Non-Smoker Population

smokers. Whereas, Red cell distribution width and Mean Corpuscular Hemoglobin levels were found to be extremely lower than normal values in smokers than non-smokers. Furthermore, the levels of Hemoglobin depict the remarkable increase in smokers as compared with non-smokers. Therefore, it is concluded that smoking not only affect the hematological variables but also the overall health of the individual. IJFMP

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REVIEW ARTICLE

Cognitive Impairment and Dementia in Frontal Lobe Syndrome Leading to Violent Behaviour: A Review Literature

Apoorva Tiwari

ABSTRACT

Studies have shown that, according to reports, people with frontal syndrome have anti-social and violent behaviours.[1] There are many neurobiological and neuropsychiatric reasons behind the increase in criminal activity. Frontal lobe syndrome can occur in stroke, head injury, degenerative changes, and multiple sclerosis and so on. Frontal lobe syndrome can damage the amygdala or orbital cortex, leading to the behaviour of people with mental illness.[1] The purpose of this review is to focus on the need to identify executive dysfunction, cognitive impairment, and dementia that often lead to violent behaviour. This review was conducted through exhaustive researches in different databases using the keywords frontal syndrome, violent behaviour, and cognitive dementia. The conclusion is that frontal syndrome plays an important role in the execution of violent and anti-social behaviours.

KEY MESSAGES: Frontal lobe syndrome cause loss of functions like judgment power, cognition, dementia which causes personality change leading to antisocial behavior.

KEYWORDS | frontal lobe syndrome, cognition, dementia

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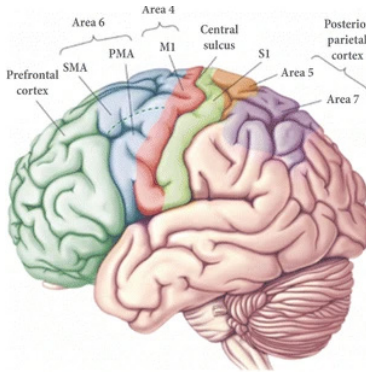
INTRODUCTION

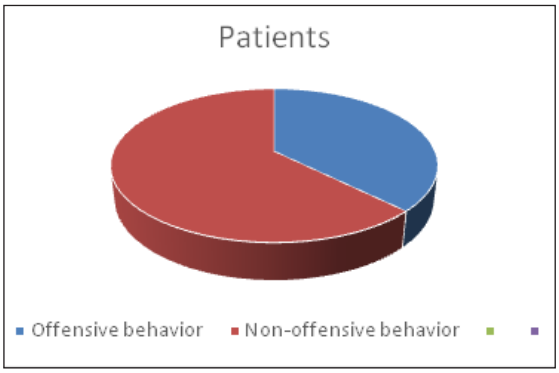
FRONTAL LOBE SYNDROME IS A CLINICAL DISORDER that causes damage and dysfunction of the prefrontal cortex and interferes with higher brain functions (such as social behavior, motivation, planning, and judgment).[1] It is characterized by the behavior and personality alterations with time. Cognitive decline and dementia are reported to be present among patients and can lead to behavior changes, which are anti-social and violent and enhances the tendency of criminal activities, large personality changes can be seen in patients with frontal lobe syndrome.[1] It is a rare clinical disease where forensic psychiatric evaluation has not been yet concluded completely and still going on, therefore it is necessary to review the research on offensive behavior patterns in patients with frontal lobe syndrome.

Pathophysiology and characteristics of Frontal Lobe Syndrome

There are multiple reasons which causes frontal lobe syndrome such as head injury, Cerebrovascular event, infection, neoplasm, many degenerative disorder specifically Pick's bodies in which patients complain of dementia.[2] The prevalence of frontal lobe syndrome is found to be 19% due to degenerative disorders.[2] Its characteristics features are following discussed. Patients are reported to be present with decreased lack of spontaneous activity in which planning executions, interest in activities were in process of deterioration and there was increasing periods of restlessness. Such patients are easily distractible and are present with loss of memory.[3] Also change of affect in which patient either behaves apathetic and flat or becomes over exuberant and childish or uninhibited with possibly inappropriate sexual behavior.[4]

Cognitive Impairment





Source: Google image

Cognition is the ability to think, calculate, and create abstract thoughts, judgmental behaviour, attention and orientation towards time place and environment. Mini mental status examination is used as measuring scale to check or quantify patients' cognitive ability. It is due to ability to think which supports our behaviour system in social as well as in personal life. It has been reported in studies that there is random decline in cognitive ability of patient in case of frontal lobe syndrome. Although in cognitive function of brain all four lobes that is frontal, temporal, occipital and parietal participates and help us in providing the thinking ability, but in frontal lobe specifically ventromedial part of frontal lobe contributes for the formation of emotion, judgmental ability, personality development and behaviour. According to studies it has been shown that cognitive impairment has been directly found to be involved in psychological disorders leading to various kinds of criminal activity. Cognitive impairment is found to be directly co related with schizophrenia which cause a person to suffer with mental disability,[5] decline in behaviour execution abilities, and presence of stress factor leaving the patient with depression or anxiety. Cognitive ability is also found to be decrease in bipolar disorder, in which patient for a time period is in state of depression and for other moment suffers from anxiety disorders. There are different scales and parameters which are used to establish co relation between cognitive impairment and neuropsychological disorders. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) is one of the important scales which are used to check the neuropsychological states in cognitive impaired patients.[4] In various studies it has been reported patients suffering from cognitive impairment and offending different crimes were used to score high on scale of Repeatable Battery for the Assessment of Neuropsychological Status scale. Hence in absence of proper cognition patients are found to be involved in various criminal acts leading to be involved criminal acts leading to anti-social and violent behaviour.

Demographic aspects of criminal behaviour

Frontal lobe syndrome can lead to dysfunctions in neural structure which results in impaired judgment, executive function, emotional processing, sexual behaviour and violence such dysfunctions can lead to antisocial and criminal behaviour. As shown in fig. 1, in a case study it was found that out of 171 patients 64 (37.4%) frontal lobe syndrome were presented with complain of criminal behaviour during the phase of their illness.[5] Specifically behavioral variant of frontal lobe syndrome were more found to be involved in offensive behaviour. Different types of criminal activity have been reported in patients of frontal lobe syndrome few examples are homicide, hyper sexuality, traffic violations and theft. The behaviour pattern of patients poses huge burden on family, hospital staff and society.

Dementia

Frontal lobe dementia is an umbrella term

which involves different kind of brain disorders. Patients with frontal lobe dementia have dramatic changes in their personality and becomes socially inappropriate, impulsive and emotionally indifferent. The common behavioral changes are following when a patient is suffering from frontal lobe dementia.

- Remarkable increase in anti-social behaviour.
- Reduction of interpersonal skills that is having sensitivity to another's feelings.
- There is phase of apathy.
- Lack of judgment and loss of inhibition

So all such behavioral changes are enough to provoke a person to perform antisocial activity and violence in society and hence increasing the criminality rates.

Executive Functions and Aggressive Antisocial Behaviors

Frontal lobe syndrome leads to impaired cognitive function associated with aggressive antisocial behavior.[6] Executive function is the ability to control thought processes and behaviors in an adaptive and goal-oriented manner. Therefore, exaggerated antisocial behavior can be conceptualized as the result of executive function deficits, especially impaired ability to suppress violent impulses. Studies have found that juvenile offenders and people involved in criminal activities related to frontal lobe syndrome also have intellectual disabilities and key intellectual functions. The orbitofrontal cortex or prefrontal cortex is damaged. This part of the frontal and limbic system can cause severe depression, expression of fear, and lack of confidence and complexity. This personality change can lead to violent and anti-social behaviors, as well as responsible and kind behaviors that lead to emotional, impatient and disrespectful behaviors, thereby increasing social crime. The prefrontal cortex controls the limbic system related to the control and correct execution of emotions. Any damage to this part of the brain will lead to increased anger and anger. Therefore, damage to the frontal cortex can lead to changes in certain social and personality characteristics.

CONCLUSION

Frontal lobe syndrome found to cause dramatic changes in personality, judgment, cognitive decline, and dementia. The limbic system plays an important role in the control and execution of emotional behaviors, frontal lobe injuries have been found to involve the limbic system, causing patients to become irritable, depressed and lead to antisocial behaviors. Damage to the ventromedial orbitofrontal cortex leads to dramatic changes in behavior patterns, leading to impulsivity and poor judgment. Figure 2 is representing brodmann area 10-frontopolar prefrontal cortex, broadmann area11-orbitofrontal cortex, and broadmann area 47-lateral surface of frontal lobe are closely related to the loss of inhibition of emotional responsibility and the inability to function normally in social interactions.[7] This review is limited and focuses more on the relationship between frontal lobe syndrome and antisocial behavior. A research based review that includes comprehensive mental health and its importance in controlling behavior patterns is the future scope of this research. IJFMP

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REVIEW ARTICLE

Significance of Medical Imaging in Forensic Science

Amit Pratap Singh Chouhan

ABSTRACT

CONTEXT: Forensic radiology is an area of expertise in medical imaging that uses radiological techniques to assist physicians and pathologists in matters related to the law. The forensic application of clinical medical radiology can be applied in many fields; The primary target of evaluation is the skeletal skeleton, but the soft tissues and abdominal and thoracic viscera may provide the predominant findings. Technological advances in clinical radiology offer many potential tools for forensic radiology, allowing a broader field of applications in this field. In the event of a massive disaster, the identity of the individual is of utmost importance. To do this, forensic investigators use different methods to identify the dead. They consider the skeletal remains of the dead to be the initial stage of identification. Radiographs have great evidence to serve as an ex-mortem record and also help to identify the individual, age, gender, caste, etc. Forensic dentistry is also emerging as a new branch of forensic medicine. Therefore, the forensic dentist must know the various techniques, developments and resources to incorporate technology to achieve success in human identification. Therefore, our aim of the present review is to focus on the various radiological techniques and new developments available for the successful identification of the dead. Since X-rays can capture their distinctive physical characteristics, they become an invaluable tool in forensic science. Radiographic identification has been used for a long time and the technique is efficient, comparatively easy, both living and dead records can be obtained, and it is inexpensive compared to DNA technology. Therefore, expert knowledge and proper application of maxillofacial radiological techniques play an important role in forensic identification and resolution of medico-legal cases.

KEY MESSAGES: Medical Radio-Imaging Techniques Shows a significant measure in forensic identification and Determination of MLCs.

KEYWORDS | forensic radiology, medical imaging, radiographs, dentistry, MLC

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INTRODUCTION

Forensic is a word the word defining the term forum, public in other word forensic is the procedure of investigating and identifying the facts in crime criminal or civil court of law by utilizing science and technology. The forensic have been much helpful in today's world by successfully identifying small abuses, murders, to mass disasters.[1]

Similarly, "Forensic Science" description includes the behaviour which are perturbed with the exercise of the power, law enforcement, and civil, legal and judicial areas in detecting illegal actions in Table 1.

The person who discovered the forensic science was Sir Bernard Spilsbury. This pioneer looked beyond the crime and to lead the science in a new way, which is very important in today's century.[2] The area included in forensic science are; Forensic Odontology (Dental), Forensic Radiology (X-Rays), Forensic Entomology

(Zoology), Forensic Toxicology (Poisons), and Forensic Psychiatry (Psychopathology), etc. (fig.1) So, the term forensic science is a vast subject including all these areas.[3]

Here, Forensic Radiology is the field of X-Rays where the rays are used to identify the suspected crime. The radiologist are fundamental part off forensic science.[5] The role of radiologist in forensic science involves identifying the corpse through the anatomical bones and x-ray images, during post-mortem, radiographs are taken which will help in assessing the foreign particles in the body.[6]

The two radiographs of same individual is taken one is ante-mortem (before dissection) and another is post-mortem, so that it is more specific in determining the person.[7] So, it is equally important in odontology as well as anthropology. When the case comes of claiming the death of a person through external force, then the radiographs of those injured area will be helpful in determining any fractures or internal trauma and the reason why the person died can also be evaluated.[8] This is essential in women and children in case of abuse like homicide. Therefore, following the court law is equally important in forensic radiology. Hence, the key role played by radiologist in forensic science has major advantages in future generations, as well.[9]

History of Forensic Radiology

- In the year 1895, November 8, Sir Wilhelm Conrad Roentgen accidentally discovered X-Rays which increased its importance throughout the decades.[10]
- These X-Rays were then first used for investigating a murder case in North America. However, the errors were observed as it took around 70 min. in taking a single radiograph.[11]
- Finally, in the year 1919 (after 24 years of discover of x-rays) the government of North America accepted legal use of radiology for solving forensic crimes.
- In year 1940, W. Koenig took the radiographs of teeth which was used in the identification of Adolf Hilter, this started the Forensic Odontology.
- The establishment of CT scan, MRI and other imaging tools set the 'Gold Standard' for comparing the post and pre-mortem radiographs.[12]

Scope of the Forensic Radiology

- The forensic radiology have increased its advancement in imaging as well as identifying the crimes.[13] And it is expected to further improve the technology and techniques in future.[14]
- B.G. Brogdon, classified the scope of forensic radiology into four categories along with its sub types:
- On the basis of service – Identify discovery, Assessment of injury and death (accidental injury/trauma, non-accidental injury/ trauma, foreign bodies, criminal lawsuit –fatal, non-fatal, civil lawsuit, etc.); On the base of Edification; on the base of Investigation; on the base of organization cases.[15]According to the types;
- Identify discovery includes; determining the age, sex, stature, whether animal or human remains and so on.
- Assessment of death and injury covers the types, depth of injury, origin of fracture, internal bleeding or not, trauma relating to death, the type of device used to injure and shape and size of injury.
- Criminal litigation- Fatal (murder, suicide, abuse and terrorism) and Non-Fatal cases (fraud, faking, kidnapping, smuggling, etc.)
- Civil Litigation – Violation of civil rights, personal injury, wrongful death or birth, etc.)[16]

Radiology in Forensic Dentistry

The forensic radiology involving dentistry deals with the evaluation of dental confirmation with proper investigation of dental findings.[17] The field of forensic odontology is concerned with evaluation, indulgence of dental evidences, findings and so on.[18] This field have a basic role in forensic science and evidence because the teeth is the only art of the body which takes

certain time to decay even if the other parts of body are highly damage due to accidents like trauma, burning, etc.[19] However, the dentition remains undamaged and will provide a solid evidence in determining the age, sex, and nationality of the deceased person.[20] (Fig. 2)

Through the stage of teeth eruption also we can identify the victim. In case of bite marks, through the shape of teeth it can be analyzed. The error in charting of teeth of dead victim can be corrected by numbering teeth;[21] In case of adjacent tooth migrating into the space of extraction, this can be joined using other radiographs. During the post-mortem inspection, the X-ray image appearance of teeth and facial bones is a perpetual data of these tissues even if teeth's and bones are being detached from histopathologic examination.[22]

The forensic odontology is very essential in anthropology as well. Through the dentition the age and life span of the individual can be identified. Hence, the necessity of dentistry in forensic radiology is increasing. The advancement in modalities of identifying and solving the crime incident is also improving. [23]

Anatomical Identification of Suspect

For the identification of victim only the surface of dental evidence will be insufficient sometimes. So, the experts rely on recognising through the surface landmarks of the teeth and making comparison between pre-mortem and post-mortem images.[24] The surface landmarks of the teeth involved in assessing the characteristics like crown morphology, the size of tooth, pulp morphemes, etc. These features will provide some particular information on the individual. In case where this morphology's are damaged then spatial connection of posterior teeth can be analyzed. Still, the identification through facial bones are much tough as the bones are overlapped mainly in maxillofacial region.[25] So, for facia evaluation, the only landmark that can be used in comparing post-mortem ante-mortem radiographs is through frontal sinus which is present in underneath the forehead, reaching above the eye sockets and eye brows. The radiographs having various sinus can be examined for further identification of individual and case solving. In some reports when the teeth's are already lost due to age or other incident, the anterior teeth can again be reconstructed so, that assessment and numbering will be possible. [26]

Medicolegal Cases

The Medicolegal Cases (MLC) is defined as a case involving injury or illness where the investigation is carried out legally through law enforcement.[26] In Forensic Radiology, the identification of MLC cases can be done through Forensic Maxillofacial Radiology.[28] This is done in case, where the dental radiology fails to attend the mass casualty cases. For example, when the victim comes with external injury like swelling of face, bruises claiming of being attacked then this will be Medico-legal case where forensic radiology will be helpful in investigating whether the injury is intentional or accidental (Fig. 3).[29] The injuries in the head can be assessed by looking through the direction of injury and point or origin of impact. If any kind of metal objects or other tools is used to harm the victim then it can also be identified. This things can be analyzed through advanced modalities of imaging like CAT scan, Micro-Computed Tomography, etc. When the person is strangulated then the evidence can be collected looking through radiographs of hyoid bone fractures or cornu of thyroid cartilage.[30]

In determining the age, the profile of victim can also be created. The soft tissue of face can be reconstructed through CT scan and the image of person can be created in case of burning incident. The use of radiology is boundless in forensic dentistry, evaluated through anatomy morphology of the teeth as well as major landmarks of maxillofacial bones. So, the difference in ante-mortem and post-mortem radiology will help in finding the identity of the individual.[31]

Utilization of DTI of Brain for investigation in Forensics

The MRI have also major role in forensic field. The Diffusion Tensor Imaging (DTI) in MRI

S.NO	STUDY	YEAR	REFERENCE
1.	Forensic radiology as an instantaneous branch of science.	2017	Tarani et al., 2017
2.	Problems, dispute and advancement in forensic radiology as well as MLR	2015	Guglielmi et al., 2015
3.	Role and importance of forensic radiology in odontology (dentistry).	2015	Manigandan et al., 2015
4.	Issues of forensic radiology; case of Computed Tomography of patient having gunshot injury in Head.	2018	Giffen et al., 2018
5.	Importance of Computed Tomography imaging in cross-sectional digital examination in post-mortem case.	2014	Higginbotham et al., 2014
6.	Correlation of forensic radiology with forensic odontology.	2013	Silva et al., 2013
7.	Characteristic features of Imaging radiation in forensic odontology.	2011	Chandrasekhar et al., 2011
8.	Examination of ancient child mummies having head trauma by utilizing forensic radiology.	2016	Davey et al., 2016
9.	MRI Diffusion Tensor Imaging (DTI) of Brain playing an essential role in Forensic radiology.	2015	Berkovitz et al., 2015
10.	Character of MRI imaging in post mortem of an adult.	2014	Ruder et al., 2014
11.	Application of AI in clinical Forensic Medical Imaging.	2020	Pena-Solorzano et al., 2020
12.	Utilization of forensic radiology in veterinary.	2017	Watson et al., 2017
13.	Employment of computed tomography (cone beam) in field of forensic radiology.	2014	Sarment et al., 2014

Table 1: Case studies on applications of radiology in forensic investigation.

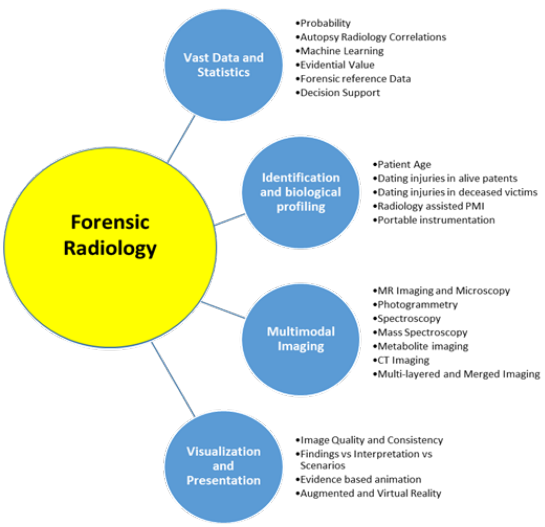

Figure 1: Showing Forensic Radiology and Medical Imaging Research outline.

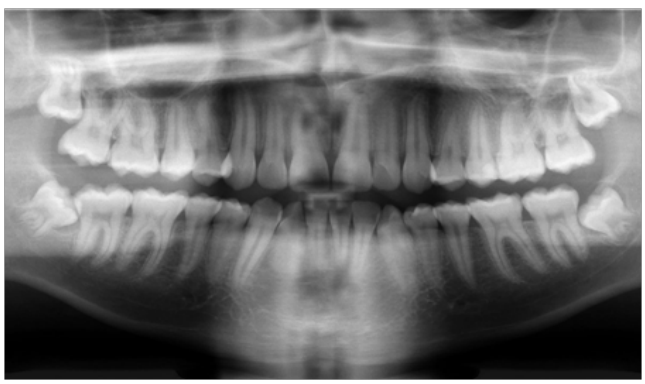

Figure 2: Showing images of Cone Beam CT

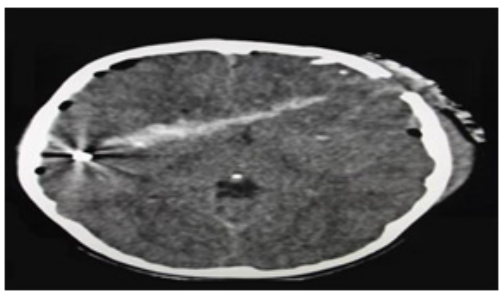

Figure 3: Showing CT images of gunshot wound

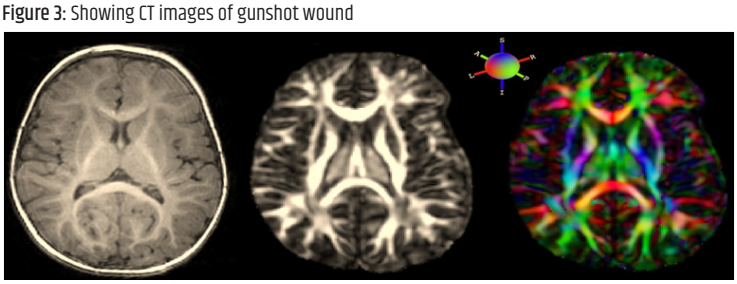

Figure 4: Showing MRI images of brain DTI

is elucidated as the sequence of MRI which gives knowledge or biological activity of tissue at microstructural level.[32] This is done by evaluating diffused water in various tissue. The DTI will provide information on neurological tissue either damaged or normal by computing the magnitude and direction of diffusion.[33] Where there is a case of cell death in stroke this is identified by decreased diffusivity.[34] The PM (post-mortem) DTI assessment is essential in differentiating MRI derived records with histological and forensic examination. (Fig. 4)

Hence, by reading the magnitude of diffusion and direction the forensic evidence in an individual can be found.[35]

CONCLUSION

Hence, the forensic radiology have played a significant role in the forensic science field. The radiographs are much more helpful in identifying and investing the cases and evidence.[36] The human identification can be relayed through radiographs of different parts.[37] The age, sex, race, and stature of an individual can be easily identified. Along with human identity the weapons of murder, injury can also be examined through Forensic Radiology.[38] The dentition is also valuable in providing information through bite-mark analysis and morphological analysis of teeth. The dental radiography should be given more importance by the forensic experts. These modalities, used along with other forensic methods will be able to provide most accurate reading in short time. In the developing countries like India, the forensic radiology and odontology are regarded as the important aspect for investigation.[39] However, the advancement in protocols are still lacking. As, the country have raising case of murders, illegal doings, the experts are focusing on all methods for solving the cases.[40] In future it will have more bright way. The value of maxillofacial radiology will also increase in coming decades.

So, in the upcoming decades Forensic Radiology will be an ideal contrivance for the investigations and it is also regarded as an integral part of forensic sciences. And with the advancements in the imaging modalities the role of radiology will also increase in the field of Forensic Science.[41] IJFMP

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REVIEW ARTICLE

Impact of Physical Activity on Forensic Psychiatric patients: A Rehabilitative Approach

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ABSTRACT

CONTEXT: Forensic psychiatry is comparatively a new upcoming and developing field in India. Substance use and mental illness have been independently associated with violence, and sometimes violent crimes. Forensic psychiatric patients, detained under sections of the mental health act, are particularly prone to developing poor physical health during the time it takes to stabilize and improve their mental health. A large and growing body of evidence suggests that physical activity (PA) may hold therapeutic promise in the management of mental health and metabolic disorders. Incorporating physical activity as an integral part of treatment strategies would appear to go a long way toward reducing the adverse health impact in forensic psychiatric care.

KEY MESSAGES: Modern forensic psychiatry has benefited from the evolution in the medico-legal understanding and appreciation of the relationship between mental illness and criminality, evolution of the legal tests to define legal insanity and the new methodologies for the treatment of mental conditions that provide alternatives to custodial care. Few interventions exist whereby patients can hope to achieve improvements in both psychiatric symptoms and physical health simultaneously without significant risks of adverse effects. Physical activity offers substantial promise for improving outcomes for people living with mental illness and substance use disorder under forensic psychiatric care.

KEYWORDS | X-ray fluorescence, Scanning electron microscopy, Dynamic light scattering

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INTRODUCTION

Forensic psychiatry is a particular branch that emerged a few decades ago, and since then, its role has constantly risen in importance. According to the American Board of Forensic Psychiatry and the American Academy of Psychiatry and Law, it is defined as 'a subspecialty of psychiatry in which scientific and clinical expertise is applied to legal issues in legal context, embracing civil, criminal, correctional, or legislative matters'.[1] There is a dynamic relationship between the concept of mental illness, treatment of the mentally ill, and the law. The aim of forensic psychiatric care is to care for, treat and rehabilitate patients back to independent life outside of hospital without recidivism into serious crime. Although the legal regulation of forensic psychiatric care differs from country to country, these patient groups are often distinguished by severe mental illness, a high risk of recidivism, complex rehabilitation and long hospital stays. These forensic psychiatric patients often have a psychotic disorder, combined with substance use, and are receiving treatment with antipsychotics.[2] Treatment often continues for several years[3] and there is a high risk of criminal recidivism

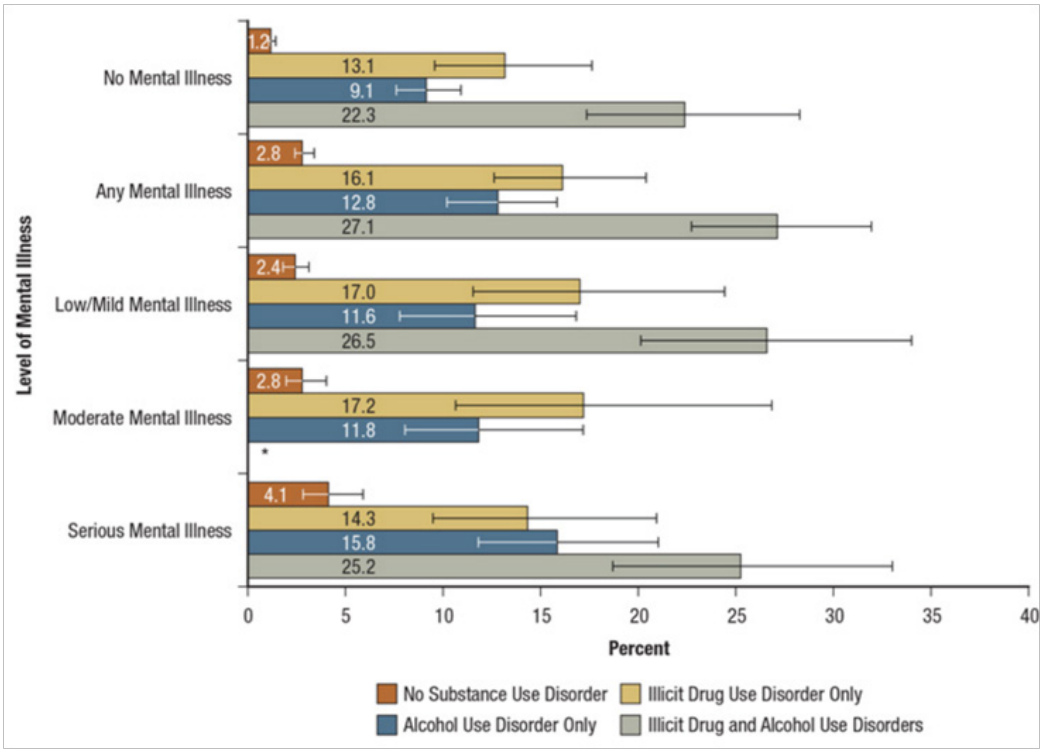

Figure 1: Prevalence of arrest in the past year among persons aged 18 or older with any mental illness, by level of mental illness and substance use disorder. **Source:** SAMHSA National Surveys on Drug Use and Health (NSDUHs)

after the end of treatment.

In forensic psychiatry cases, it is necessary to ascertain the risk of recurrence of violence. It is always important for a clinician to aid the legal system in balancing the rights of the individual to live freely in the community and the community's safety. It becomes important in cases where patients are acquitted of their charges on the grounds of an insanity plea. A risk assessment indicated that it could be dangerous to others because of any underlying brain pathology, mental illness, alcohol or drug dependence syndrome, poor family support and relationships, a prior history of poor compliance to medication, and a history of violent crimes, all of which suggested that there is a possibility of recurrence of intoxication/violence. It was opined that the patient would require treatment in a supervised setting in a long-term continuous-care home.

A person with a severe mental disorder, who commits a serious criminal offence, is sentenced to compulsory forensic psychiatric care. A majority have committed violent crimes, such as assault and arson. Of these patients, 70% have previously been in contact with mental health services. The most common diagnoses are psychotic disorders. In addition, 63% have a history of substance-use disorders[2] (Figure 1). Patients with mental disorders have a life expectancy of at least 10 years shorter than the general population.[4] The predominant cause of this shortened lifespan in severe mental disorder is cardiovascular disease and other physical disorders. Patients with severe mental disorder are at a higher risk of developing diabetes and metabolic syndrome. They live a sedentary lifestyle compared to the general population and have low levels of physical activity, all of which increase the risk of cardiovascular disease (CVD). Furthermore, patients with severe mental disorder have low maximal oxygen uptake (VO2max), which is an independent risk factor for cardiovascular disorders in the general population. Exercise, on the other hand, acts as a protector against

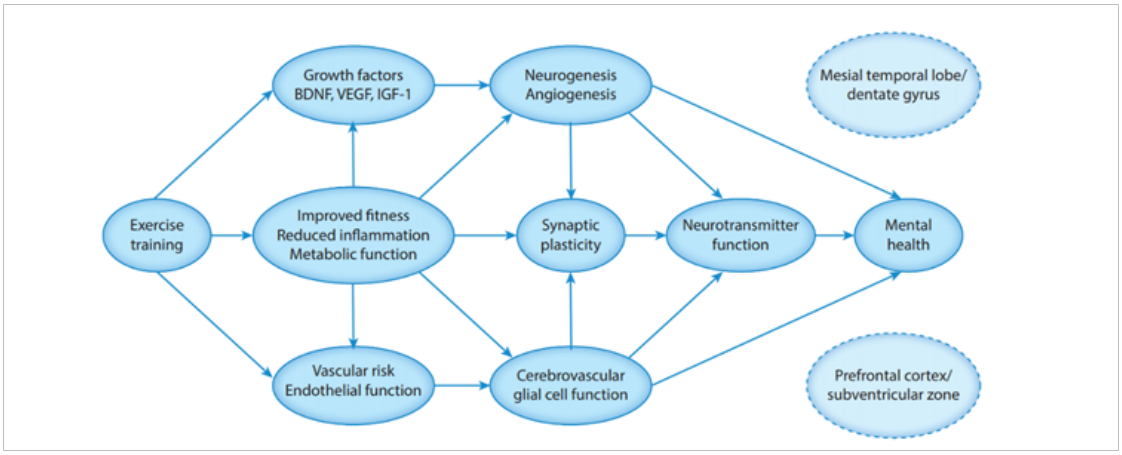

Figure 2: Mechanisms by which exercise training improves mental health outcomes. Abbreviations: BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor 1; VEGF, vascular endothelial growth factor.

cardiovascular disease.[5]

There is a dearth of research concerning effective interventions for improved physical and mental health for this client group, perhaps because security and staffing issues present significant challenges to conducting research in this environment. Also, despite the established health benefits of physical activity, the impact that physical activity may have on physical health outcomes in people diagnosed with mental illness and substance use disorder is unclear and a neglected component of previous literature reviews and interventional studies. Thus, this study is to provide an overview on the use of physical activity for the rehabilitation of forensic psychiatry patients that have been published and to sum up the current research evidence as well as future directions for incorporating the physical activity as an effective adjunct therapy in rehabilitative practice that could enhance the treatment outcomes.

Physical activity and Forensic Psychiatry: Experimental Evidence

Physical activity has been recognized as a key component of a holistic approach to recovery within mental health services, with the potential benefits ranging from a reduction in symptoms to an improvement in service engagement and utilization.[6] Physical activity is a broader concept than exercise and encompasses exercise as well as non-exercise interventions.[7] Impaired brain health in forensic psychiatric patients may have a number of different causes and result in different effects on the body, the psyche and on behaviour. Some of the obvious causal factors that are often seen in patients include early and long-lasting, extensive substance use, and a psychotic disorder with persistent negative or cognitive symptoms. The brain is affected in various ways by physical illnesses that can often be investigated and treated. The risk of developing diabetes[8], metabolic syndrome[9] and cardiovascular disease[10] is heightened with psychotic disorders. Cardiovascular disease, diabetes and metabolic syndrome are all linked to poorer cognitive functions.[11] The causes of compromised cardiometabolic health within this population are multifactorial and include low levels of physical activity and higher prevalence of smoking as well as weight gain, dyslipidaemia, and insulin resistance, particularly associated with the use of second-generation antipsychotic medication. Structured interventions for lifestyle changes, with targets such as the patient giving up smoking or losing weight, can have beneficial effects on physical and mental health.[12]

Evidence supporting the promotion of exercise within acute inpatient settings leads experts to suggest this should now form a part

of treatment.[13,14] Mutrie (2000) recommended aerobic and anaerobic exercise to alleviate depression. Biddle (2000) has shown emotion and mood is improved during effort toward the mastery of skills in PA and PA conducted in group climates. Fox (2000) says that 'global' esteem is improved as 'physical esteem' is enhanced through exercise, whilst Taylor (2000) shows that anxiety and stress is reduced by moderate exercise,[15] also illustrated how PA interventions independently aid attempts to quit smoking.[16] Positive social experiences may be gained by mental health service-users from group-based PA[17] and PA and sport generally have the potential to offer a sense of purpose and meaning to the lives of people with mental health problems.[18] Many service users understand and value the potential benefits of PA, although clear barriers to becoming more active exist for this group.[19] The benefits of PA and exercise on mental health symptoms, such as depression, anxiety and psychosis are well known. In addition to mental health benefits, exercise can attenuate antipsychotic-induced weight gain and improve cardiometabolic profiles in people with mental illness. More importantly, exercise can improve cardiorespiratory fitness, an independent predictor of all cause mortality.[20]

A patient may have difficulty in controlling feelings of aggressions, anxiety and frustration with the new restricted environment. These may lead to some form of acting-out behaviour. In these circumstances exercise can be used constructively to channel release of those feelings in an acceptable and positive way. Physically based, muscle relaxation technique proved to be effective. Four-week Tai Chi intervention reduced sensitivity and attentional bias to drug-related cues in individuals with MUD, suggesting that mind-body exercise might enhance recovery from methamphetamine use disorder (MUD) via attention control and induce similar beneficial effects on the abstinence rate, withdrawal symptoms, anxiety, and depression levels in subjects with SUD.[21,22]

Physical Activity and Forensic Psychiatry: Explanatory Mechanisms

Several researches linking exercise to mental health suggests that exercise training is beneficial for a broad array of mental health outcomes, although the strength of treatment benefit appears to vary across populations and training modalities. The exercise training likely improves mental health through synergistic influences of both neurobiological and behavioral learning mechanisms. Neuroplasticity is increasingly characterized as a central mechanistic component of mental health improvements and is highly influenced by PA. Within this framework,[23] training improves neurobiological systems critical for adaptive learning, as well as affective and cognitive control processes, resulting in synergistic improvements in the regulation of both cognitive and affective responses through a "virtuous circle" of reinforcement[24] (Figure 2).

Low oxygen uptake ability is an independent risk factor for cardiovascular disease and premature death. Low oxygen uptake ability has been demonstrated in patients with a psychotic disorder and in patients in forensic psychiatric care. Aerobic exercise offers one possibility for improving patients' general health and their cognitive functions possibly via activation of neurotrophic factors, such as BDNF (brain-derived neurotrophic factor), and brain repair although the mechanism is not fully understood. In patients with schizophrenia, aerobic exercise has positive effects on psychotic symptoms, cognitive function, general functional outcomes and quality of life. Aerobic exercise can also be expected to reduce the incidence of metabolic syndrome in forensic psychiatric patients and thereby reduce cardiovascular morbidity, diabetes and premature death.[25]

Regular exercise can help to reduce weight, reduce blood pressure, and improve lipid disorders, including raising HDL (high-density lipoprotein) and lowering triglycerides.[26] Among the physiological systems that respond favorably to physical activity, it has been argued that one source of insulin-mediated glucose

uptake and fatty acid oxidation. The exposure to exercise evokes adaptation in skeletal muscle in a multitude of signaling pathways, the functional response to which is determined by training volume, mode of training, intensity and frequency. With persistent exercise exposure, there is mitochondrial biogenesis, fast-to-slow fiber-type transformation, changes in substrate metabolism, and angiogenesis. Moreover, a host of myokines are released from active muscles providing communication throughout the body. Enhanced fitness is associated with high levels of insulin sensitivity/insulin action. While glucose homeostasis at rest is insulin-sensitive, exercise with muscle contractions increases glucose uptake from the circulation that is not reliant on insulin. Indeed, GLUT-4 (Glucose transporter type 4) is responsive to both insulin and muscle contraction independently. The increased glucose disposal associated with resistance exercise was the result of the increase in the quantity of lean body mass, without altering the intrinsic capacity of the muscle to respond to insulin.[28] On the other hand, endurance training enhanced glucose disposal independent of changes in lean body mass or VO2max, suggestive of an intrinsic change in the ability of the muscle to metabolize glucose. Moreover, abdominal fat and fat-derived mesenchymal stem cells are responsive to physical activity; both high-intensity aerobic and resistance training decrease visceral fat effectively while the molecular expression of fat-derived mesenchymal stem cells is significantly altered with exercise preventing adipogenesis.[29]

There is evidence suggesting that PA increases peripheral insulin growth factor 1 (IGF-1) levels and elevated serum IGF-1 levels are associated with improved cognitive performance. It is therefore likely that IGF-1 plays a role in PA induced improvement of cognition. Other neuropeptides such as neuropeptide Y (NPY), ghrelin, galanin, and vasoactive intestinal peptide (VIP) could mediate the beneficial effects of PA on cognition.[30]

Studies showed that physical exercise can regulate the gene transcription of endogenous opium brain-derived neurotrophic factor (BDNF) by activating the cyclic AMP response element-binding (CREB) protein and synaptic plasticity, which is critical for rehabilitation for patients with substance use disorders (SUD) via promoting repair of drug-induced neuronal damage and improving corresponding brain functions. This neuronal structural change induced by exercises might contribute a long-lasting effect on SUD.[31]

A multitude of studies have been conducted showing a relationship between physical activity and overall well-being. It has been repeatedly shown that an inverse relationship exists between physical activity and the occurrence of CVDs (i.e., with increased physical activity, the relative risk of developing CVD is decreased. With regard to specific surrogate markers and biological factors pertaining to CVD risk factors (e.g., high BP, and increased cholesterol and triglyceride concentrations), clinical and laboratory evaluations have been performed to show the benefits of physical activity. Such quantitative measurements were performed to determine the influence of exercise on blood coagulation and fibrinolysis, vascular remodeling, BP and blood lipid profiles.[32]

CONCLUSION

The review concludes that physio-therapeutic interventions, including individually adapted physical activity and exercise, if monitored and incorporated into forensic psychiatric care, could improve the patients' physical status and thereby lower the risk of cardiovascular disease. Importantly, it could ameliorate psychiatric symptoms and improve overall cognitive function making it an interesting and promising treatment option in forensic psychiatric care. From a medical-psychiatric perspective, the patient should be investigated and treated for physical illnesses with particular focus on metabolic syndrome and other risk factors for cardiovascular disease and diabetes. IJFMP

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REVIEW ARTICLE

Smoking: A Toxic Influence in Young Adult Population: A Retrospective Study

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ABSTRACT

There are about 1 billion smokers in the world, with 80 percent of them living in poor countries. Tobacco use causes lung cancer, COPD, atherosclerosis, Peptic ulcer illness, intrauterine growth retardation, spontaneous miscarriage, antepartum hemorrhage, female infertility, male sexual dysfunction, and a variety of other disorders are among them. Factors leads to smoking initiation are Parental smoking cessation, Low socioeconomic status (SES), Peer and family influence, Access to tobacco, Depression and mental health conditions, Genetics, Unemployment, Fashion/Up gradation of status and Influence by marketing. This study aimed to review the toxic influence of smoking in young population. This review was conducted through exhaustive researches in different databases. This article concluded that school pupils, parents, instructors, and the general public should all be educated about the harmful effects of smoking. Therapeutic approaches for smoking cessation are quite important. Along with more well-known approaches including cognitive behavioral therapy, social support, medications, and nicotine replacement therapy, e-cigarettes (ECs) have emerged as a viable and distinctive aid in smoking cessation therapies. This study discusses the impact of smoking in today's society.

KEY MESSAGES: The study discusses the impact of smoking and its influence in young adult population. Additionally it also portrays the factors causing the deviant behaviour among youth.

KEYWORDS | smoking, young population, anxiety, nicotine

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INTRODUCTION

INDIA HAS THE WORLD'S SECOND LARGEST TOBACCO user population. Tobacco usage among Indian youngsters is fairly high: 14.6 percent of those aged 13–15 years used it in 2009, and 12.4 per cent of those aged 15–24 years used it in 2016–2017. Tobacco is utilized in a variety of ways.[1] There are around 1 billion smokers in the globe, with 80 per cent of them living in poor nations. Tobacco use causes lung cancer, COPD, atherosclerosis, peptic ulcer illness, intrauterine growth retardation, spontaneous miscarriage, antepartum hemorrhage, female infertility, male sexual dysfunction, and a variety of other disorders are among them.[2] Adolescents in this age group are more likely to have a number of risk factors linked to early onset and persistent cigarette smoking, such as mental problems and other substance abuse. In the general population, teenage cigarette smoking has been linked to mood, anxiety, and trauma-related problems.[3]

The immune system is harmed by cigarette smoking in a variety of ways, including decreased white blood cell function

(leucocytosis), decreased immunoglobulin levels in the blood, decreased antibody response to different antigens, reduced T-cell response, and proliferation to mitogens, reduced lymphoid tissue mass and cellularity, suppression of T-lymphoblasts in the cell cycle, and impairing immunity.[4] Tobacco smoking is well recognised to raise the chance of developing a number of illnesses and health problems, such as Cancers of the lung, bladder, colon, esophageal, kidney, larynx, mouth, and throat, as well as respiratory infections, diabetes, and coronary heart disease. The number of packs of cigarettes smoked increases the risk of hair loss, skin wrinkling, and erectile dysfunction, as well as harmful effects on microvasculature.[5]

Furthermore, young people are more vulnerable to peer pressure, and identity formation is a key problem for them. Tobacco smoking is predicted to grow increasingly common among university students, owing to the perceived reduction of stress, life difficulties, peer pressure, social acceptability, smoking class history, parents' lower educational levels, and the desire to rise in society class. Nearly one-third of the world's population, aged 15 and above, smokes, and the incidence of smoking is rising, especially in developing countries. Smoking is being started by a large number of young people at an early age, which is a serious public health problem.[2]

Factors Contribute to Smoking Initiation

Parental Smoking Cessation

Smoking by parents exposes children to harmful substances through second- and third-hand smoke, therefore quitting is especially important for them. Child healthcare practitioners may use behavioral stage-based treatments that generally match the Trans theoretical model of change to assist parents quit smoking. According to this concept, persons who want to stop smoking go through several phases of motivation, including precontemplation, contemplation, preparation, action, and maintenance.[6]

Low Socioeconomic Status

Low socioeconomic status is linked to high rates of cigarette smoking. Young adults who have not attended or are enrolled in college smoke twice as much (30%) as their college-educated peers (14 percent).[7]

Peer and familial pressure, and lack of Parental Support

Teenagers who have cigarette-smoking parents and classmates are more likely to follow in their footsteps. Teenagers will be encouraged to follow the media's lead if they see adolescent idol figures smoking cigarettes. Adolescents' smoking habits might have a negative impact on their academic performance. The availability of teenage pocket money has an impact on smoking behavior; for example, if a person has a lot of pocket money, he or she is more likely to smoke.[8]

Access to Tobacco

Nearly half (44 percent) of young smokers (15 to 18 years old) obtain cigarettes for free from family, friends, or other individuals. It's worth noting that a significant proportion of people in this age range (16%) say they receive their smokes from "other" sources, such as friends or illicit providers.[5]

Genetics

"When societal sanctions against smoking are eliminated and social temptations to smoke arise, genetic impacts on smoking should increase". [9]

Unemployment

The impact of unemployment on smoking might be positive or negative. On the one hand, unemployed people may smoke more as a supplement to their leisure activities (leisure effect). Unemployed people would also be free of the limits imposed by workplace smoking bans.[10]

Fashion/upgradation of status

Adolescence and young people nowadays are more prone to engaging in these types of behaviors. Marketing has a greater impact on children than it does on adults, and if their friends or family use tobacco, they are more likely to try it. It was also shown that the

majority of the subjects consumed merely for the sake of having pleasure.[11]

Influence by marketing

According to Qureshi A *et al.,* 70.4 percent of students consumed due to a pleasant taste, while 17.7% were influenced by advertisements. Advertisements for various tobacco products can be seen in a variety of media, including print, television, and roadside billboards and banners. "Tobacco advertising and promotion successfully target young people by portraying smokers as fashionable, athletic, and successful. Cigarette smoking is frequently shown as a daily practice by characters in movies and television shows. They will even show you how to light a cigarette using various methods. These situations frequently entice the adolescent's sensitive mind to try similar techniques or adopt comparable behavior."[11]

Smoking by Teenagers and their Effects

Nicotine Dependence

Nicotine is a highly addictive substance, and young individuals are more likely than adults to get addicted. Nicotine dependence is a key element in predicting which people become habitual smokers following a period of experimenting. Adults had more severe withdrawal symptoms than adolescents in some studies, whereas adolescents have less severe withdrawal symptoms in others.[5]

Nicotine's Impact on the Adolescent Brain

Nicotine causes long-term changes in neural connectivity in several brain areas, including the nucleus acumens, the medial prefrontal cortex, and the amygdala, all of which are involved in emotion regulation, according to several studies on the effects of early smoking on the developing teenage brain. Chronic nicotine smoking throughout adolescence has also been related to brain epigenetic changes that make it more susceptible to other substances and raise the likelihood of future substance abuse.[5]

Strategies for Smoking Cessation

Individual Counseling

According to a recent Cochrane study on smoking cessation in teenagers, individual counseling, motivational enhancement, and cognitive behavioral therapy are the therapies with the most evidence to support them . The '5 A's' method is the most often used framework (Ask-Advice-Assess-Assist-Arrange). It should just take 3 to 5 minutes to complete and can be used to guide a quick counseling session.[12]

Pharmacotherapy

Nicotine substitution treatment (NRT), bupropion, and varenicline are among the principal line pharmacological medicines for grown-ups. The frequently suggested items are nicotine gums and transdermal patches, with capsules and nasal showers limping along. Mouth and skin disturbances, quicker pulses, and worse hypertension readings are the most regularly revealed unfriendly impacts among young people.[12]

Mind-body Interventions

In the adult literature, mind-body treatments such as mindfulness, yoga, hypnosis, and biofeedback have been regarded as promising.[12]

E-cigarettes

E-cigarettes are battery-fueled nicotine conveyance frameworks that give nicotine while mimicking the tangible engine results of smoking (inward breath and hand movements) without the utilization of tobacco.[13]

School-based Cessation Programs

Because a diverse group of students from various socioeconomic situations attend school for a substantial portion of the day during the age range when most individuals begin smoking, the school environment has been largely regarded as an appropriate location in which to intervene with adolescents. All children are affected by school-based interventions, regardless of their smoking status (vulnerable never smokers and current smokers. Students exposed to the preventive program were considerably less likely to be vulnerable to future smoking, according to a review of this school-based tobacco prevention

program in India. Tobacco control policies, in addition to tobacco prevention initiatives, may be implemented by schools to limit tobacco use on school grounds.[14]

CONCLUSION

In this article, we look at the toxic effects of smoking on the young adult population. Educational achievement is a notable financial indicator of well-being, and our discoveries on family smoking in adolescence and people's educational achievement as youthful grown-ups are in accordance with past research. Furthermore, studies have shown that high school students with poorer academic achievement engage in considerably more health-related risk behaviors.[15] The processes relating to teenage smoking and poor educational performance are bi-directional and negative in nature.[16]

The current concentrate additionally explained the connection between youth home smoking status and current smoking status among youthful grown-up members, showing that current smoking was more predominant among people from families where smokers resided. This finding is in accordance with past research showing a solid connection between guardians' smoking and their kids' momentum smoking [17,18,19] just as Szabo *et al.,* [2006] discoveries that smoking boycotts at home diminished the probability of teenagers exploring different avenues regarding smoking. Furthermore, an earlier report that took a gander at the causal connection between instructive fulfillment and smoking status found that having more schooling prompted a lower shot at beginning to smoke, a lower measure of smoking, and a higher probability of stopping smoking among smokers.[20]

As per Tabuchi *et al.,* (2017) the level of association between poor educational accomplishment and smoking was more prominent in more youths than in established ones.[21] High educational accomplishment can decrease smoking status coherence among young people across ages.

Efforts made to be made to debilitate more youthful age not to start these propensities and to perceive their potential wellbeing perils. The mindfulness projects ought to be intended to instruct younger students, guardians, instructors and overall population to debilitate smoking propensities. Foster preventive systems to lessen tobacco utilization. Preventive methodologies particularly engaged towards youngsters and youths should be started on emanant premise. Utilizing the '5A's' strategy gives a functional system to distinguishing and helping teenagers who smoke. Significant holes in the exploration writing remain, nonetheless, and there are many inquiries still to reply around smoking discontinuance in youth. There is a critical need to make successful strides, particularly on dispatching local area mindfulness programs for the younger students and public to instruct them about the outcomes of tobacco use, and on evaluating their viability in checking the issue. Absence of mindfulness among individuals having a place with poor financial layers of the general public, cultural impact, and helpless execution of against smoking laws could be the potential purposes behind its far reaching occurrence. Subsequently, more thorough enemy of smoking efforts and far reaching execution of against smoking guidelines are the need of great importance. Smoking end remedial mediations are critical. With more settled techniques, like intellectual conduct treatment, social help, drugs, and nicotine substitution treatment, e-cigarettes (ECs) have arisen as a potential and novel guide in smoking discontinuance mediations. IJFMP

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REVIEW ARTICLE

DNA Technology as Forensic Tool in Advancing Justice

[1]Lovepreet Kaur, [2]Shivani Guleria Sharma

ABSTRACT

Forensic biology is an application which involves a union of people to firstly suspect the crime location, artifact's and person on basis of the data provided by the victim. The provided data is analyzed by various investigations to solve criminal and civil cases. Various techniques are implemented that can be used in forensic science such as ABO blood grouping, Variable number tandem repeats, polymerase chain reaction, short tandem repeat and single cell analysis. These techniques play a significant role to completely study the samples. The entire process of the DNA analysis is divided into 4 major parts. Nanotechnology is another major field of biotechnology is currently under study for the developments of further more techniques that can be utilized in on the spot detection of sample in no time. Distinct portable devices are already developed to carry out detection and few are under study. In the forthcoming times, the process of investigation might be at rapid rate which will prevent contamination as well as mishandling of samples. The main requirement is of bioinformatics tools in laboratories in order to handle vast samples.

KEY MESSAGES: Investigation is key step of criminal cases and DNA technology will make this step rapid and error free. Thus, Development of authentic, portable and user friendly molecular techniques is necessity of present era for fast and accurate process of justice.

KEYWORDS | DNA fingerprinting, RFLP, nanotechnology, genetics

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INTRODUCTION

THE PROCESS OF ANALYTICS STUDY, SEQUENCING, as well as cutting and pasting of DNA with incorporation of various techniques forms the backbone of the DNA technology. Techniques such as DNA sequencing, polymerase chain reaction followed by cloning and further processing via electrophoresis plays a significant role in various experimentations of the DNA. A vast number of techniques are developed that include DNA fingerprinting, gene therapy, DNA microarray and gene therapy (Thanakiatkrai *et al.,* 2013). Each technique has its own important role in science research. DNA comprises of genetic instructions in the form of molecules of nucleotides in all living organisms. It is organized into chromosomes in a very structural manner. DNA techniques are helpful in tracking the ancestors of species, to determine the culprit in case of forensics and to find out the paternity of a child. Likewise, various pathogens can be identified through the biological remains recovered from the archaeological sites which can be helpful in proper identification of the remains (Ian *et al.,* 2014). Thus, these techniques are very helpful in proper analysis of the forensic laboratory samples.

Forensic science is a branch of science that conducts investigations from the evidences collected at the site of incident, from suspects

and victim. It involves the use of scientific methods in which the samples collected are analyzed using different DNA techniques (Kashyap *et al.,* 2004). The results obtained are then represented in the court to punish the culprit. Similarly, the inferences are utilized to acquire knowledge about the dead remains in case of the archaeological studies. In order to resolve legal matters various evidences are collected, studied, experimented and then interpreted to create a link between them so that an investigatory lead can be provided. Different fields such as toxicology, engineering, computing and pathology form the term forensics (Lowe, A. *et al.,*. 2003). Earlier, the ABO blood grouping method was used for the detection purposes, but this approach was not successful. In 1981, DNA (deoxyribonucleic acid) was used for the testing in criminal justice. It has become a vital part of the forensic science to carry out investigations over different samples. The process of DNA technology was initially used in 1985 for the study of casework in United Kingdom. Later on, in 1988 the Federal Bureau of Investigation (FBI) started using forensic analysis to investigate crimes. During the investigation process the genetic material is the epicentre (MacKnight, 2004). With the advancements in technology the repeated sequences came into practice in 1985, under which restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR). Following it, the technique of polymerase chain reaction discovered which help in amplifying the segments of DNA in less time, this approach provide a great benefit in case of low quantity and quality of samples. The research on DNA techniques to be utilized for forensic studies was on peak for a decade. From 1985-1995, distinct approaches were introduced such as microsatellite and simple sequence repeats (SSRs) in which short sequences of 2-6 base pairs long was taken as target sequence (Shanly *et al.,*. 2018). In 1990, this technique was successfully used in forensic DNA analysis for the investigation purpose as they represent those alleles which are distinguishable from each other, in case of evidence loci is stable and even small amount of sample can be used as short length of fragments is required. Later on, low copy number technique developed to deal with sensitive samples and single nucleotide polymorphism is used extensively to solve rape cases (Murray, C *et al.,*. 2001).

In order of on spot detection the need of time demands the development of investigation tools such as Sci-Fi which is a hand-held device. Sci-Fi comprises of a chip which is enough to test the samples at the crime scene in order to generate their sequences of the DNA, it is one of the kind of lab on the chip (Gill, 2001). For this purpose nanotechnology serves a great role as it has already been used in detection of illicit drugs. The nanoparticles have been used to develop a smart system for the detection of codeine sulphate, the citrate-stabilized gold nanoparticles (AuNPs) was used as a probe. Additionally, with aid of micro fabrication of capillary electrophoresis development of a single integrated platform is in process. Single integrated platforms will safe time as the extraction; amplification and sequencing of the DNA will be carried out at one space (Hariprashad *et al.,* 2021). The use of three dimensional computer automated techniques for morphological analysis of skull has already been implemented in forensic biology. Under the next generation technologies of the DNA fingerprinting, the determination of the color of skin, hair and eyes with help of the various techniques of gene sequencing is the main focus.

Steps in DNA Fingerprinting

The process of DNA fingerprinting involves the collection of sample, analysis; examination or experimentations and then observations of sample are done. The genetic material can be in the form of hair, skin and blood collected from the suspects, victims and crime scene. The collected material is then analyzed by DNA techniques such as RFLP, VNTR, STR, SNP (single –nucleotide polymorphism), Low copy number and Y- chromosome analysis (Manteen

et al., 2020). Following analysis, the results of obtained gene sequence or fingerprints are compared to the DNA databases. These databases comprises of a large number of profiles worldwide or area wise maintained they serve as a great purpose for the depiction of the diseases related to genes and genealogy purposes. Scientifically, the three major parts namely, serology, DNA technology and genetics form the backbone of testing of samples using various techniques (Bruce R. *et al.,* 2019).

Serology – It is the main step in which the investigators report the crime site registered by police administration. They collect samples such as semen, hair, skin patches, blood or saliva from the site. Additionally, photography is done of the entire location (Alves *et al.,* 2007). It is important to collect and mark the sample properly to prevent any cross- contamination. Then, the sample is carried out to laboratories for further investigations.

DNA technology – The extraction of the DNA is done from the sample collected from both the victim and the suspect. Following it the quantification of the sample is done using spectrophotometer. Then the amplification of desired bands is done with help of polymerase chain reaction performed under controlled conditions. After amplification with help of the STR markers the regions are located and the final analysis between the two sequences is done (Bhatt *et al.,* 2020). For making out comparisons with databases, the human and non-human DNA are separated following it genome sequences are compared.

Genetics – The final report is generated after statistical interpretations obtained from the set of data collected from the crime scene. From the date of crime registered to the final test reporting date, every minute detail is mentioned in the report presented in the court room (Pandya *et al.,* 2018). The information in a report consists of the photographs from the crime scene taken as evidence, list of samples collected as evidence, reason why the a specific person is taken as suspect and analysis of the DNA genome sequence that shows the matching of the two sequences giving clear information about the accused.

Various Techniques used in Solving Criminal Cases

After 1985, the advancements in DNA profiling increased extensively, the modern techniques of the forensic science evoke from the first application taken from the work of Alec Jeffrey's. In 1985 while working on the myoglobin gene, Sir Alec Jeffrey from the University of Leicester U.K. introduced with the new modern technique of the DNA fingerprinting that can be used for solving various cases in a short time period (Rahiman *et al.,* 2010). With advancements in technology initiating from the exploration in which the utilization of the restriction fragment length polymorphism was done to reaching the use of STR kits a lot of techniques came into practise (Shukla *et al.,* 2016). Each technique has its own specific role such as polymerase chain reaction helps in amplification of small amount of DNA to billions of copies. Likewise, detection of diseases which are specific for a locus with help of RFLP. (Table 1).

Role of DNA Technology in Forensic Science

- *Generation of DNA Data Banks* – In forensic science various techniques are used on daily basis and numbers of genome sequences is generated from the samples collected. These genome sequences forms a library or database of sequences which can looked later on for sample comparisons. Each sequence plays a significant role due to its individuality in nature (Angers, A. *et al.,* 2019). In order, to preserve the generated sequences the data banks are generated by the FBI.
- *Determination of Drug* – Examination of the toxic substances isolated from the critical samples such as hair, blood, saliva and fingerprints collected from the crime scene can be done by nanotechnology. For instance, the quantification of cocaine has already been done with help of nanoparticle titanium. The psychotropic substances given to the victim can also be

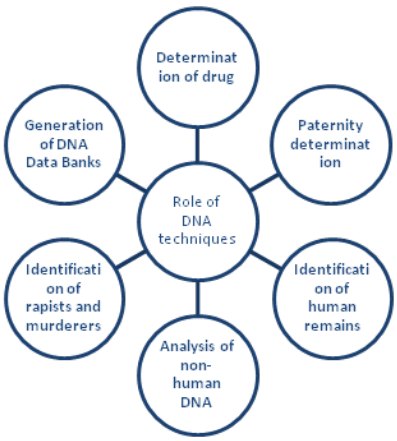


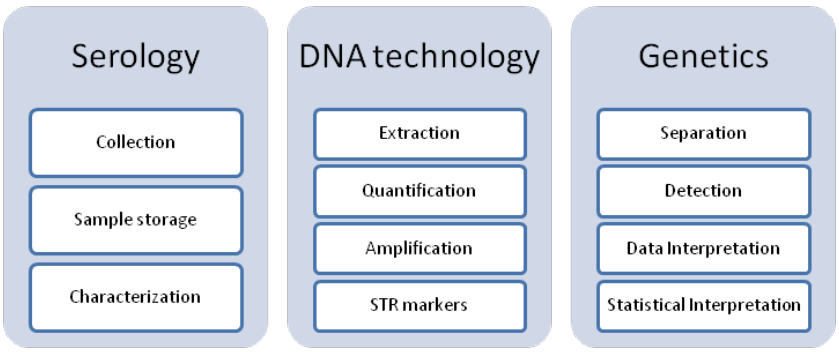
Figure 1: Role of dna techniques in forensic science

S.NO.	TECHNIQUE	ADVANTAGES	REFERENCES
1.	RFLP	Detection and diagnosis of diseases, specific for locus thus helping in detecting the gene responsible for a particular disease.	Thotakura et al.,, 2016
2.	PCR	Replication of specific nucleotide sequences from low levels of DNA or degraded DNA, Require small sample for analysis.	Chauhan et al.,, 2017
3.	STR	Use of separate chromosomes in STR markers makes the technique more simple and unique thus preventing any problem related to linkage between the markers.	Yang et al.,, 2014
4.	LCN analysis	The chances of the error are more as compared to other techniques.	Chaudhary & Jyoti 2017
6.	Y- Chromosome analysis.	Helpful in forensic analysis in case of sexual assault cases.	Alonso et al., 2004
7.	Single Cell DNA Fingerprinting	The DNA of the sperm cell is highly conserved and compacted in a protein head thus the technique of single cell DNA fingerprinting is effectively implemented in solving rape cases.	Pandya et al., 2018
8.	VNTR	To study genetic diversity and breeding patterns in population.	Shukla et al., 2016
9.	mtDNA analysis	In cases of highly damaged sample either due to burn samples or hair without root from which DNA is to be isolated the technique of mitochondrial DNA analysis is used.	Hariprashad et al., 2021

Table 1: Different molecular technoques and their applications in forensic science

analyzed with nanoparticle. Additionally, nanoparticles in detection save time and gives effective results (Bhushan *et al.,* 2015). Nano sensor has already been developed to detect the drug clonazepam from the samples of the blood and skeletal with help of the melamine modified nanoparticles of gold.

- *Paternity Determination* – To solve the paternity dispute cases of various offspring's, the technique of the DNA analysis plays a crucial role. In case of completely burnt individuals, the bones can be used to identify the individual from bone samples (Budowle *et al.,* 2009). Various cases have already been solved in paternity dispute, a man doubted that out of two daughters he is not the biological father of second one, he filed a case on his wife for cheating. Later on, the DNA fingerprinting was carried out and results in court room declared that the fingerprinting shows completely matching of father to both the daughter, thus the officials proved him his doubt as wrong (Narajo & Avais 2012). This is how the paternity cases are effectively solved via DNA technology and this method is widely accepted on legal basis.
- Analysis of non-human DNA – In cases bodies are recovered from damp forest area and their conditions is terrible which makes it difficult to analyze. It is advised to firstly analyze whether the isolated sample from such conditions contains a human DNA or non- human DNA. A forkhead box (FOXP2) can be used to determine the type of DNA. In addition to this; short tandem repeat can easily determine the presence of non-human DNA (Asplen *et al.,* 2004) (Fig. 1).
- Identification of rapists and murderers – In cases of sexual assault, the samples are collected by expertise from the victim either through vaginal swab or through semen found at the site of the crime. Semen, blood, hair or nail sample is taken from the suspect (Ballantyne Jack *et al.,* 1991). Additionally, the separation of cells is done by geneticist so that sperm cells can be separated from women cells collected via vaginal swabs. Moving further, the DNA is determined



and fingerprints are matched with that of the suspect's to declare the real culprit. For instance, on July 6, 2017, a sixteen year old girl's body was found in a dense forest. The case was taken over by Central Bureau of Investigation. They reported that numerous marks were found over her body, she has been gang-raped and murdered. To find out the culprit, approximately 200 blood samples were taken from nearby areas and various items like liquor bottle and clothes collected from crime scene (Butler, John M. 2007). Initially, the team didn't get any lead then they did percentage and lineage test and luckily a match was found with a family from Kangra. Then, samples were collected from family and a boy named Anil was turned out to be the main culprit.

Nanotechnology in DNA analysis

The use of nanoparticles in the field of the research and development give rise to the term nanotechnology. Nanoparticle of titanium has already been widely used for the detection of drug cocaine which is illegal to consume and supply. They are also used for determination of various narcotic drugs given to the victims in case of murders, kidnapping and sexual assault. Nanoparticles can help to detect the drugs very effectively due to their high mobility, specificity and stability (Pandya *et al.,* 2018). For instance, gold nanoparticles (AuNPs) are used to increase the specificity of the polymerase chain reaction. In addition to the gold nanoparticles, the carbon nanotubes and the silver nanoparticles are also used to enhance the specificity of the PCR (Alonso *et al.,* 2004). Various other uses of nanoparticles in forensic analysis is discussed as follows –

- Creation of biosensors using nanoparticles in order to perform on the spot analysis to save time.
- Death time estimation with help of fluorescent nanoparticle which can determine the level of amino acid (Bruce R. *et al.,*. 2019).
- Detection of codeine sulphate drug with help of citrate-stabilized gold nanoparticle smart camera and phone, this provide quick and qualitative results.
- Thin films analysis to be used for the biomedical purposes, it provides in depth analysis of the various samples collected from the crime scene (Rahiman *et al.,* 2010).
- In order to generate fluorescent in nature Nano-composites are used in combination to the hybrid calcium sulphide quantum dots, this fluorescent helps in the better analysis of the fingerprints of the DNA present on a surface and hence nanoparticles can be used to detect surface DNA prints. (Gill, P. 2001). IJFMP

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REVIEW ARTICLE

Entomotoxicological Studies: A Review Report

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ABSTRACT

Toxicology and forensic entomology are mixed to determine the minimum postmortem time (PMI-min) and circumstances of death in cases where toxins and hazardous substances are suspected causes of death. Forensic entomotoxicology proves the existence of toxicants in insects feeding on corpses, but it also investigates their impact on insects' bio-morphometry and growth rate. Isolation of larvae and pupae of real flies (Dipteran) and/or adult forms of, for example, beetles (Coleopteran) located on or around the corpse can provide information about harmful compounds potentially present in the body. This review aims to examine current knowledge in the subject of entomotoxicology, including cases from the research, and to demonstrate the effects of various toxic compounds and medications on the growth of insect larvae.

KEY MESSAGES: Insects are significant in forensics because their larva feeds on dead bodies. Insects (larvae) are also a suitable toxicological sample because they are present in high concentrations on the cadaver and the puparia case remains unchanged and unaffected for an extended period.

KEYWORDS | X-ray entomotoxicology, post-mortem time, insects

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INTRODUCTION

Forensic entemology is a discipline under forensic science concerned with the investigation of numerous insects and arthropods found on human and animal cadavers. It is the application of entomology in the case of legal investigations, to solve a crime.[1] Insects are involved in the decomposition process of a cadaver and can be an important aid in evaluating the various phases of decomposition. Because of the known history of insects, their developmental stages, and the habitats in which species live, the investigation of insects to determine criminality is helpful. These factors aid in the identification of the scene of the crime, as well as for determining the possible cause of the crime and identifying postmortem and antemortem injuries.[1] The most typical kind of civil lawsuit using forensic entomology is those involving bug infestations in metropolitan areas or pests of stored commodities. The majority of these insects are located in man-made constructions. In medico-legal instances, insect inspections are conducted to determine the period since the death. The medicolegal investigation includes determining if the crime was homicide, suicide, or an accident (Merritt and Higgins, 2000). Since the 1990s, forensic entomology has been employed to solve crimes (Jamieson *et al.,* 2009). A forensic entomologist aids in the assessment of the postmortem interval, which is defined as the time between an individual's death and the body's recovery.[6] The post mortem interval can be calculated by estimating the pattern of insect succession on corpses and calculating the developmental period for each stage of an insect's life cycle. Fresh, bloated, decay, advanced decay, and dry stages of decomposition are observed as per insect activity and are useful in predicting the post mortem interval, based

on the pattern of insect succession.[6] The fresh stage ends with the bloated stage, which involves the degradation and metabolism of proteins, fats, and carbs. This stage is expected to last between 0 and 3 days. Adult blowflies, flesh flies, muscid flies, and yellow jackets were the most frequent insects found in this stage. Putrefaction happens in the bloated stage which leads to the evolution of gases and the swelling of the corpses. This time lasts between 4 and 7 days. Adult and larval blowflies, flesh flies, muscid flies, rove beetles, hister beetles, and yellow jackets are the most frequent insects found. The body deflates and ruptures during the decomposition stage, releasing unpleasant fumes. This step is expected to take 8-18 days.[6] Adult and larval blowflies, rove beetles, carrion beetles, and cockroaches are among the insects discovered. The dominant group of insect families present during different days of decomposition was as follows: early succession by insects of dipterans family Calliphoridae, sacrophagidae and Muscidae families, and late succession is done by insects of histeridae, Cleridae, Dermestidae, Scarabaeidae, and Staphylinidae families. The succession patterns and the presence of the insect families were vary in different regions. The skin becomes dry and just cartilage and bones remain in the advanced deterioration stage.[7] This stage lasts between 19 and 30 days. Dermestid, hister beetles, mites, and other insects are commonly found at this stage. Only the bones, cartilage, and hair remain in the dry stage. This stage lasts for more than 30 days. Dermestid beetles, soldier bugs, and ground beetles are some of the insects discovered at this stage.[7] The blowflies are the first to colonize the corpses, laying thousands of eggs in the natural orifices and around the cadaver's wounds. Flesh flies leave tiny larvae in the same area. The flies' larvae go through three stages of development. The size of a larva upswings after each instar due to feeding on the cadaver mass, and by the third and final instar, the larva assumes ten times larger size than the first, indicating the start of the dry stage of decomposition.[10] After the third instar, the larvae metamorphose away from the decomposing site and enter the pre-pupal stage. During the prepupal stage, the larva immobilizes and darkens, and the pupa begins to develop within the hard exoskeleton covering. The pupa goes through metamorphosis and becomes an adult fly before emerging like a flying adult beginning its new cycle (Fig. 1). Many factors influence insect growth and colonization, including temperature, weather conditions, food exposure, burial, photo duration, and so on.[10] Insects are cold-blooded animals, which means their temperature is affected by the temperature of their surroundings. The temperature of the environment should be ideal for both the insects' growth and their development. The optimal temperature is closely related to insect development. Maximum insect development can be observed at optimum temperature, because if the temperature is either too hot or too cold, the rate of development of the insects becomes slow, and the rate of development cannot be observed below the optimum temperature range. Insect developmental rates differ from species to species and geographical location to location. The rate of metabolism of insects increases as the temperature rises, and thus the rate of decomposition rises with each successive stage of insect development.[11] For a more accurate estimation of the post mortem interval, forensic entomologists focus on determining the accumulated degree hours of different species, which was unique to each species.[8] The accumulated degree hour (ADH) is defined as the amount of thermal energy required by the insect to progress from one stage of its life cycle to the next. It can be calculated as the product of time and the difference between the average and threshold temperatures. A hot summer day will have more ADH than a cool autumn day. The post mortem interval index can be calculated in two ways: the first is to determine the age of the fly by calculating the growth stage of each stage of the insect's life cycle.[8] The ADH can be calculated and compared using the same method under

temperature-controlled laboratory conditions. The second method is to identify the pattern of insect succession at each stage of decomposition. The arrival of various insect groups can be determined by the body's natural decomposition process. During the first stage of decomposition, flies arrive, lay eggs, and the larvae develop. After that, many types of beetles arrive, feeding on eggs, maggots, and soft tissues of the carcasses. After that, omnivore insects such as ants and wasps arrive on the body to feed on other insects or vegetation, and lastly, indigenous insects such as spiders come. Rain, darkness, varying temperatures, and delayed oviposition are among the elements that influence post mortem interval estimation.[9] Many circumstances, including burial below, submersion underwater, wrapping or enclosure in a freezer, building, or closed vehicle, have been blamed for the delayed oviposition and colonization of insects. Seasonal and habitat interest were used to estimate the season of death and a second location of the remains (Dekeirsschieter et.al, 2011). While Forensic Entomology is concerned with the investigation of numerous insects and arthropods found on human and animal cadavers, Entomotoxicology refers to the application of entomology to the detection and estimation of drugs or toxins. The field of entomotoxicology is separated into two subfields: entomological analysis of compounds that alter insect developmental rates and the post mortem interval index. The second is a toxicological examination of medicines, toxins, or drugs in the body of insects3. Because of the bioaccumulation of medications inside the insects, the concentration of drugs will be higher inside the insect's body than in the surrounding areas. Benzodiazepines, antidepressants, amphetamines, cocaine, organophosphate, barbiturates, heavy metals, and other drugs or toxins are some of the commonly detected toxins in entomotoxicological cases. Insect samples are usually gathered from natural orifices, wounds, under the body, and wrapping materials, among other places. The toxins are examined from the body of the insects from the adult, larvae, pupa, and skin of the insects discovered on or around the cadavers[3] Several studies have been done in forensic entomotoxicology such as quantification of Phenobarbital in blowfly larvae and its detection by gas chromatography (Beyer.et.al, 1980), quantification of mercury in larvae of various blow flies' species (Nuorteva and Nuorteva, 1982), detection of the arsenic in the insect's species Muscidae, Piophilidae (Brahy et.al). Goff and Gunatilake (1989) in their study showed the detection and quantification of Malathion in the Calliphoridae species using gas chromatography. Studies have shown that the rate of development of the insects and their survival were affected by the presence of drugs (Magni et.al 2014, 2016b). The rate of development and survival of the insects was also found to be dependent upon the varieties of tissues or diet on which the rearing of the insects was done.

One of the studies which were done by Clark et.al (2006) compared the development rate of Lucilia sericata on different tissues such as the lung, liver, and heart of animals like cows and swine. Their study showed variations of larval growth rate between lung, liver, and heart. The entomological evidence determines the cause of death, manner of death and medico-legal aspects of not only humans but also in wildlife investigations. In wildlife investigations, the death of the animal is caused due to illegal cruelty, trade, possession, and poaching. The accuracy of the post mortem interval was more at the advanced decay stage of decomposition because the changes in the insect succession were slow and most of the body mass was lost. The sites of trauma on the body were estimated by measuring the larva activity on areas of the body except for eyes, nose, ear, and mouth before the decay stage of decomposition. Few of the fly species lay eggs on the living tissue and their presence help in determining the duration of being alive before death.

Entomotoxicological Researches

Gunn *et al.,* (2006) determines morphine in

Figure 1: Life cycle of Blowfly

the larvae of Calliphora Stygia using HPLC chemiluminescence detection. Potassium permanganate was used to detect the morphine in larvae in low concentrations and the chemiluminescence detection was used for the effectiveness and robustness.[2] Strong chemiluminescence was detected when acidic potassium permanganate reacted with morphine. Results of the study showed that the minimum limit of detection for morphine was 2500ng/g. The control samples tested negative for morphine. The presence of morphine was reported at high concentrations. The detection of morphine was negative or absent at low concentrations. No chemiluminescence was detected when the substrate was reared with the potassium permanganate. The concentrations of morphine increased with the increased concentration of morphine in the larvae.[2] The concentrations of drugs in the insects were stable and several kinds of research were also done for detecting the drugs in the larvae, pupa, and puparia of insects. The quantification of drugs in the larvae helps in the estimation of drug effects on insect development. The most investigated insect sample for the quantification of the drug was the third instar larva. Various instrumental techniques were used for the quantification but because of the heterogeneity of insect tissues, the G/LC coupled with MS was used for their sensitivity and selectivity.

Matthias Gosselin *et al.,* (2010) in their research determine the concentrations of methadone and its metabolites in third instar larvae using LC-MS. The quantification of the methadone and its metabolites were done in the feeding and post-feeding stages of Lucilia sericata insects.[3] The findings of their study showed that the feeding stage marked the higher presence of methadone's metabolites than the post-feeding stage because of the different chemical structures and physiological properties. The more excretion of metabolites was because of higher polarity and more solubility resulting in less concentration in a post-feeding stage. In both the feeding and post-feedingstages, the concentration of methadone was similar because of the bioaccumulation in adipose tissues. The pupal stage marked the low concentration of methadone and its metabolites.[3]

Magni, Paola *et al.,* (2019) describes the method for the quantification of the Endosulfan and its isomers in the Calliphora vomitoria using the HPLC/MS technique. The results showed that the quantification of the Endosulfan was different in the test samples when compared with the control samples.[4] When the Endosulfan concentration was less than 10ng/mg and 25ng/mg in the food substrate, no effect was noticed in the development time and survival of insects but when the concentration was 50ng/mg, the developmental stage of the larva was observed.[4] The larvae and pupae length of the insects in the test sample has no differences when compared with the control samples. When the concentration of Endosulfan was 50ng/mg, the larvae length was smaller and they don't undergo pupation.

Paola et al. (2019) describes a method

for the quantification of the ketamine in Calliphora vomitoria using HPLC/MS and its validation based on the various parameters. The findings of their study showed that the concentrations of ketamine were absent in all control samples, L2 and adult samples.[5] In terms of developmental time especially from oviposition to eclosion, the time was different between control and larvae. The ketamine also affects the survival of the instar's development but during the metamorphosis this effect was significant. During the instar development, the survival was 15% and in metamorphosis, this survival was 85%.

Thomas *et al.,* (2016) describe a study held in Texas, the USA to determine and estimate the temperature and tissue types on the development of various stages of the life cycle of the insects. The larval length and growth rate of insects were examined on the different temperature ranges and tissues types. The method was validated to determine the accuracy by estimating the post mortem interval index. The findings of their study showed that the development rate of the insect was affected by temperature range. An increase in temperature promotes faster development.[7]

The study was done by Harnden and Tomberlin (2016) for studying the effect of temperature and tissues types on the developmental stages of H. illucens with a comparison between laboratory and field reared larvae. The results showed that the development rate of the concerned insect was directly affected by the temperature and the tissue diet. The pork and beef-based diet estimated the larval age in most of the sampling units but it was impossible by grain-based diet.[8] When compared the larval length of laboratory-reared larvae, it was less than the larval length of the field-reared larvae in most of the samples. In the case of pupal development, the temperature and tissue diet have no effects on accumulated degree hours.

The present study done by Wang et.al (2020) mainly focused on determining the developmental stages of H. spinigera at seven different temperatures ranging from 16, 19, 22, 25, 28, 31 to 34°C, and developed the various models that help in determining the post mortem interval index based on the developmental data of the insect H. spinigera. Their study showed that with the help of the Isomorphen diagram it was estimated that the rate of development of insects from egg-laying to adult emergence decrease with an increase in the temperature range. With the help of the Isomegalen diagram, it was determined that with an increase in the temperature range, the larval development rate also increased.[9]

The development of insects was depending mainly on the temperature ranges. Research has shown the effect of temperature on the developmental stages of flies. Previous many types of research also showed that the time of colonization of different flies was also affected by the temperature ranges. The geographical ranges and the densities of blowflies were also affected by the temperature. There is less information about the threshold temperature required for oviposition for an individual blowfly species. The temperature variations on the probability of oviposition behavior of individual blowfly species remain undetermined and also to determine how the temperature of the remains affects the oviposition behavior and the survival of the eggs lying.

Considering all these points, Ody, *et al.,* (2017) describe a study for the determination of various temperature ranges of oviposition in individual blowflies and also the probability of oviposition behavior and survival of eggs in these temperature ranges.The colonization of the species in the cadavers occurs predictably. The necrophagous dipterans are the primary colonizers of the remains colonized in the early stages of decomposition while the coleopteran was the late colonizers of the remains colonized in the late stages of decomposition. The insects were found in every habitat and at all times on cadavers, whether it may be outdoor/indoor, open/forest habitat, high/low elevation, cold/warm, and small or large cadavers.[10] The changes in the composition of the insects and

their succession patterns were associated with the changes in the geo-climatic conditions and ecological features of the crime scene. The patterns of a succession of necrophagous insects help in the estimation of post mortem interval index. The succession patterns of the insects and their developmental stage were found to be affected by the local geographical and environmental conditions, so their estimation was an important part of investigations.

The studies done by Abd El-bar and Sawaby (2011) to determine the stages of decomposition and insect succession pattern of cadavers in Egypt and compared these data with the data of stages of decomposition and insect succession patterns of cadavers killed with organophosphate poisoning.[11] The results of their study showed that the rate of decomposition was rapid in the control carcass and takes around 19 days to reach the skeletal or dry stage. The time taken by the test carcass to reach the decay stage was 40 days but only the lower part gets decayed and the upper part remains as it is because of the slow rate of decomposition. The presence of toxins in the decomposing body changes the succession patterns of insects and their developmental stages but it does not affect the arrival of the insects.

CONCLUSION

From the past, many years of research have been done on entomotoxicology to establish a relation between the concentration of drugs and toxins in the substrate to their concentrations in the insects reared on that substrate. The detection of drugs and toxins in the human remains by using various analytical techniques helps estimate the post mortem interval of cadavers.[1] Various methods and procedures have been systematically developed and validated for entomotoxicological analysis. The effectiveness of methods and procedures depends on the ability to detect and identify the drugs present in the insects. The most accepted technique used for detection was chromatography coupled with mass spectrometry. The fate of drugs or toxins in insects depends on their feeding activities and developmental stages.[6] Every insect has different feeding habits because of its diet and life histories that affect the accumulation of the drug in different species of insects. With the help of entomological evidence, the post mortem interval index can be estimated in cadavers by using two primary ways – one by determining the insect succession pattern since the patterns of their succession depict the various stages of decomposition. The other one is depending on the rate of development of immature insects on the corpses. The rate of development of immature insects depends on various natural and anthropogenic factors like climatic conditions, the temperature of the cadavers, humidity, nature of burial, clothing, presence of drugs or toxins, and many more.[8] The thermal growth of flies is a useful technique in forensic entomology, and more research will improve its utility. Because of the intricacy of insect growth and the numerous factors that affect it, determining insect development is inevitably an estimating process. With more research, we can expect our estimates to become more consistent. However, until we have highly exact temperature measurements, thermal development predictions are unlikely to ever yield correct post-mortem interval estimations.[7] When combined with other data, such as successional patterns or direct age marking of insects, temperature growth estimations are a powerful line of independent evidence. The predictable pattern of insect succession on a body has long been regarded as a reliable way to calculate the time after death. However, a variety of factors may affect the frequency and species richness of the carrion fauna. It's critical to be aware of all the circumstances that can affect insect colonization of remains and to account for them while examining a death.[11] Studies need to be done, in particular, to establish more spatial datasets of insect succession on carrion in a wide range of habitats and circumstances across all countries where forensic entomology is applied. IJFMP

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REVIEW ARTICLE

Psychological Profiling: A Tailored Forensic Investigation

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ABSTRACT

Forensic Psychology is a vast field which comprises of many subdivisions. It focuses on the study of personal, behavioral and psychological characters of the criminal who have committed the crime. Criminal Profiling is the method which includes the entire behaviour and psychology of the culprit. It does not directly leads towards the individual, but it gives the idea regarding the traits or the characteristics of the offender. Criminal Profiling is the emerging field and is used especially in the violent homicidal cases such as murder, rape, arson. This method is useful in investigation and examination of the criminal personality and behavioral descriptions and aids in the identification of type of the person who have committed the crime. Therefore, Criminal Profiling is a standard weapon for the forensic identification. This review paper covers almost all the aspects of criminal profiling and how this method has potential to investigate the heinous crimes.

KEY MESSAGE: Psychology is an emerging field in forensic science. This study focuses on the procedure and application of criminal profiling in the forensic investigation.

KEYWORDS | forensic psychology, criminal profiling, investigation, criminal behaviour

INTRODUCTION

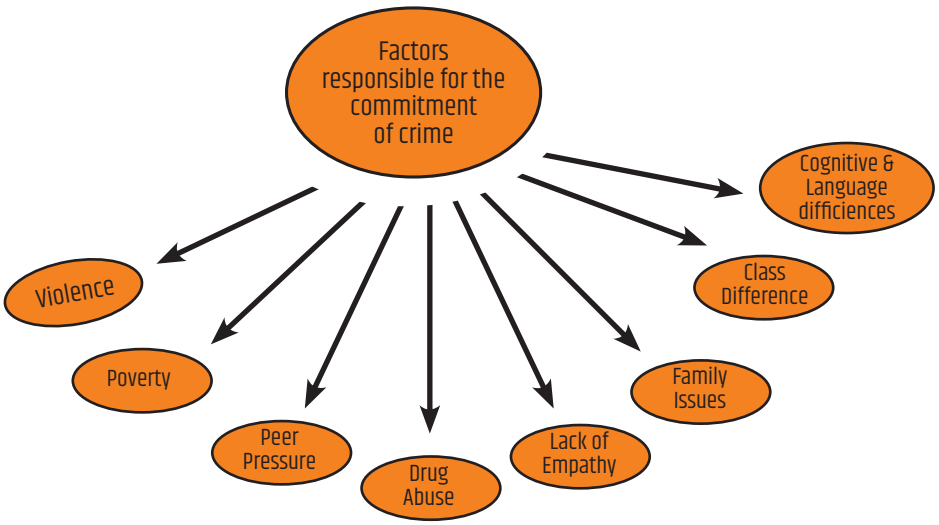
FORENSIC PSYCHOLOGY HAS BEEN DEFINED as the sub-discipline of psychology which focuses on the psychological research which aids in the addressing of legal, administrative and contractual matters. This branch is associated with the collection, examination and presenting the evidences for the legal purposes.[1,8,12] This branch of science aids in investigation by studying the behaviour and mental process. Psychologists have started including this study to study the mind of the culprit, witness or the victim and infer what the cause of the crime is. Forensic psychologists provide important information and guide the officials in the legal system. Like other forensic experts, they also act as expert witness. Their role is to treat and diagnose the criminals and they also administer psychological tests.[10]

The field of forensic psychology is generally categorised into two sub fields:

- Legal Psychology
- Criminal / Criminological Psychology

Legal psychology is related to the psychology which is concerned with the law including vulnerable witnesses including juveniles, legal decision making, testimony and memory of the witness, interactions in the court and the role of expert testimony. Criminological psychology is concerned with the investigation, assessment, explanation of the criminal behaviour. Other disciplines that are a part of forensic psychology are victimology and police psychology.[12]

Forensic psychology has immense potential to grow rapidly in the area of investigation. It is an undeniable fact that science brings justice only if it is applied and shared.[8] The



imperative part of forensic psychology in the criminal investigation is to study the culprit psyche, description of the offender and this includes the parameters like personality traits, behavioral patterns, psychopathologies and the demographic variables including the age, race and geographical location.[4,9] The main application of this field is to narrow down the number of suspects and to figure out how to interrogate the suspect already in custody.[10]

How they become criminals?

Crime is basically an act which is deviant from what the society has already constructed in the past. This act is against the social norms and thus makes a person a criminal. Crime has a devastating effect on the society irrespective of the deceased. Crime and the criminal intent include series of convoluted psychological decisions which influence the criminal unconsciously and the criminal/offender is unaware about the same. Any human being does not acquire this criminal intent during birth. These characteristics are developed over the passage of time. Human is a creature who imitates others and learn.[2]

The most imperative role is played by sociological criminology in mounting the psychological criminology in the mind of the culprit. There are certain factors that convince an individual to opt unlawful activities for fulfilling the needs and desires.[2,7]

Criminal Profiling

Criminal profiling is conducted on the perpetrators who are involved in violent crimes and serial killing. This technique is not used in every case, it is used only when there is an extremely violent crime. The main objective of this technique is the identification of the characteristics of an unknown culprit by analyzing the scenario of the crime scene as well as the victim characteristics. Furthermore, the case scenario is then compared with the prior cases which are somewhat identical. The technique of criminal profiling was first used by the Federal Bureau of Investigation (FBI) in 1971.[3,4] The technique provides valuable information to the investigative agencies which will aid in seeking attention on the individual who possesses the same or identical traits with that of other perpetrators who are accused of similar criminal offense. One cannot get the exact details of the criminal but it is helpful in narrowing down the number of suspects.

The assessment of the culprit in the

psychological profiling is made on the basis of various factors including age, sex, marital status, employment, education level, the possibility of confessing the crime etc. Profiling makes the use of combination of psychological socio-legal theories and sociological concepts in order to establish the linkage in the characteristics of the culprit. The behaviour and the motivation of the specific offender is studied on the basis of physical evidences at the individual crime scene. Criminal profiling is unchallenged in some cases. On the other hand, some cases are quite obscure. The profilers look for the similarities and dissimilarities to get more information regarding the offender.[1]

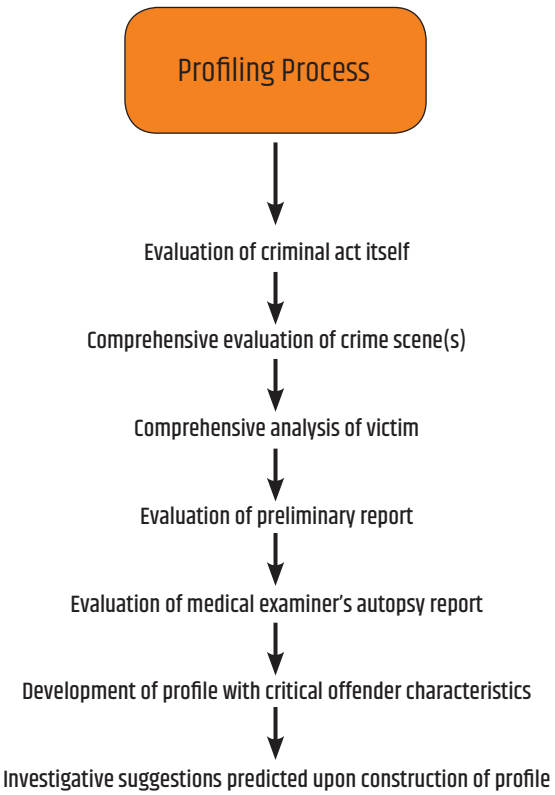
Profiling

A profile of a criminal is an assemblage of the inferences that are made on the basis of qualities of person who are under suspicion of committing the crime or series of crimes. The profiling process also focuses on the issues involving political, artistic as well as criminal behaviour. Psychological profile is also known as "Paper Tiger". This profiling is forensically important in the investigation and sometimes is destructive in some cases when it diverts the investigation procedure and mislead it.[4,6] The parameters which are required for the creation of the profile include:

- Photographs collected from the crime scene
- Demographic data
- Medical reports including autopsy
- Travel history of the deceased prior to the crime
- Comprehensive investigation of the crime scene
- Entire background and life history of the deceased or victim.

The process of profiling involves the following steps:

- Evaluation of criminal act itself
- Comprehensive evaluation of crime scene(s)
- Comprehensive analysis of victim
- Evaluation of preliminary report
- Evaluation of medical examiner's autopsy report
- Development of profile with critical offender characteristics
- Investigative suggestions predicted upon construction of profile



Purpose of Profiling

The main objective of psychological assessment of the crime scene is to reduce the scope of police investigation as it helps in exclusion process and aids towards the successful resolution. It suggests the effective ways of interviewing and assists the criminal justice system to fight against the crime. Further more, it helps in eventual identification of the culprit and other investigative processes. In addition to this, it is helpful in unfolding facts and facets of the offence by focusing the investigative resources in one particular direction. One can identify eventually the offender in conjugation with other investigation procedure.[1,11]

S. NO.	INDUCTIVE REASONING NOMOTHETIC APPROACH	DEDUCTIVE REASONING IDIOGRAPHIC APPROACH
1.	Time efficient	Time consuming
2.	Inexpensive and less laborious	Expensive and laborious
3.	Less effective	More effective
4.	No skills are required for behavioral studies	Expert is required for behavioural studies
5.	Encourages egocentricity and investigative short-cuts	Encourages deliberation, competency and thoroughness

Table 1: Difference between Inductive and Deductive reasoning

S. NO.	ORGANIZED CRIME SCENE	DISORGANIZED CRIME SCENE
1.	Average or above average IQ	Below average IQ
2.	Employed, usually quite skilled	Unstable employment record, unskilled
3.	Self-competent	Socially isolated
4.	Uses alcohol in commission of crime	Lives close to crime scene
5.	Uses car to drive to crime scene	Strict discipline as a child
6.	Obsessed with media coverage of his crimes	Extremely anxious

Table 2: Difference between Organised and Disorganised crime scene suggested by offender

S. NO.	ORGANIZED CRIME SCENE	DISORGANIZED CRIME SCENE
1.	Body is hidden.	Body is not hidden.
2.	Weapon is removed from scene.	Weapon is present.
3.	It appears to be well-planned.	It appears to be spontaneous.
4.	Victim is specifically targeted.	Victim may be an acquaintance.
5.	Aggression takes place before death.	Aggression or sex post-mortem.

Table 3: Difference between Organised and Disorganised crime scene

Different Types of Profiling

Profiling is of three types. These are as follow:
Crime scene profiling involves the analysis of crime scene for drawing the conclusions about the culprit. Psychological profiling involves the use of expertise of a professional psychologist or profiler who provides information regarding the personality traits of the offender. While offender profiling use combination of all available information from crime scene as well as from the psychologist/profiler.[1,9]

Different Phases of Profiling

The four different phases of profiling are explained below:

Methods of Profiling

Various methodologies are employed to develop the criminal profile. The methodology either focuses on the development of offender's profile or on the complete understanding of the modus operandi (MO).[1,9,11] By analyzing the modus operandi, the investigators get the events lead to the crime scene. There are basically two methods of profiling:

Nomothetic Approach / Inductive Reasoning

In this method of reasoning, the characteristics are observed on the basis of broad generalizations and statistical analysis. Thus, leading to the development of the hypothesis. It is generally regarded as an average. Inductive reasoning is the set of characteristics of the culprit which comprises of co-relation, experiential as well as statistical inferences.

Idiographic Approach / Deductive Reasoning

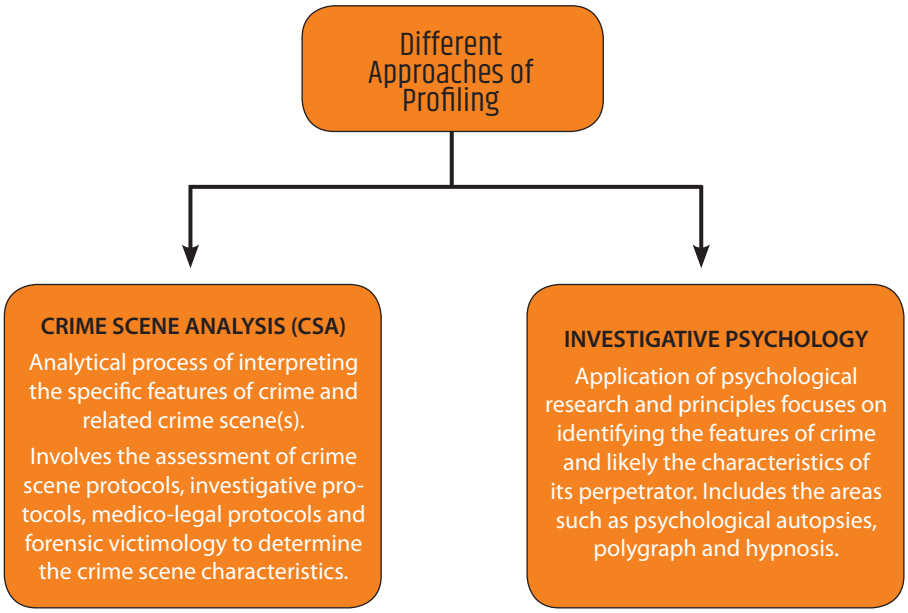


Figure 1: Different approaches of Profiling

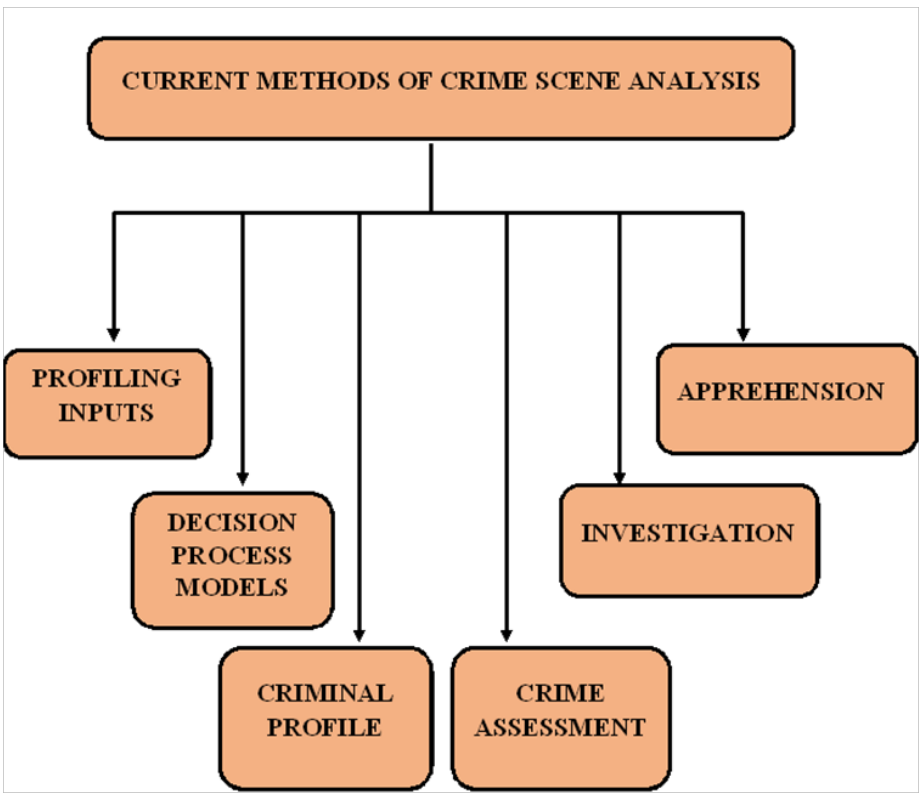
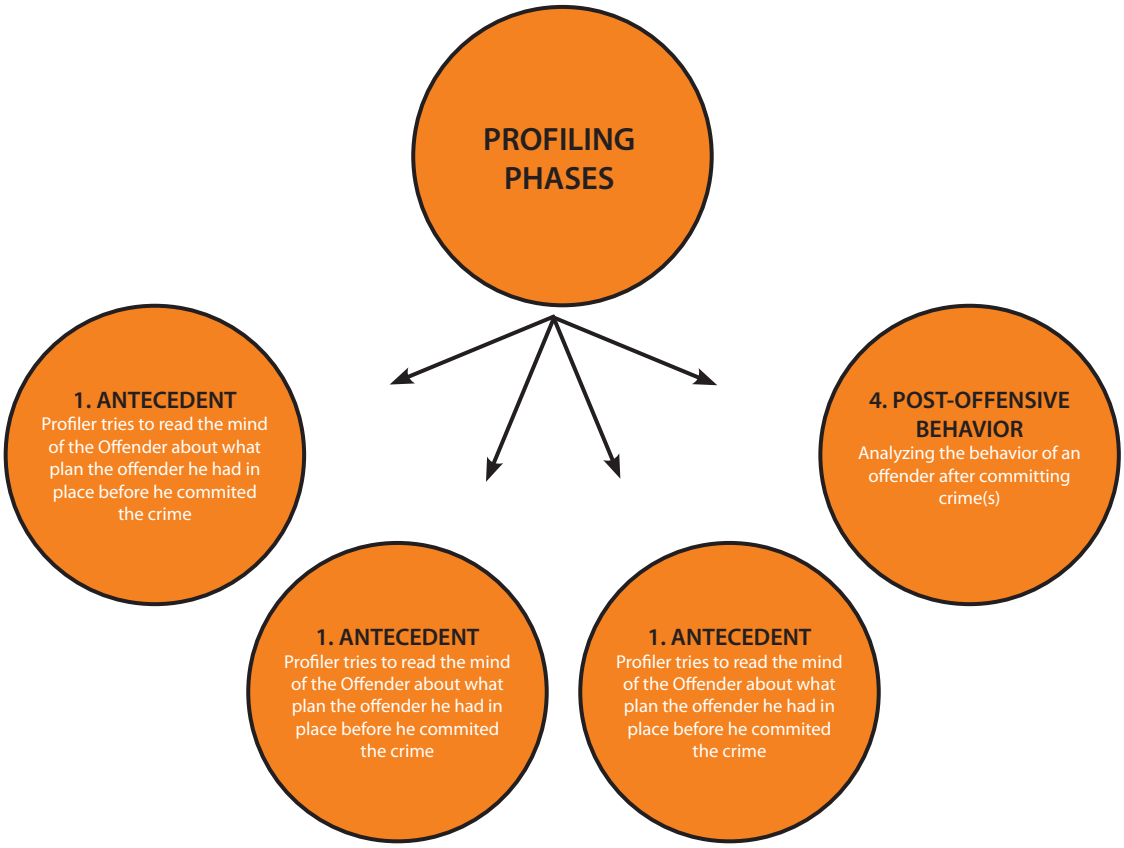


Figure 2: Current methods of Crime scene Analysis



In this reasoning method, a set of characteristics are observed that might be reasoned from convergence of physical and behavioral actions or patterns. These actions also include the modus operandi. This is also termed as "Behavioral Evidence Analysis". The behavior of the culprit at crime scenes such as emotions, individual patterns, personality and offensive behavior can be deduced. Deductive reasoning is dynamic and hence, re-examined when new information is available. The difference between inductive reasoning and deductive reasoning are described in Table 1, above.

Different Approaches of Profiling

Criminal profiling is usually a vague process.[1,3] This technique is majorly categorized into two types as depicted in Figure 1.

Crime scene analysis made popular by the Behavioral Science Unit of the FBI (Federal Bureau of Investigation) for solving violent crimes in 1970s. There are different methods of crime scene analysis which are currently used during analysis process.[1,3] These methods include:

According to the Federal Bureau of Investigation (FBI), there are several stages of investigation for the generation of profile of the culprit, as depicted in Figure 3:

Crime scene classification is the second stage of profiling process. According to this classification, crime scene has two types: Organized crime scene and Disorganized crime scene.

These types of crime scenes reveal different aspects regarding the psychology of the offender. The differences between the offenders on the basis of crime scene are described in Table 2 and 3.

Applications Of Criminal Profiling

Criminal profiling is an imperative tool in criminal justice system and is used in crimes

Figure 3: Stages of Investigation

which are extremely violent. Such crimes create terror and fear among the society because of their randomness as well as an ambitious nature. Violent homicide cases are very challenging to solve because of their randomness. Crimes such as assaults, murders, serial killing etc. are investigated with criminal profiling.[1,3] The more adverse the crime is, more would be the likelihood of the profile to be used. Criminal profiling is used in cases such as:

- Sexual assaults and homicide
- Other homicides such as torture, mutilation, ritualistic violence
- Arson and explosive cases
- Assassination
- Extortion

This helps in leading the investigation by narrowing down the number of suspects and by making use of personality, behaviour as well as psychopathy to interrogate the offender. It also suggests the investigators with investigative and proactive strategies as well as the trial strategies.[1,5,3]

Case Study: A Serial Killer

Jeffery Dahmer was a 31 years old white man who was a serial killer and was also known as "Lust Killer". He was having repressed hostility, frustrated acceptance and was rejected by his peers. He was going through intense loneliness and then attributed his motive of action towards lust. He usually had homosexual as well as homicidal fantasies. Jeffery captured 15 young men and killed them brutally, some of them were even raped by him. Dahmer was profiled by a psychologist to infer his psychology regarding his criminal intent at the time he was committing the crime. While he was interrogated by the criminal psychologist, he was found calm and free from emotional liability, his answers were logical and relevant and he was confidently answering them.

After analyzing and interrogating him on the basis of his psychology, he was then undergone through psychological testing, MRI, EEG and chromosomal analysis. These psychological and medical testing methods resulted that Jeffery Dahmer was suffering from mixed personality disorder along with sadistic, obsessive, necrophiliac, cannibalic as well as fetischistic characteristics and hence

he was stated as an organized, non-social "Lust murderer". He was consequently sentenced to life imprisonment.[11,12]

CONCLUSION

Criminal Psychology is a valuable tool for combating the heinous crimes by using psychological and behavioral aspects of the culprit. This technique is not specific and does not give assurance as it sometimes provides the investigative lead or sometimes mislead the investigators. Criminal profiling is a multi-disciplinary field in the forensic scenario as the expert must have keen knowledge in psychology, criminalistics and medico-legal death investigation. The expert who interrogates the offender uses variety of techniques and methods to get ample knowledge about the culprit and the crime. The expert firstly evaluates the crime scene and then gathers information from the victim and the witnesses. Finally, he assembles the entire information which is collected to apprehend the criminal. Moreover, new and advanced databases are required to be prepared for all such crimes. These databases would definitely help the investigators as well as the law enforcement agencies to solve the cases in a more efficient way. IJFMP

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REVIEW ARTICLE

A Recent Advancement in Techniques for Investigating Cybercrimes, Digital Crimes and Audio Forensics

[1]Abhinav Singh, [2]Sally Lukose

ABSTRACT

The illegal activities using mobile phones, computers, and the internet are rising, including pornography, online prostitution, identity theft, phishing, sniffing or snooping attacks, spamming or malware attacks. Internet crime or cyber-attacks play a pivotal role in impacting the system since we started using the internet. In this era, of digital world crimes are increasing at par. The advancement in the technology for detection of these crimes has revolutionary affected the forensic field. Starting from, the detection of digital crimes from small scale like Email Bombing to Denial of Services (DOS) at large scale. Furthermore, the sensitivity of detection is quite decisive hence, it is cardinal responsibility of forensic experts to investigate these types of crimes critically. An attempt has been made in this paper to percolate the significance of digital crimes, cybercrimes, and audio forensics. Additionally, it also focus the investigative tools and techniques for such crime.

KEY MESSAGES: This paper elaborate the key features for investigation of Digital crimes, cybercrimes & Audio Forensics.

KEYWORDS | digital forensics, audio forensics, cyber-attacks, ethical hacking

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INTRODUCTION

As technologies are blossoming daily, industrialization and digitalization are facilitating these days. The extension of digital technologies creates a domain for performing various tasks quickly and effortlessly. Nowadays, 90% of the work is going online, whether it is classes, conferences, offices, webinars, official meetings, state or political meetings; everything is online now; hence there is a tremendous rise in digital crimes and cybercrimes. On counting, the User of android phones and Apple iPhone devices are immense in number. About 1.7 billion people are Android users[2] and 700 million banks upon the iPhone devices.[1]

In contrast, Windows users are significantly less in numbers.[3] Modern technologies eased living and made it easy to connect with our friends and families via SMS, WhatsApp, Skype, Telegram, Facebook, Instagram, and other social media platforms. With the advancement in different instant messaging applications with various features and the introduction of the internet, it became an easy task to convey your messages from one end to another in the form of encrypted text messages, documents, or any video and audio file format. As per the record, in April 2015, there were about 800 million users on WhatsApp.[4] These unlawful activities usually remain unnoticeable as encryption technology transfers information from one end to another.[5] Usually, a single message is sent to a large number of victims.[6] The attacks grasp the individual's security by different social media platforms, instant messaging applications, e-mail, and scanning the QR codes.[7] The attackers mimic the electronic environment to steal the victim's private information or

recipients.[8]

Phishing: Phishing attacks are tremendously rising and acting as a critical component of cyber-attacks. The word phishing means "password harvesting fishing," which is used to steal a victim's identity via computer networks.[9] The attacker gains access to the username and passwords of the victim's identity and illegally exploits them. There are several types of phishing attacks malware-based phishing.[10] deceptive phishing,[11] hosts file poisoning phishing,[12] search engine-based phishing,[13] and other types of phishing classified based on their attacks. The hacker creates a fake page and tries to steal the personal information of the victim.[14] The primary phishing e-mails which are flourishing these days are related to the topic of competent, legal activity, commitment, security, perceptual contrast financial loss, health, retaliation, socialization and social proofs. On the basis of the evidences, it was reported the URLs tempt the attackers to perform phishing attacks, and the web shortening service bit.ly is used to mask the URL in the browser.[15]

Malware

The word malware denotes malicious software.[16] The malware tends to breach the system's device and policies in the integrity of the files, breaking the confidential data, and stored data. The malware is categorized into various types according to their attack, propagation, or exploitation of the targeted device.[17] The malware is assigned as harmful depending upon the different protocols of the antivirus vendors; if one antivirus vendor considers a file as malware, it may be possible that the same file is not malware for another antivirus vendor.[18]

Viruses

The prevalence of the virus tends to reproduce, propagates within the system files, duplication. The viruses lack independent movement and require a host for their functioning.

Worms

The worm self-replicates or shows self-propagation; hence no host is essential for their functioning. Trojan programs are standard files; they create vulnerabilities for attackers and act as malicious files for the system.

Spywares

Spywares attacks the users and spies on them without their knowledge; spyware comprises six types: adware, keylogger, Track ware, cookie, riskware, and sniffer.

Denial of Service (DoS) Attack

The Denial of Service creates congestion because of the continuous request, which ceases the system's working or server. The server remains busy working on the same type of files which leads to the overloading of the server due to the processing of the same request, and the whole process is known as a Denial-of-Service attack.

Mobile & Digital Forensics

The mobile forensic investigation or digital forensic investigation aids in the investigation of digital devices which are under the suspicion of cyber attacks. These types of investigation are done on the basis of raw data recovered by using hex dumps. Seldom the data is being recovered from the memory chips with the help of specific tools and software.[19]

On the other hand, Autopsy 4.1.1, MOBILedit, Cellebrite Universal forensic extraction device (UFED), XRY software, Andriller, Oxygen Forensic Suites are used for investigation of audio, video, emails, browsing histories, credential information or any third part application install in the suspected mobile and cell phone devices. Likewise, Access data F'TK imager, EnCase, and SQLite software play a pivotal role in digital crime investigation(s)[20,21] as shown in figure 1.

Audio Forensics:

Linguistic and voice authentication

Authentication of voice is quite crucial for personal identification of an individual. As the offenders try to impersonate a voice note or call,[22] there is an urgent need to compare the questioned audio with admitted exemplars as shown in figure 2.

Voice: Decipherment and detection

The decipherment and detection of the voice is feasible by analyzing the audio signals retrieved from the suspect. The manipulation

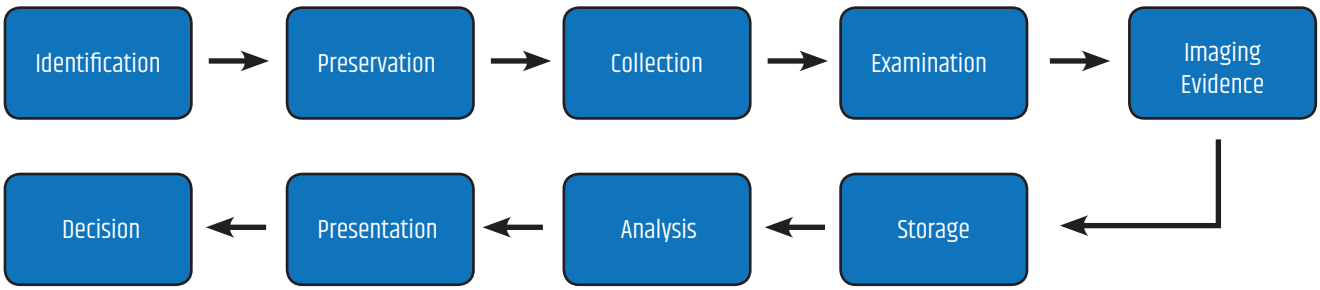

Figure 1: Framework for Mobile and digital crime investigation

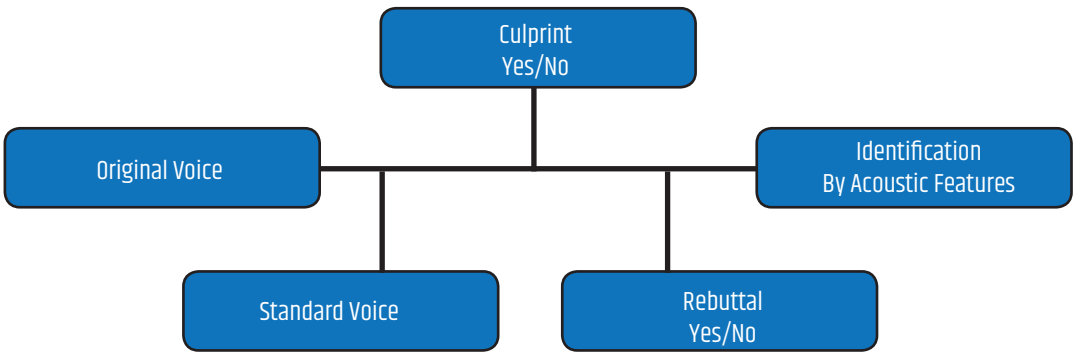

Figure 2: Framework for Audio Forensic investigation

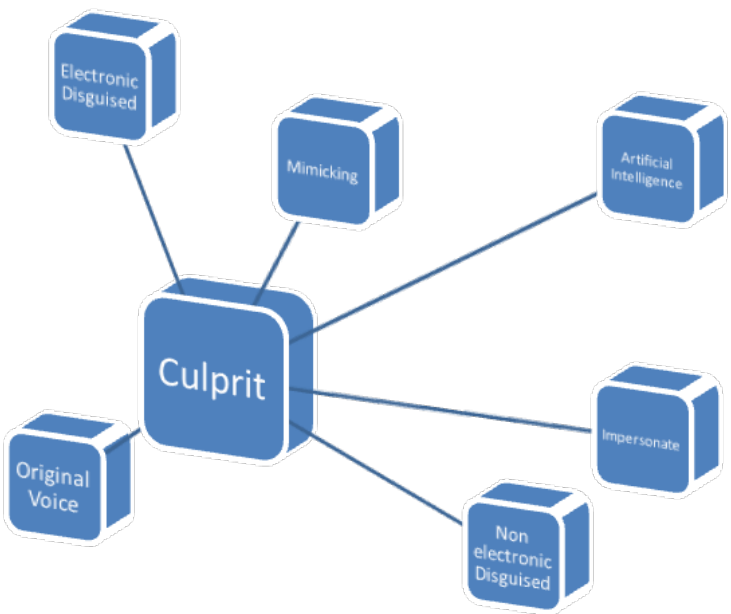

Figure 3: Type of cases encountered in Voice Authentication and Identification

impersonation, transplantation, electronic or non-electronic disguised, etc., of audio samples are investigated with the help of certain software such as, Praat, GoldWave, Audacity, Computer Speech Lab (CSL).[23]

The Contemporary, methods for identification and detection of GANs (Generative Adversarial Networks), supervised and unsupervised method like change in the pitch, formants, spectral envelope, intensity, pulses, spectrogram are cumbersome tasks therefore, requires strong expertise[24,25] as shown in figure 3.

CONCLUSION

In a nutshell, the detection and apprehension of the cyber-attacks, audio authentication, and mobile forensics require sound methodology, procedures, and protocols. There is a strong interlinkage between direct, and indirect investigation proceedings. These identification and detection procedures are quiet crucial for better justice. Even though the price of these original software are quiet expensive, there is no other alternative to investigative protocols. There are many open-source freely available software which are used for the said purpose however, the reliability is scarce. IJFMP

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REVIEW ARTICLE

Perspective of Entomotoxicology in Forensic Investigations: A Critical Review

[1]Jasmeet Kaur, [2]Ekampreet Kaur, [3]Tilak Raj

ABSTRACT

CONTEXT: A Forensic Entomotoxicology is a contemporary technique which makes use of insects as an alternative samples for forensic investigation. In case of recovery of degraded cadaver, in which body fluids and tissues are vanished, the toxicological studies of insects aid in forensic investigation to some extent in such legitimate cases. The drugs consumed by the corpse and further ingested by the insects feeding in the corpse affect the life cycle and development of the insects. Various analytical methods are employed for proper identification, detection, as well as quantitative analysis of the corpse. This paper summarizes the definition, applications, analytical techniques and the future perspective of forensic Entomotoxicology.

KEY MESSAGE: This article discusses the importance of insects as the toxicological evidence in forensic investigation.

KEYWORDS | forensic entomology, Entomotoxicology, insects, corpse, putrefaction, PMI

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INTRODUCTION

Criminal investigation involves diverse factors. Although ascertaining cause of death is one of the crucial factors. It incorporates the discernment amid natural, accidental, suicidal and homicidal death. There are numerous practices in a criminal investigation to resolve the root of death. The united collaboration professionals of distinctive curriculum for instance crime scene technicians, death investigators, anthropologists, entomologists, forensic pathologists and many other frontline professionals are key requirement for competent death inspection. The death scene should be thoroughly handled to bring out the facts which are helpful to re-establish the incidents that ascertain integrity of proclamation by witnesses. The delayed recovery of cadaver under suspicious conditions makes the death investigation complex in concluding the cause and manner of death. All the dermis and vitals are severely putrefied in disintegrated and skeletonized corpse. Under such conditions the toxicological samples are neither available nor useful for analysis. In such situations the exclusive and mere approach to find out the cause and time since death is entomology.[4] The study of insects and arthropods is interpreted as Entomology which is evolved from to Greek words entomon and logos meaning insect and word or reason respectively. When the study of entomological evidences like insects and their arthropod counterparts

populating on decaying remains is enforced to legitimate proceedings is described as Forensic Entomology or Medicolegal or Medicocriminal entomology.[3] Insects are extensive constituent of kingdom *Animalia* which are plausibly diverse and remarkably prosperous entities on earth. These are significantly versatile class of approximately 1 million species presently well recognized. Insects have successfully oppressed around whole credible environment and the immense mass of insects have ambiguous link to humans or are valuable to us. Toxicology is the field that amalgamates the assorted features of biology, chemistry and medicines that targets the detrimental consequences of chemicals, drugs and other substances on human body. Toxicology is further extended to clinical toxicology (therapeutic approach), analytic toxicology (laboratory testing and analysis) and forensic toxicology (for provision of justice in legitimate matters in both ante mortem and post mortem medico-legal cases. Entomotoxicology is described as the sampling of insects and arthropods (flies and beetles) that feed on cadaver as a positive substitute for testing of toxins and drugs. Entomotoxicology is composite of distinctive concepts of ancient Greek words entomon (insect), toxikos (poisonous) and logous (subject matter) which means study of xenobiotics affecting insects. The insects are utilized as redundant source for drug identification when traditional source like blood, urine, vitals are not feasible. Entomotoxicology investigates the presence of drugs in corpse at death time. The positive identification of insects is the key constituent for death investigation. Data like nature, growth rate, developmental records and geographical assortment is utilized.[9] Forensic Entomotoxicology is utilized for detecting xenobiotics and post mortem interval (PMI). Numerous arthropod species primarily Diptera and Coleoptera (flies, beetles and their larvae) are the first visitors to the cadaver. These species feed, reside and propagate on and in the cadaver according to the stages of decaying. By utilizing the information of developmental stage, time since death or post mortem interval is computed. PMI is the approximate value of time since death which is known as Colonization interval for forensic entomology analysis goals.[6] Various techniques like GC-MS, LC-MS, HPLC and immunoassays have been used in routine xenobiotic detection.

HISTORY

The insect importance of facilitating decay and assisting usual organic matter depletion has been studied in preceding centuries. It was initiated in the 13th century in China when the first medicolegal case was reported and solved with the help of entomology. In 1767 decomposition by insects was studied. In 18th and 19th centuries in French, buried bodies consumption was witnessed and PMI calculations were initiated. Further studies include utilization of forensic entomology in famous Buck Ruxton Case in 1935. In 20th century, new species of discovery, their life cycle study was conducted. Now new trend to describe life cycles of forensic insects to rule out cases of murders and assaults, negligence, deceiving was fruitfully achieved, but it still needs further research. It is the historical background of forensic entomology. The history of Entomotoxicology dates back to 1980 when a 22-year-old female cadaver was recovered in skeletonized stage. The cadaver was recovered 14 days after her last sight. No toxicological sample was available for analysis, thence the fly larvae were utilized as substitute for toxicological samples and phenobarbital was successfully detected. Afterwards numerous toxins, drugs, narcotics, for instance, Benzodiazepines, Morphine, Amitriptyline, Nortriptyline[1], Acetaminophen, Cocaine[2], Malathion, Bromazepam, Diazepam, Nordiazepam[5], Temazepam, Propoxyphene, Trazodone, Methylphenidate, Levopromazine, Nicotine, Fluoxetine, Barbiturates and Meprobromate, Clomipramine[8], Trimipramine, Opiates And Opioids, Phencyclidine, Codeine, insecticides and pesticides for instance Terbufos (OPs), metal toxins, for instance, lead, mercury, Aluminum phosphide, and alcoholic

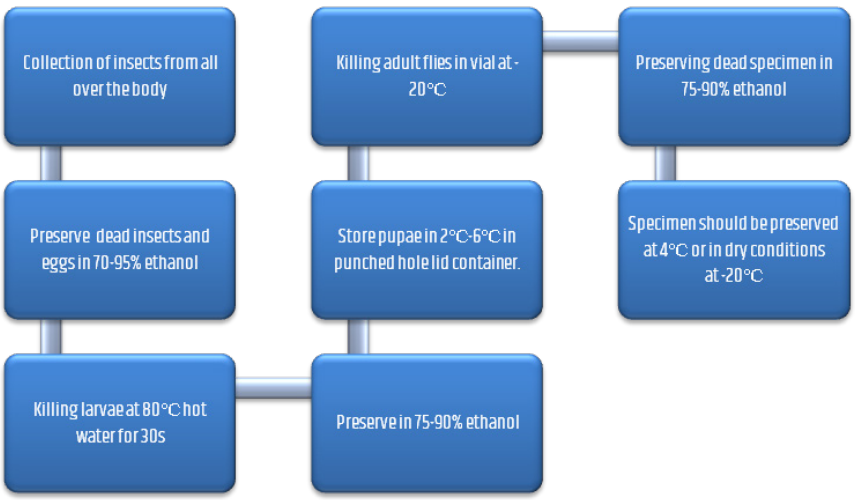

Figure 2: Sample collection and Preservation protocol

beverages.[10]

Insects as Toxicological Sample

The progression of decomposition commences right after the death of an individual. The decomposition proceeds in five stages: fresh, bloated, decay, post-decay, and skeletal. Putrefying remnants caters an interim micro-territory extending dynamically altering food source to numerous organisms such as bacteria, fungi and vertebrate scavengers. Insects are the foremost invitees to invade the cadaver onset putrefaction. They were intrigued to the body fluids from natural orifices and blood from wounds immediately within minutes. The majorly encountered insects are flies and beetles which belong to Dipteran families (Calliphoridae, Sarcophagidae) and Coleopteran (Histeridea) respectively that construct a compound food web within the corpse. Arthropods are the key constituent of the kingdom Animalia insects as predominant taxa in terrestrial surroundings worldwide. These insects were fascinated due to the strong odor from corpse. Flies arrive before beetles to the cadaver and feed on liquid oozing out of fresh cadaver but unable to feed on fresh cadaver's tissues. The prevalent flies are blow flies despite of this other insect flies, beetles and arthropods are also present. Blowflies provide the most accurate estimate of time since death. Beetles prevails at extreme stage of decomposition. The materials for entomo-toxicological investigation are larvae, pupae, adult insects, puparial cases, beetle fecal material, cast beetle skin, and fly predators. Some of the species that are investigated thoroughly on which toxins have been recovered auspiciously. The most recovered insect species are Calliphora vicina and Lucilica sericata. About 63 papers relevant to Entomotoxicology were reported to be published in the year 2016. Approximately 73 research papers were known to be published during 1980-2018. The authors have tried to include all articles till 2020.[10]

METHOD

Sample Collection and Preservation

For the decisive and authentic assessment of PMI assorted procedures for collection and preservation of the entomo-toxicological evidences have been devised. Sample collection and preservation of insect specimens is the integral constituent of toxicological analysis. Because of the redistribution of drugs, sample is collected from different area of cadaver.[10] The samples were collected mainly from the

key organ (liver), muscles, and head area as well as in the environment surrounding the cadaver. Skin surfaces can also be considered as sampling site where no key organ is present. The investigators must sample the specimen by keeping in mind that the source of the insects can be divergent from the cadaver. There are numerous standards and guidelines published for entomological sampling in forensic Entomotoxicology. Sampling can be done in diverse developmental stages at systematic lacuna of time. Subsequently the preservation is initiated on the completion of sampling procedure. In one experiment, specimen samples are rinsed with normal water froze at -68°F to -39.2°F. The organic samples were rinsed to remove human fluids, crushed and heated at 650°C for 24 hours. In other experiment, samples collected were placed in hot water (780°C) for 30s and stored in 75% ethanol 60°C for at least 2 weeks. In another experiment, larvae were directly stored in preservative (10% formalin, 80% ethanol and 95% ethanol) or hot water killed at 80°C and 100°C for 1s, 30s, 60s, 90s and stored in preservative. 80% ethanol was considered as best preservative medium. But it is not applicable to beetle larvae thence it is suggested that beetle larvae should be measured alive.[7] Keeping in mind the aforementioned experiments, a summarized method for sampling and preservation of insects is depicted in figure 1.

Sample Extraction

The removal of xenobiotic material from the entomological specimen is advantageous than human tissues. Sampling procedure is effortless and no interference in analysis is encountered unlike the human samples. Quantification was achieved in larvae sample but not in human tissue sample was explained in an experiment. Extraction procedure of insects is similar to human tissue samples. Diversified extraction protocols for instance solid phase extraction and liquid-liquid extraction are utilized for extraction of different drugs and toxins. Amidst aforementioned protocols solid phase extraction administers utmost organic decomposition from aqueous concentrate of entomological exhibits.

Toxicological Analysis

Numerous animal models and substrates had been utilized by diversified researchers for identification, quantification of toxins from insects and larvae. The extraction protocol and its efficiency affect the successful detection and redemption of drugs from insect specimens. There is a systematic analysis layout of diversified chemical compounds in entomo-toxicological samples of interest.[10] Analysis can be further categorized as qualitative and quantitative.

Qualitative Analysis

Insects can serve as the sample for the detection of any drug of toxin presence in the cadaver. There are numerous research articles that have proved the successful drugs for instance, cocaine, methamphetamines, Malathion, nicotine by using GC-MS. Many analytical techniques such as HPLC, GC, GC-MS, LC-MS, LC-MS/MS, GC-MS/MS, immunoassays re used for qualitative analysis of insect sample for toxin detection because of the accuracy, sensitivity, selectivity, and reproducibility.

Quantitative Analysis

In addition to determine the kind of drug present, the quantitation of drugs is also considered as a crucial aspect of forensic entomo-toxicological analysis. It was found that the drug concentration is less in insects and larvae when compared to substrate. If the substrate was treated with high doses than the concentration of drug is also increased in the insects and larvae. Also there is occurrence of drug elimination when the maggots mature, which in turn results in reduction of drug concentration exempting antidepressants whose concentration is high in post feeding stages because of the bioaccumulation. Immunoassays and HPLC techniques are not capable to quantify low levels of drugs, thence LC-MS and GC-MS techniques are utilized for best output. By utilizing these hyphenated

techniques quantification of diversified body parts of humans and animal models by numerous researchers. Quantification of diversified concentration levels of drugs in liver, heart, lungs, blood, brain, urine and skin have been reported. However liver is considered as the utmost vital in reference to other vitals as the metabolism of numerous xenobiotics occurs there. Thence, attentive perception of quantitative output should be executed.

Determination of Post Mortem Interval (PMI)

The prevailing usage of insects in forensic entomology is to assist the determination of PMI. Flies are utilized to evaluate the PMI in legitimate proceedings, it is achieved through collection of immature larvae, pupa and insects from the body which aids the identification of insects well as the size and stage.[10] There are numerous measures to be considered for estimation of PMI. For instance, stages of succession of arthropod species on carrion which varies corresponding to geographical location, age dependent changes in the intestinal contents as insect life cycles acts as precise clocks which initiates immediately after death (it is reportedly efficient method for PMI estimation), on stage invasion as entomological protocols is statistically reliable, developmental pattern of blowfly larvae as age is utilized to estimate minimum time since death, weight, length, width of larvae, isomegalen/isomorphen diagrams, fly eggs. Insects in gut content, simulation model and many more. PMI calculation is affected by the climatic conditions, seasons, geographic region, substrate type, location and position of body, altitude, latitude, cause of death, inter and intra specific competition and larval migration amid others. The growth rate of insect larvae vary from one species to another species, thence estimation of PMI through age of larvae can be accomplished by thoroughly studying life cycles of insects. In forensic Entomotoxicology in addition to PMI detection of presence of toxins, xenobiotics is also considerate. As xenobiotics affect the growth rate of insects thence insects are beneficial for forensic entomo-toxicological studies. For instance presence of dimethoate enlarges the life cycle duration of calliphoridae flies as dimethoate lengthens the feeding, post feeding and pupal stages of development of blowflies. Whereas malathion decreases the growth rate and alters the PMI estimation by 36 hr and 28 hrs.[1] lead toxicity in low concentration accelerates the development of immature L. cuprina whereas at high concentration delay the developmental rate.[6] As the toxins alters the growth rate efficiently, therefore wrong estimation of PMI can culminate if the type of species, their succession pattern, and their life cycle knowledge is not considered.

Future Perspective

The research and studies conducted on forensic Entomotoxicology is restricted. More research needs to be done on various aspects of this field, establishing the link between the insects and drugs including their interaction, insect metabolism etc. Moreover, bioaccumulation studies can be done. For analyzing the insects and detecting the drug samples from the corpse, proper data or reference library should be constructed, digital databases should be made depicting the morphology of insects, geographical location as well as the toxicological aspects for unchallenging detection of such evidences. Presently, DNA based identification, digital protocols are of great interest in forensic scenario, these applications can also be employed in the future trends of forensic entomo-toxicological investigation.

CONCLUSION

Forensic Entomotoxicology is captivating and exhilarating discipline. The preeminent intent of the study is the utilization of insects such as flies and arthropods for determination of cause of death such as the deceased was intoxicated before death or not. It can also be beneficial for determination of the manner of death for instance natural, accidental,

suicidal, homicidal or other unidentified manner. Amidst aforementioned details the utmost crucial factors are to determine cause of death and estimate PMI. It is useful when there is complete putrefaction of the cadaver but for precise estimation of PMI all important measures should be considered for instance climatic conditions because in high temperature and humidity decomposition process fastens and vice versa. It is beneficial tool for toxicological analysis but it is time consuming, needs thorough knowledge about insect species, their growth rate and succession pattern. Pharmacokinetics of drugs in insects is not thoroughly known. There is a drawback of interpretation of detected drug concentrations. There are not standard protocols established for analysis. This field is still emerging and needs more research for its proper beneficial usage.

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REVIEW ARTICLE

Microbial Forensic: An Update on Advancement and its Applications

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ABSTRACT

Microbial Forensics (MF) is an interdisciplinary branch utilized in various microbiological methods to study elaborately various pieces of evidence involved in criminal cases focusing on forensic attribution, bioterrorism, frauds, outbreaks, toxin or biological agent release. Microbial forensic investigations characterize nonbiological and biological (toxins, fungi, bacteria and viruses) evidence. Recently DNA sequencing and next-generation sequencing technology have given us the opportunity with advancement in genetics and molecular biology and have been applied in fields including disease diagnosis in forensics. Metagenomics is a culture-independent method to sequence microbial DNA which is collected from mixed community samples and environment. Several studies which shows applications for analysis of metagenomic sample for investigations in forensic science i.e., cause of death, detect time since death, biological fluid characterization, outbreak investigations, environmental samples and bio-surveillance. The major microbial diversity which is unaware of and so the present database does not accurately show the diversity which exists and thus various programs have started that sequence the reference genomes. Application of bioinformatics deals with the analysis of biological data and phylogenetic reconstruction. The analysis and interpretation of microbial forensic science data need to compare the data with a complete reference genetic database. Proper training and education in this field are essential for the scientists of the next generation to protect society from various causative harm which results from the act of biocrime and bioterrorism. Thus the development of various resources and infrastructure related to education must be aimed at practitioners of next-generation, policymakers, researchers and communities related to enforcement of the law.

KEY MESSAGE: This review provides insight into microbial forensic and investigations based on next-generation sequencing technology, metagenomics, and bioinformatics. With adequate training and education in this field, scientists can protect the society from various harms.

KEYWORDS | microbial forensic, microorganisms, forensic investigation, next-generation

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INTRODUCTION

RESEARCH IN MICROBIAL FORENSIC (MF) have made many changes concerning development, evolution and expansion, incorporation of latest technologies, various tools, and implementation of various capabilities of analytical nature encouraging the cumulative preparation and its response. Microbial forensics may be defined as the scientific area which is given for explaining various shreds of evidence from the acts of bioterrorism, or the use of biological agents to kill people or make a person sick, or unknowingly microorganism release and toxin for purpose of imputation.[1] This field is employed in various microbiological methods to study elaborately various pieces of evidence

which are involved in various criminal cases particularly focusing on forensic attribution, ranging from frauds, bioterrorism, biocrime, bacterial outbreak spread from pathogens, or accidental release of agent of biological nature or/and toxin.[2,3]

Microbial forensic is involved in careful examination of suspected laboratory materials which are microbe-related found at the scene of crime suspected laboratory, for attribution to forensic science and plays a pivotal role for development and investigation leads. Attribution is termed as the sample characterization with highest specificity, which for microorganism is at the strain or species level, isolate levelt. Usually pathogen outbreak observation and toxicology are not regarded as important elements of MF. As these topics are basic to MF, because pathogens emission can be purposefully or because of medical malpractice and by applying a reliable and careful protocol of surveillance for monitoring the pathogen which would give us invaluable information to differentiate from sudden and damaged spread of microorganisms (related with biocrime or bioterrorism).

In the past decades, the number of pandemic had been escalating.[4] During a bio terror attack, the biological agents are willfully released with the intention to kill a lot of people, and cause loss to economy, which is motivated by political, ideological, and religious beliefs.[5] The agent is often used as they are present in nature or engineered genetically and improved for mass destruction, results mortality and resistant to the presently available medicines or the vaccines. If we face the chances of attack by bioterrorist weapon it is important to identify the agent which is involved, to prevent panic among the population and also to contain the mortality and morbidity which is related to the spread of these agents.

Epidemiological forensic investigations:

Biothreats received renewed attention after the 2001 anthrax letter attack in the US. Not even a month had passed when 9/11 happened, which was a bioterrorism act which was committed with the use of Postal Service of United States as a vehicle for dissemination to purposefully dissipate the spores of Bacillus anthracis.[7,8] The biological agents which can cause harm can be easily available, less sophisticated, easily distributed and relatively cheap in comparison to the various types of weapons to cause massive destruction of masses. The weapons of biological nature is a continuous warning to mankind for various biocrimes and bioterrorism cases.[9]

The center of investigations for microbial forensic to detect and characterize facilities related to both biological agents, along with the nonbiological evidence. Biological agents comprises of fungi, bacteria, toxins, protists and viruses. Evidences of not biological in nature, such as delivery devices, additives of growth media is useful forensics microbiology, to provide investigation leads which also helps to conclude the methods for manufacturing and also for dissemination of the non-biological evidences and the analysis which is an important part of microbial forensics.[10] Microorganism and its related toxin are preferable tools because are less costly compared to the cost to culture them, is easy to procure them if endemic or which occur natural way, only minute quantity of biological materials are able to cause death or spread infection. There are a number of species of microbe that might be helpful as useful threat from biological weapons such as (plant, animal and human pathogens) around 1400 microbes cause infection to mankind.[11] The microorganisms which are dangerous to national security and public health are listed in Center for Disease Control and Prevention (CDC).[9]

Toxins are produced by fungi, bacteria, eukaryotes plants, and Botulinum toxin, which are synthesized by Clostridium botulinum, which is a bacterium and a potent harmful agent which is dangerous and has lethal effects when consumed also in low doses.[12,13] There are several evidences of Phylogenetic studies which has been used in various law courts to give

relevant explanations regarding these crimes which involves infectious microorganisms. By constructing strain phylogenies and species and by utilizing the various information, such as disease transmission is concluded from an individual to another during infection and transmission of disease.[13,14] Outbreaks of disease is incident throughout the world and occur every year, and investigations in these outbreaks includes microbial forensics investigations and epidemiology. Public health and microbial forensics have similar interests regarding the genetic characterization and identification of various biological agents and how it can be dispersed in a given population.[15] A common path of microbial forensics and public health is to determine whether the transmission is accidental, intentional or natural. Epidemiology and forensics microbiology both are combined fields, which according to law focuses to attempt and individualize the toxin or agent and how it will be produced and and dispersed by law enforcement concentrate and microbial forensic scientists.

Detection and characterization with traditional methods and their drawbacks:

The primary goal in microbial forensics is to compare the data received from various evidence samples to reference samples. The evidence can be from various samples which includes water, swabs, air filter, food, soil, water, samples related to clinical specimens (e.g. sputum, blood, tissue, stool, urine) in forensic microbiology. So, an analyst should have various methods for processing sample to meet the growing needs very large number of sample possible in the present scenario.[9]

Methods to detect traditional manner in the microbial forensics can vary from microscopy culture. The best way to detect pathogen is only by culture. Drawback of culturing is that it is not able to give proper resolution, beneath the level of species or genus, and as there may be lag time that is substantial, it is also not so effective particularly safety concern point of view of the of individuals. Around 99% of the microorganism are unable to be cultured by present methods.[16] Biological evidences in addition to other threatening agents of genetic nature, like immune response of host, gives us a very valuable information related to investigation leads such as if a suspected perpetrator had taken antidotal substances or prophylactic antibiotics, decided by the manufacture, handling, possessing a agent of biothreat.[17] Traditional forensic evidence like human or animal DNA, fingerprints, hair and fibres can be analyzed. Immunoassays and culture are potent methods for sample screening ,initial testing , nucleic acid typing is quiet resolving.[9]

Emerging methods for detection and characterization:

Traditional forensic evidence like human or animal DNA, fingerprints, hair and fibers can be analyzed. Immunoassays and culture are potent methods for sample screening,initial testing, typing of nucleic acid is quiet resolving.[18] MLVA [multi-locus variable number tandem repeat (VNTR) analysis] for the analysis of polymorphic regions.[19,20] The SNP marker detection approach is the use of microarrays, which consists of many large numbers of small oligonucleotide probe. Microarrays, which can be highly efficient characterization and screening tools, have been developed specifically for viral and bacterial identification.[21,22] The marker detection SNP approach which is microarrays use, which comprises of huge numbers of probe of oligonucleotide. Microarrays, which is very efficient characterization and screening tools, have been developed particularly for viral as well as bacterial identification.[17]

Sequencing: DNA sequencing technology has given us the opportunity to enormous advances in genetics and molecular biology. However in Sanger sequencing technology the disadvantage is its high cost, low throughput and difficulties in its operation.[23]

Next-Generation Sequencing (NGS) technology: The development of next-generation sequencing (NGS) technology, which has high-throughput capacity and low cost, has averted these problems to a

large extent, and the technologies which is applicable to many infields which includes disease diagnosis forensics, ancient DNA analysis and agrigenomics.[24-26] Non-Sanger-based NGS technology refers high-throughput DNA sequence technology. DNA molecules can be sequenced in millions or billions copies, thereby increase the amount of product to minimize and substantially the requirement of method of cloning a fragment which is used in sequencing by Sanger method. Based on loop array sequencing, includes second-generation sequencing, which analyses simultaneously a number of samples, which can study the composition of base of single DNA molecule in third-generation sequencing technology.[27]

(i) *Analysis of Short Tandem Repeats* (STRs): Now a days, majority of forensic nucleic acid based tests employ capillary electrophoresis (CE)-based fragment analysis and PCR methods to identifychanges in short tandem repeat (STR) markers. In forensic science nucleic acid applications based various technology used for investigative purpose in forensic science has provided DNA analysis as an essential techniques. DNA analysis in forensic science is confronted with highly degraded DNA sequence of contaminated samples less copy number, with high reproducibility and accuracy, with cost and time considerations.[27]

(ii) *Analysis of Mitochondrial Genome*: Recently forensic mitochondrial DNA (mtDNA) analyses mostly detects in hypervariable area the polymorphism. But in case of mtDNA which may be used as haplotype marker of genetic nature, addition to loci which is polymorphic in nature are necessary to elevate the power to discriminateto identify. So, NGS technology have the ability to help in the evaluation process and analyze theentire mitochondrial sequence.[27]

(iii) *Analysis of Y Chromosome:* In forensic molecular biology the markers of genetic nature which is assumed an important role and play pivotal role is located on Y chromosome. The male component of DNA mixtures need to be resolved unambiguously, commonly Y-STRs which is used and present with high female background, or to create relationships of paternal side between different male individual. The two male individuals which is used in NGS technology had shared the identical ancestor around 13 generation before and because of this more than 10 million Y chromosome nucleotideswere comparatively studied.[28] Additional information present within the human genome may gives us an ideaof personal characteristics such asphysiological and physical characteristics, age and ethnicity.[29,30]

(iv) *Analysis of phenotypic and Ancestry inferences:* Previous studies suggested SNPs resembles hair and iris color with an accuracy of 90 per cent.[31,32] Evidences are there for which investigation on features of facewith the help of test onDNA and association were analyzed and legally approved with the results using face reconstruction.[33]

(v) *Forensic Microbiological Analysis:* forensic microbiology is current area originated by the Federal Bureau of Investigation (FBI) just after the attack by Anthrax held in USA on 18 September 2001. This area is decided on the accurate identification quick detection of microbes found in crime area of biological nature, which has the target to trace the microbe.[34] Terrorist attack by using microbiological forensic led to disastrous consequences therefore the microbiological forensic analysis had attracted a good attention.[35] With the help of sequencing by whole genome by the solid system, in which particular suspects by sequencing four strains each of Yersinia pestis[36] and Bacillus anthracis. To identify biological traces in a study of 454 sequencing systems using metagenomic analysis and deep sequencing which suggests that the technique is used to identify of forensic material of traces of traces of biological samples.[37] In another study they studied that the bacteria which is left byskinpossess enough DNA information for forensic analysis[38] of human using NGS-based metagenomic method.

(vi) Epigenetic analysis:Various studies recently have suggeststhe epigenetic markers

can also have many uses in in microbiological forensic. Example, evidences suggests that markers of epigenetic may be helpful to diffrentiate monozygotic (MZ) twins, accurately determine the age and predict tissue type of a DNA donor.[39,40] Epigenetic approaches based on NGS technology include reduced representation bisulfite sequencing and methylated DNA immunoprecipitation sequencing, methylation beadchips and whole-genome bisulfite sequencing.[41,42]

(vii) Analysis of MicroRNA: Introduction of microRNAs (miRNAs) in forensic microbiology which are endogenous RNA molecules small in size and of[18–24] nucleotide lengthwise. Due to the small size, high tissuewide diverse expression tissue specific or highly tissue wide diverse expression and resistance to degradation they are useful for post-mortem interval (PMI) inference analysis[43] forensic body fluid identification, identification of species. In astudy in 2009, miRNA profiling introduced to forensic science and found that 452 miRNAs were genotyped by quantitative PCR from forensic samples.[44] Another study shown that the expression levels of 718 miRNAs in semen, venous blood, saliva, vaginal secretions and menstrual blood were analyzed on a microarray. Out of these 14 expressed miRNAs wasrecognized, which might be act as candidates potentially identified for examination and to identify of body fluid. By the use of the technique NGS, sequences of millions of miRNA is analysed rapiedly thus provide a potential tool in analysis in forensic science.[36]

Metagenomics: Inmetagenomics applicationtosequence the DNA obtained from complex and environmentand mixed community samples. It's a culture-independent method for microorganisms study and analysis collected from environments such as soil, water and human-associated samples.[45–47] Species are not able to be cultured for identification[48] and around 1,030 bacteria exists on earth. Many studies have shown the applicability of metagenomic sample analyses for forensic investigations like for human identification, reason of death, to know the time of death, for biological fluid characterization and identification, for disease outbreak investigations, for environmental samples, and for public bio-surveillance.[49–54]

Additionally to detect target pathogen from the desired samples for metagenomic analyses, for biodefense purposes and epidemiological study in microbial community profiling in forensic microbiology utility. The types and conditions of sample is encountered in a microbial forensic investigation are fluctuating and may be added with different nucleic acid which makes the methods of detection quite challenging. Microbial forensics focusescomparative analyses and to detect and compare and analysisof important pathogen whichdetect agent veryrapidly from pure or homogeneoussamples. There are two primaryways for metagenomic sequencing, Whole-genome shotgun sequencing (WGSS) or targeting the 16S rRNA gene. Among these two methods, WGSS is more desirable for microbial forensic metagenomics analysis as species level resolution.[9]

Data analysis and interpretation: Bioinformatics deals with the methods to applyvarious computation method which analyses data biological in nature, such as massively parallel sequencing (MPS) data which is required in interpretation of data, statistical analyses, reconstruction phylogenetically and representation of data visually. With the evolution of MPS and the onslaught of data sequence many data management systems and bioinformatics software tools had been developingfor metagenomic assembly by utilizing software tools for, taxonomic classification, phylogenetic analysis, entire metagenomic analysis pipeline and database analysis and management systems.[55–59]

Challenges in result interpretation and validation of Microbial Forensics:

Microbial analyses which is of ancient originis one more branch of forensic related targets in expanding the limits to analyze samples challenging in nature. The past history

suggests that spread of disease has led to the deaths of many people across the globe. In few of epidemics the actual cause or the agents causing is known, however for some past epidemics the causative agent was controversial or stayed a mystery.[60] In recent had shown to improve extraction technique with highly sensitive detection assays, and with various sequencing technologies have been developed enhanced the ability to characterize genetically these ancient pathogens from skeletal remains and other sample types. Because of damaged properties and fragmentation of ancient DNA, novel library strategies and preparation methods and bait using comprises of nucleic acid sequences which was used.[61,62]

Proper interpretation and evaluation of forensic microbiology evidence is very important and cannot be ignored to establishconfidence, in scrutinizing the legal system to making critical decisions and policy. Though, consequences were devastatingwhich occur we can prevent it which depend on results of microbial forensic. The interpretation of standard guideline in the field of microbial forensics is lacking, thus the analysis of phylogenetic trait has association which support them and has admitted as evidence in proceedings of legal and criminal cases successfully in United States also abroad.[12] Reconstructing phylogenies has been used as a Microbial forensic tool is used to reconstruct phylogenies convicted individuals in cases of intentional infection with RNA viruses.[13,14] The girlfriend of Dr. Schmidt was injected with with a mixture of hepatitis C virus (HCV) and HIV infected blood his two patients.[63] Data study of a particular region can plays an important role in investigation of forensic microbiology investigation to check the presence of a microbes probability being due to a intentional outbreak or natural means. In 2009 injectional anthrax of many cases was diagnosed for population taking heroine in Scotland. In ScotlandB. anthraciswas not endemic. WGSS and SNP genotyping was used to determine origin and strain of the B. anthracis spore, that was introduced in Turkey or its surrounding area.[64]

Databases are an very important tool in forensic microbiology. Microbial forensic interpretations with analysis require the comparison of genetic data to completely characterized references available in databases. Databases must includes as possible as many strains of species, in addition to near-neighbors and other microorganisms representative of a range of phylogenic organisms. Metadata are the information associated with a given sample like date of collection, collection site location, tissue source, extraction and sequencing methods, virulence, assembly and annotation methods and can be used to determine endemicity and other information. Metadata are important to epidemiological investigation which provides information to aid in therapeutics ,source tracing, and essential to microbial forensic investigations provide a valuable information for investigative purposes.[17]

The microbial diversity remains in majority and still unknown so the present database does not reveal the range of actual diversity which occur and programs has started to know the reference genome.[65] In the area of microbial forensics the main target is on microbes which arepathogenic and infectious many of which are sequenced along with continuous improvementin MPS, so the total number of microbial genome which are available increases everyday. The Human Microbiome Project (HMP) consortium started theworkof sequencing new reference genomes to increase and interpretdisease state and health conditions of microbiome.100K Foodborne Pathogen Genome Project and Human Microbiome Jumpstart Reference Strains Consortium, 2010 targets to sequence 100,000 different food-borne pathogen which is helpful in various epidemiological related investigations.[66]

Role of education and training for awareness and implementation: To understand the forensic microbial field is required to determine which evidence is collected, what are the safe and proper methods related tocollect and preserve, techniques to analyze the evidences,

result significance, various supports to identify a prosecution perpetrator. Education along with training given in the field is necessary for preparing next generation scientists.[67] The applications, scientific bases, advance interpretation and lesson which is acquired by those who had actively involved in gaining knowledge on microbial forensic required to document and transfer the next generation scientistand decision maker to impart the society with better protection from potential harm resulted from act of biocrime and bioterrorism. Thus development of infrastructure of education and various resources should target to successive generation of practitioner, various research, diverse elements for the policy, and law enforcing communities.[67,68]

CONCLUSION

Forensic microbiology which is interdisciplinary branch which includes various law enforcement, scientists, public health, intelligence community, decision and policy makers. All of themhelp to give us a system which helps us to and give us protection from various disease outbreaks that occur naturally or epidemics and the act of intensely occurring biocrime and biological terrorism. Recent development in various methods at the molecular level, particularly next generation sequencing (NGS) technology and methods of whole genome sequencing, gives tools to forensic microbial scientists to find out crucial and important information with reduce them the cost than earlier. For the researchers it is imperative in the forensic microbiology to continuously carry on with their novel research in areas such as method and comparative genomic and bioinformatics software development, and to increase the databases. Continuous updates for evaluation and to quality control and assurance in forensics microbiology must be maintained and practiced up to their standard. Evaluation and result interpretation in forensic microbiology investigations should follow the right criteria with appropriate validation. Recent genome-based data and technology, databases with expanded reference genomes, validated method and endemic data together contributes to the proper interpretation of result in a forensic microbiology investigations. Confidence of results is based on high quality are important since forensic microbiology interpretation have huge impacts on our society, political policy, regarding economy and safety. Many challenges evolved and continuously exists with time in forensics microbiology but introduction and updated technology implementation and regular communication across the scientific, intelligence, law enforcement, policy makers and public heal this expected to contribute towards the advancement of the forensic microbiology. IJFMP

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REVIEW ARTICLE

Evolution of New Emerging Multimedia Tools for Crime Scene Sketching: A Review Literature

[1]Vipin Sharma, [2]Bhavna Sharma, [3]Meenakshi Verma

ABSTRACT

The documenting of the crime scene is a key duty which needs to be performed quickly, accurately and reliably, and highlights the evidence that may be used to further provide justice for victims and to assure that offenders are prosecuted. New emerging tools and soft-wares are used for creating crime scene designs viz; Sketching, Photoshop, Illustrator, Auto CAD and Sketch-Up. These tools play a crucial role in recreating the facts and figures to help and prepare the meaningful analysis of the situation. This article focuses mostly on documenting a typical crime scene and noting any potential pollution that might have affected its original look quickly. To measure and record exact positions of findings and functions, a Total Station (TS) is employed.

KEY MESSAGE: Sketching offers various instruments for manipulating, viewing and working with your model. The essay will not explore them completely, but will present the fundamental tools for the creation, modification and view of a model's sides and faces. Typical scene drawings and models give a two-dimensional image of the scene (2D).

KEYWORDS | crime scene visualisation through sketching, 2d diagrams, 3d modeling

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INTRODUCTION

RECONSTRUCTIONAL SKETCHES OF CRIME scene is useful for interpretation of information of the investigation. All relevant observations need an accurate location in investigations into the criminal arena to safeguard the custody chain for any findings or observations. Evidence placement is vital for the analysis of the spatial distribution of evidence, the support of the investigative report and the eventual rehabilitation.[1] The initial stage of the investigation and inspection of the crime scene quickly records a full, objective picture of the crime scene without incorrect information. The display of the 3D crime scene is more efficient and intuitive than previous techniques.[5]

In this paper we will try to summarize the concept of crime scene sketching including various tools that helps to depict the frame of situation cited including the modern techniques like crime scene software imaging, 2D–3D modeling etc.

METHODOLOGY

Distribution centers that were utilized in this article: Benji_02, Christopher Clepoint, Elfpainter, GE Apparatuses, Highlander, Joseph Briggs, KARE Plan/SketchUp, Kostyan Novikoff, Emblem Cabinetry, Woodsmith, SketchUp Group, FORENSICnetBASE, Criminal science Assortment (ProeQuest), Web of Science, Exploration Door including the Public Criminal Equity Reference Administration (NCJRS) Edited compositions Data set, with additional record and full-text

inclusion of academic diaries. It additionally incorporates remedial and law implementation exchange distributions, wrongdoing reports, wrongdoing sites and other applicable material for analysts or those planning for professions in criminal equity, law authorization, and related fields. The accessible distributed and unpublished writing was looked up to September 2020 comprehensive.

Dispersion focus that were used in this article: Benji_02, Christopher Clepoint, Elfpainter, GE Contraptions, Highlander, Joseph Briggs, KARE Plan/SketchUp, Kostyan Novikoff, Symbol Cabinetry, Woodsmith, SketchUp Gathering, FORENSICnetBASE, Criminal science Variety (ProQuest), Web of Science, Investigation Entryway including the Public Criminal Value Reference Organization (NCJRS). Altered pieces Informational collection, with extra record and full-text incorporation of scholarly journals. It also consolidates healing and law execution trade appropriations, bad behavior reports, bad behavior destinations and other pertinent material for examiners or those getting ready for callings in criminal value, law approval, and related fields. The available appropriated and unpublished composing was admired September 2020 far reaching.

Studies were rejected from the examination for any of the reasons: article didn't have adequate information; copy distribution of a similar report; and articles accessible in conceptual structure.

Reconstructional Tools and Investigation

Passive recording of the crime scene, such as pictures is already generally understood inadequate. A task requiring particular knowledge is necessary for active reconstruction documentation.[1] Since the 1970s, when computerised 3-D models were first produced, computer graphics were utilised to increase the visualisation of the forms and structures, Until recently, however, computer software is primarily use for 3-D modelling. To generate a useful 3D model, we need a modeling specialist and AutoCAD software. In the view of the time demanding and expensive nature of the 3D modeling procedure, researchers use free trial version of 3D modeling software for every investigation, until the investigation team or crime situation not demanding.[6]

In certain situations it is more evident than in other cases that it's important to make a map of the crime scene, indicating where the evidences are placed. For instance, situations with bloodstain patterns already have apparent evidentiary dispersion. For situations with invisible traces in the image and invisible evidence, it is less common to visualise their geographical dispersion even though a map is important to comprehend where they have been located. An investigator into the crime scene utilises four main documents: reports and note-taking, photographs, and videography, as well as mapping or drawing.[7-8] There are advanced ways for documenting, such as 360° photography, 3D laser scans and a Total Station (TS) and/or photogrammetries, but require particular know-how. In the Netherlands, the Visualization and Reconstruction Team of the National Police (ETVR) is using these approaches. Investigators from the criminal scene and visualisation specialists document two separate elements of the crime scene, each one of which has evidence. The context of the evidence and its relationship. Both are necessary for rebuilding. But it is impossible to deduce the exact location of the proof from pictures or to make it evident in the 3D scans. This is where a hiatus in crime scene documentation arises.[1,3] There is a fairly high learning curve with many modeller tools, thus it is frequently not enough to devote time to master tools to get even simple results. [9-10]

Fortunately, as researchers have made interaction with their computers more comfortable and graphical software easier to use during the past few years, the typical investigator has new alternatives accessible. One instrument in particular, Sketch-Up (version 8), truly flattens the learning process and gives any researcher wanting to spend some

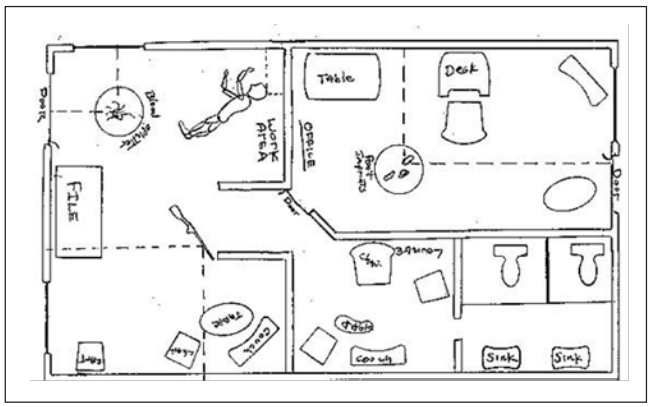

Figure 1: Rough Sketching: Crime Scene

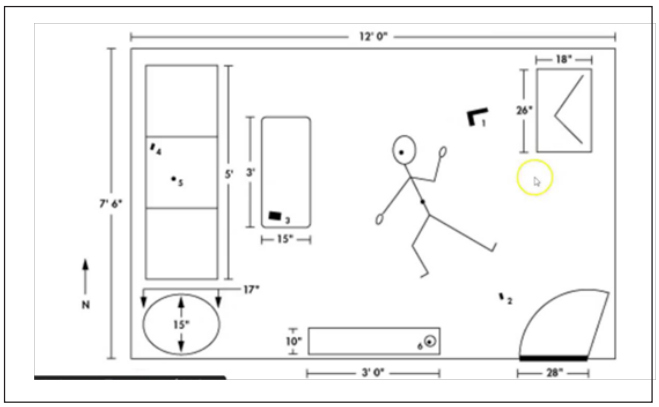

Figure 2: Crime Scene 2D Sketching: Adobe PhotoShop/Illustrator

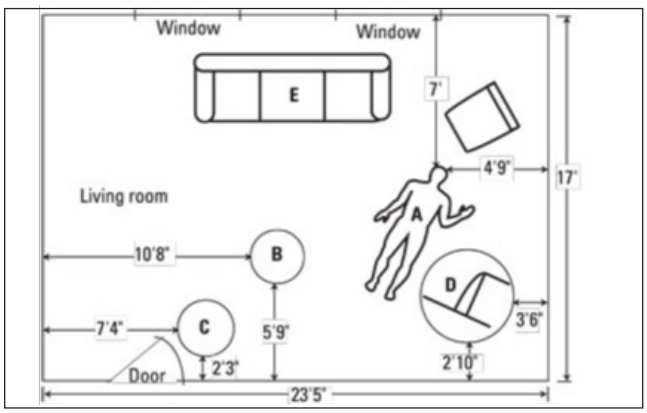

Figure 3: Crime Scene Sketching: Triangulation Method

Figure 4: Crime Scene 3D Model (SketchUp)

time studying the programme a fundamental 3D modelling.[2,7]

The graphics of a crime sight are visual designs designed to convey details seen in a real place. They include basic, hand-drawn (often termed rough sketches), complicated, complex designs and interactive, realistic reproductions. The two-dimensional (2D) top-down representation of the scene is presented in typical scene designs and model models. Although 2D drawings and models are valuable to show space information acquired at scenes, 3D models can communicate such information more effectively. With Sketch-Up, the authors suggest that researchers who have no professional expertise in graphics may build realistic models for the 3D crime scene without changing the way they are doing the scene and even construct to-sized 3D models provided enough measurements are given. When Sketch-Up is started, a popup will show the user that allows the project to pick a drawing template. Templates are not changing the manner in which sketches are done; they just offer the default scale, style and color for new projects. Any parameters that can still be modified following the creation of the project. Sketch-Up has many integrated templates for both imperial and metric units and may build and save user-specific templates for future use.[2,8]

In Sketch-Up, the authors suggest that researchers that don't have professional graphics education are capable of producing realistic 3D crime scene models, without modifying the processing of this scenario.[2,4]

Sketch-Up offers several tools for manipulating, viewing, and using your model. This paper will not explore them completely,

but will present the most fundamental tools for creating, modifying and viewing the borders and sides of a model. The Large Tool Set palette had all the fundamental tools for the example provided in this article, but most are intended for specific work. When you pick a simple tool, you will receive instructions on how to use the status indicator at the end of the window. The question mark icon next to the status indicator will launch the instructor window, which helps researchers learn about the fundamental and advanced functions of each tool.[2,9] The authors highly recommend new users to use the free lessons on the Sketch-Up website (http://www.sketchup.com/ intl/en/training).

All these instruments allow the modeler to draw 2D forms on a certain plane. What's the 3D translation like? If the borders of a 2D form are closed (there are no gaps), SketchUp tones the form surface to show that the face may now be modified. The Push/Pull tool allows the user to click any face, to push it or to pull with the mouse to turn a 2D form into a 3D one. Typing a number into this tool will alter the size of the form to allow the user to produce cylinder and boxes with certain proportions.

CONCLUSION

Modern techniques like Rough Sketching Layouts, Adibe PhotoShop, Illustrator, AutoCAD and SketchUp are the most used method for 3D Portrayal of entire crime scene in the court room. On the contrary, this is not being applied in the conventional method of sketching. So, it is concluded that the modern techniques like crime scene software imaging etc., are creating revolutionary benchmark in criminal justice. IJFMP

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CASE STUDY

Forensic Psychological Investigations for Corporates: A Case Study

[1]Deepti Puranik, [2]Rukmani Krishnamurthy

ABSTRACT

Forensic Psychology is the only field of Forensic Sciences that deals directly with live human beings and hence is a unique field in the entire Forensics. This field explores the brain mind interactions and how these interactions give birth to various crimes. In India more emphasis is given on scientific techniques such as test for Detection of Deception or Investigating interviewing and Psychological Evaluations which are mostly conducted in Forensic Laboratories and mostly in Criminal Investigations. However, these psychological tools for Investigation can be used even in Corporate crimes or even by Vigilance Department for their internal investigations and can be extremely crucial. This study highlights the application of Forensic Psychological Investigations in Corporates through a single case study and further discusses the necessity of these Psychological Investigations for Corporates Internal Investigations.

CONCLUSIONS: It was concluded that titanium is already present during the manufacturing of rexine and paint therefore, the nanoparticle coating of TiO2 doesn't create a large difference. Besides, there was a significant difference in

KEYWORDS | forensic psychology, polygraph, investigative interview

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INTRODUCTION

Forensic psychology is a unique field as it is the only field of Forensic Sciences that deals directly with live human beings. When a Professional Psychologist applies the psychological principles of various subfields such as Clinical, Developmental, Neuropsychological, Social, cognitive, etc. for the purpose of assisting the legal system, would come under the purview of Forensic Psychology.[1] Forensic Psychologist plays various roles in assessments of Mentally ill offenders, selection of jury and consulting with lawyers, exploring the factors leading to crime as well as work in civil areas such as guardianship or in family court, sexual harassment cases in Organizations.

All crimes originate in the brains of the perpetrator and the brain is one of the most complex systems in the entire world. One of the important roles of a Forensic Psychologist is working along with Investigating officers for unraveling crimes by exploring the modus operandi, motivations, and plans about the crime. Crime is something that has an impact on each and every individual's life either directly or indirectly and an observable increase in the crime rate is seen in our country. Criminal investigation has then played a very significant role in curbing the offenses to a large extent. The exposure of media and other resources has created much awareness even for offenders for destroying physical evidence left on the crime, and thus making it relatively difficult for the investigating agencies to gather evidence which

will lead them to the offender.

A crime generally encompasses a body or a property, but sometimes even organization can be victim of attacks or crimes and the crimes in organizations may be known as "Corporate Crime" "Corporate Frauds", "Business Crime", "White-Collor Crimes", Corporate misconduct", "Corporate misbehavior". The crimes happening in a corporate setup can be detrimental for the progress of the organization, the employees, the clients and the society at large.[2]

A number of scientific studies on Forensic Psychological cases have been published in various journals highlighting the importance of Forensic Psychologist in Criminal Cases. [3-5] However, role of a Forensic Psychologist in Corporate or Organizational Behavior have not been studied much in India.

The research in Forensic Psychology have had great interest in methods of detecting deception. To ascertain whether subjects are lying or hiding something related to the event took place. The first approaches to deception detection relied on anxiety induced autonomic indicators, using polygraphs measuring pulse, blood pressure, respiration, and galvanic skin response. An executive summary report by American Polygraph Association, 2011 found that Polygraph Technique intended for event-specific diagnostic testing produced an aggravated decision accuracy of 89% (confidence interval of 83%-95%), with an estimated inconclusive rate of 11%.[6] A study by National Research council in 2003 concluded the accuracy to be 81% to 91% which certainly provides strong scientific basis of its applicability in detecting deception.[7] Polygraph was used even for pre-employment screening or credibility assessments in organizations. The Employee Polygraph Protection Act 1988 protects the employees from being dismissed, as the results themselves cannot be solely considered to be admissible as evidence in the courts. However, the Act permits the Employers are permitted to use Polygraph Test on Employees in cases of Financial Loss due to suspected data theft, damage to the organization's brand, sabotage, etc. with the consent of the person.[8] Even in India, the voluntary 'Informed Consent' of is the pre-requisite for conduction of Polygraph Examination.[9]

Another technique which is used globally in the area of Forensic Psychology is Psychological Interviewing. Generally, the investigating officer interrogates his suspects while a Forensic Psychologist uses interview as an authentic tool for gathering information from his or her client. The Interrogation technique focuses on extracting confession, whereas Interviewing technique focus on being more open ended. Certainly, Forensic Psychologist needs to elicit information from the subject without coercing into making a false confession. There have been various models derived for Investigating interviewing of which structural model, peace model and Cognitive Interview are commonly used. The structural model focuses on minimizing resistance and focusing on increasing the internal stress on subject by applying the suspect's perception about the availability of evidence against him.[10] The PEACE model focuses on "planning, preparing, engaging, explaining, account clarification, closure and evaluation" while the Cognitive Model focuses on Memory and cognition for better retention of information.[11] Hoffman (2005) observed Empathy, Communication and Professionalism as imperative skills for investigating interviewing techniques. [12]

This paper highlights the combination of Forensic Psychological tools such as Polygraph and Investigation Interviewing in an organizational setup for aiding corporate investigations.

CASE STUDY

An organization having its global presence suffered a major financial loss because of some sort of confidential data theft which was shared with its clients as well as competitors. Such an event directly cost a major financial loss and reputation of the organization. All the confidential information was passed without the use of technology and sent via regular post

office.

Investigations pointed towards a particular person 'A', however, few days later another person 'B' came and confessed to have done this act under the pressure of person 'C'. He further stated that he is extremely guilty for his actions as he did not intend to damage the organization's reputation.

The complications in this case were scrutinized and Forensic Psychological techniques helped in gathering lot of inputs on this matter. After the initial interview with investigating officers, Psychological Profiling and Polygraph Examination was conducted following all the protocols. 'B' came out to be clearly Deceptive on few questions, while 'C' came out Truthful on all the questions. The focus was shifted to 'B', who underwent Investigating Interview for the purpose of further exploration and understanding.

The interview was conducted in 3 phases.

Phase 1: Confrontation:

This phase focused on minimizing resistance by presenting the report of the Polygraph Report and followed by an open-ended Interview. The open-ended interview was aimed at getting more aspects of the entire case with miniscule details about this entire incident. This interview phase helped in establishing the fact that 'B' was the only person responsible for the entire incident and 'C' was in no way responsible or involved in this incident. This was also supported by the Polygraph report of the individual.

Phase 2: Exploration

This Phase was exploration phase and the Forensic Psychologist focused on understand the links between 'B' 'A' and 'C' which could give insight into the roots for the cause of this entire incident which led the entire organization to bear loss of crores. This phase of interview helped in revealing the revenge angle that had cropped up.

Phase 3: In-depth Analysis and Closure

The third phase was the most important phase as it was this phase that helped in putting all scattered pieces of the puzzle and giving holistic understanding, figuring out modus operandi and motivations by analyzing and linking various psychological behaviors and work environment combined with the Employee's perception and cognition.

DISCUSSION

In many cases, Forensic Psychological techniques can provide vital clues for resolving a crime especially when lack of physical evidence. Polygraph Examination clearly ruled out the involvement of 'C' thus helping the Investigating agencies focus on the involvement of 'B'. Though Polygraph Examination helps in differentiating a Deceptive person from innocent person, it could not help in gathering information on the root cause of a specific event. Helping understand the rationale for a crime occurrence can help further in prevention of similar event. Thus, the investigating interview technique could help in gathering this information. Application of thematic analysis helped in the emergence of ample of data which are discussed here.

The confrontation and exploration threw light on the retaliation and the revenge angle which was not revealed in the initial interviews. The factors such as animosity at workplace, no appreciation, being bullied were discovered. The fact that B was feeling left out, and C was taking all the limelight even for the work done by B was not acceptable to him. Rather than finding a solution to this issue, he tried to get back to C in an inappropriate manner. Walter, Brown and Weidlitzka found retaliation is one of the most important motivations for deviant behaviors.[13] The frustrations faced by the person at workplace are reflected sometimes through deviant behavior. This phase also revealed the friend turned foe angle which further created fire in the heart of B. The fact that someone i.e. 'A' who was extremely closed now hates and makes fun could not be accepted. Further, the fact that 'B' always perceived

himself as a loyal employee and well-wisher of the organization but not getting the deserved recognition or appreciation from his seniors worked as fuel. Failures at workplace increase stress levels and can lead towards deviant behavior. One major factor, which was explored through this interview was that 'B' perceived himself to be harassed by all his co-workers and Managers. He was always laughed upon for his behavior by his colleagues. Though ample of attention has been paid to cyberbullying or children getting bullied, workplace bullying is an area which is completely ignored especially in India.

Further individual factors such as family and personality have positive correlation with deviant behaviors observed through research studies.[14,15] An exploration into B's family history and family interactions revealed lack of conducive social interactions. B did not share good relations with his father or any other relatives. He was always considered as a nerd or a geek. He only shared positive relationship with his mother. He never had any friends and hence he was more hurt by the fact that his only friend A has also turned into rival which was unacceptable at any cost. Further, no marital relations also indicate dysfunctional family interactions. Hirishi's social control theory emphasized the increase in deviant behavior as a determinant of weak social relations of the individual with significant others.[16] A thorough analysis of this case revealed lack of assertiveness, low problem-solving skills as some traits that may have been negatively influenced the deviant behavior. However, since this was a case study and cannot be generalized to normal population. Risk assessments by Forensic Corporate Psychologist at organizational levels can prove to be helpful especially when there were multiple factors contributing to the deviant behavior.

CONCLUSION

Forensic psychological techniques is an authentic tool that can be used as an aid to investigation. When analyzing any case from forensic psychological viewpoint and to get holistic understanding of a multiple technique can be used for the purpose of assessment. This would help in identifying truthful responses as well as highlighting the modus operandi as well as the motivation for the crime. Forensic Psychologists can bring out those aspects of a case that technological advances cannot, because human brain is wired differently. IJFMP

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