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Original Article

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Study of Sickle Cell Anemia in Tribal Area of Thane Region of Maharashtra

Raviraj Naik[1], Sarita Dakhure[2]

Author Affiliation: [1]Assistant Professor, Department of Biochemistry, Indian Institute of Medical Science and Research (IIMSR), Warudi, Jalna, 431202, Maharashtra, India. [2]Assistant Professor, Department of Pathology, GMC, Aurangabad, 431001, Maharashtra, India.

Abstract

The cross sectional study was performed to find out the prevalannce of sickle cell anemia in tribal population of Thane district; Maharashtra from July 2012 to December 2013. The study was conducted at GMC & Sir JJ group of hospitals ; Mumbai. In this study we screened 1524 tribal subjects comprising adult men & women as well as children. Whole blood Samples were collected in EDTA bulb in different camps organized in PHC & RH centres located in tribal region of Thane as well as samples were also transported by health workers from these tribal areas health centres to Biochemistry special investigation laboratory. Samples were analysed by Automated High Perfomance Liquid Chromatography analyser. We found that 11% of tribal population was having sickle cell trait & 0.5% of were suffering from sickle cell disease.

Keywords: Sickle Cell Anemia; HPLC; Tribal Community; Chromatograph.

Introduction

Indian tribal populations constitute 8.2% of the total population [1]. In all, 461 scheduled tribes have been listed and they have their owncharacteristic cultural patterns, languages and social systems, by and large keeping to themselves [2]. However, Reich *et al* [7] concluded that "several thousand years ago, the entire subcontinent underwent a period of massive intermarriage, shuffling its population's genetic deck so thoroughly that it left clear traces even in the genomes of today's most isolated tribes [3]. Many population groups have been screened and the sickle cell gene has been shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes in India [4,5,6]. The prevalence of sickle cell carriers among different tribal groups varies from 1 to 40 per cent [7]. In Maharashtra, the sickle gene is widespread in all the eastern districts, also known as the Vidarbha region, in the Satpura ranges in the north and in some parts of Marathawada.

The prevalence of sickle cell carriers in different tribes varies from 0 to 35 per cent. The tribal groups with a high prevalence of HbS (20-35 %) include the *Bhils*, *Madias*, *Pawaras*, *Pardhans* and *Otkars* [8]. Sickle cell disease(SCD) is one of the most common monogenic disorders globally with an autosomal recessive inheritance [9]. Sickle cell anaemia is an autosomal genetic disorder caused by a defect in HBB gene (β 6Glu→Val). The beta (β) globins gene is located on short arm (i.e., P-arm) of chromosome 11 and there are over 475 allelic variants. HbS (Haemoglobin S) is responsible for sickle cell disease, one of the most prevalent genetic diseases, affecting millions of people in India. Individuals who are sickle cell carriers are referred as sickle cell trait and do not express symptoms of sickle cell disease. Either double

Reprint Request: Raviraj Rajan Naik, Assistant Processor, Department of Biochemistry, IIMSR Medical College, Warudi, Aurangabad. University, Aurangabad-431005, Maharashtra, India.
E-mail: raviraj_40@yahoo.com

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copy of the HbS variant on both the chromosomes or one copy of HbS and one copy of HbC variant on different chromosomes results into disease manifestation. In addition SCD results in combination with mutation for beta thalassaemia on other chromosome [10].

The sickle cell gene in homozygous condition was found to be lethal in Africa [11]. The homozygous apparently suffer from severe anaemia and die before attaining reproductive age without contributing to the gene pool of the population. Majority of the children born with SCA die before the age of 5 years [12]. The incidences of SCT are higher among the tribal groups than other caste populations [13]. It was found that the trait was present to the extent of about 10% in African origins. The absence in other racial group led to the belief that sickling was an exclusively an African gene. Lehman and Cutbush reported the presence of the trait in considerable frequencies in some of the tribal populations in and around Nilgiri Hills in South India [14]. Buchi was of the opinion that "the sickle cell cannot be a character of Weddis as a whole". He further pointed out that "the possibility of direct contact with the African for the introduction of the trait in India than independent mutation". SCD is by no means an African characteristic alone [15]. Ingram obtained the molecular change in the haemoglobin molecule of SCD – Chemical structure Position of amino acid [16]. Normal HbA H3N – Val – His – Leu – Thr – Pro – **Glu** – Glu Sickle HbS H3N – Val – His – Leu – Thr – Pro – **Val** – Glu The primary pathophysiology is based on the polymerization of deoxyHbS with formation of long fibers within the RBCs causing a distorted sickle shape which eventually leads to increased haemolysis and vaso-occlusion of sickle red cells. However, the clinical presentation of SCD patients is extremely variable and there are several events that may trigger vasoocclusion. Recent work has shown the importance of red cell dehydration,abnormal adhesion of RBCs to the vascular endothelium, imflammatory events, and activation of all the cells in the vessel and abnormalities of nitric oxide metabolism in the pathophysiology of this multi-organ disease [17]. Most of the early studies on epidemiology of sickle haemoglobin in different parts of the country used the sickling or the solubility test and in many reports this was followed by Hb electrophoresis to determine the phenotypes. However, in recent years, high performance liquid chromatography (HPLC) analysis has been used in many large programmes to identify carriers of both sickle haemoglobin as well as â-thalassaemia. Capillary electrophoresis has also now been introduced at some centres Nonetheless, even the simple and cost-effective solubility test has been shown to have a sensitivity and specificity of 97.4 and 100 per cent, respectively in comparison to HPLC and could still serve as a good first line screen for sickle haemoglobin in remote areas where other facilities are not available [18].

Tribal populations also have a high prevalence of β-thalassaemia. The role of these genetic modifiers in reducing the severity of the disease in tribal groups was first shown by studies done in Odisha [19]. Subsequently, studies have shown that tribal groups in Gujarat and Maharashtra have a milder presentation than non-tribal populations with the rates of painful crises, infections, acute chest syndrome and hospitalizations being fewer in them. This has partly been attributed to the very high prevalence of β- thalassaemia (90 to 97 %) in some tribes and/or much higher foetal haemoglobin (HbF) levels [20,21]. There are little data on the maternal and perinatal outcomes of women with sickle cell disease in India. A prospective study from Odisha showed that neonatal outcomes such as low birth weight, perinatal mortality rate, admissions to the neonatal care unit, intrauterine growth retardation and preterm births were significantly higher in sickle cell anemia mothers with successful pregnancies being achieved in 84.44 per cent of cases [22].

Maternal and perinatal outcomes were also evaluated retrospectively from patients' case files in women with sickle cell disease in a tribal population in Madhya Pradesh. There were 25 deliveries to women with sickle cell disease and preeclampsia and disseminated intravascular coagulation were common problems. There was no maternal mortality; however, there were five intrauterine foetal deaths and one early neonatal death [23].

Although there are significant advances in the management of sickle cell disease, yet increased morbidity and early death are not infrequent. Thus, prenatal diagnosis remains an important option for couples at risk of having a child with homozygous sickle cell anaemia, sickle-β-thalassaemia or HbSD disease despite the fact that it is impossible to predict the severity of the disease and many individuals may have a milder clinical presentation. With increasing awareness in the community more couples are opting for prenatal diagnosis [24,25]. Most of the tribal populations where sickle cell disease is common rely on the primary health care facilities in rural and often remote areas. Thus, the goals of medical genetic services should be to help these people with a genetic disadvantage and their families to have access to quality care as well as social and genetic counselling support to make informed choices for reproduction

to have healthy children with the availability of prevention programmes when needed. The Indian Council of Medical Research (ICMR) under its Tribal Health Research Forum (THRF) activities as well as other programmes under the National Rural Health Mission (NRHM) in different States have initiated programmes to enable advances in genetics to reach these communities.

Aims & Objectives

Identify high risk population and to create community awareness followed by counseling to the affected individuals/ families about sickle cell disease for its prevention and management.

Material and Methods

Study Design

The current cross sectional study was undertaken from Mumbai; Maharashtra.

Study Period

July 2012 to December 2013.

Ethical Approval

The study was approved by the GMC Mumbai & Sir JJ group of hospitals; Institutional Ethical Committee.

Inclusion

All males and females aging less than 40 years.

Site of Sample Collection

PHC and RH hospitals of Thane Vasai taluka. (Tribal Belt)

Site of Sample Study

Special Investigation Biochemistry laboratory, department of Biochemistry, J.J hospital, Mumbai.

Material

EDTA bulbs; 3 ml venous blood sample ; Sickle cell short program recorder pack,contains(whole blood primer, wash solution – deionised water, elution buffer 1, elution buffer 2, analytical cartridges, retention time marker set of retention time marker 1 (FAES) and of retention time marker 2 (FADC) contains lyophilised human red blood cell hemolysates with preservatives.

Method

Sample collection was done by venepunture withdrawing 5ml blood inn to EDTA bulbs.The sample was stored in refrigerator at 2-80 c till further assay.

Sample preparation – A dilution adjustment of the sample may be required due to variation in sample collection, transport and storage. 5 micro lit of umbilical cord sample is taken in a vial each time with the help of a pipette. A 0.5 micro lit deionised water is added to each sample vial which is allowed to stand for 30 min at room temperature. Each sample vial is mixed by inversion. The sample vial is placed into the sample tray.

Sample annalysis is based on the principle of cation exchange high performance liquid chromatography (HPLC - BIORAD VARIANT TM). All steps are automated.Diluted specimens are maintained at 12 +/- 20 c in the automatic sampler chamber. Each specimen is sequentially injected into the analysis stream and then separated by the analytical cartridge. Two dual piston pumps and a pre-programmed gradient control the elution buffer mixture flow through the analytical cartridge.

The ionic strength of the elution buffer mixture is increased by raising the percentage of elution buffer 2. As the ionic strength of the mixture increases, more strongly retained haemoglobin elute from the analytical cartridge. A dual wavelength filter photometer (415 and 690 nm) monitors the elution from the cartridge. As the haemoglobin elute from the cartridge and pass through the photometer flow cell, changes in the absorbance at 415 nm are detected. The secondary filter at 690 nm corrects the baseline for changes caused by the buffer gradient. Changes in absorbance are monitored versus time, producing a chromatogram (graph of absorbance versus time).

Each haemoglobin has a characteristic retention time. Retention time is measured from the time of sample injection to the maximum point of each peak. Identification of unknown haemoglobin is accomplished through the comparison of the unknown haemoglobin's retention time with the retention time of known haemoglobin, analyzed on the same system. A built in integrator performs reduction of the raw data collected from each

analysis. At the end of each sample analysis, a copy of the chromatogram and report data is automatically printed [26].

Results

The screening was completed over a period of 18 months during which venous blood samples 1524 subject was collected, analyzed by HPLC and finally results were obtained as chromatograms.

Out of total sample screened; total number male tribal subjects screened was 990 while total number of female tribal subjects screened was 534. More delineation was made by separating total number of tribal children screened which was equal to 476.Out of this 330 were boys and 176 were girls.

Table 1:

Type of Sickel Cell Anemia	Male tribal subjects affected	Female tribal subjects affected	Tribal Boys affected	Tribal Girls affected
Sickel Cell Trait(Heterozygous)	87	58	22	8
Sickel Cell Disease (Homozygous)	0	1	3	4

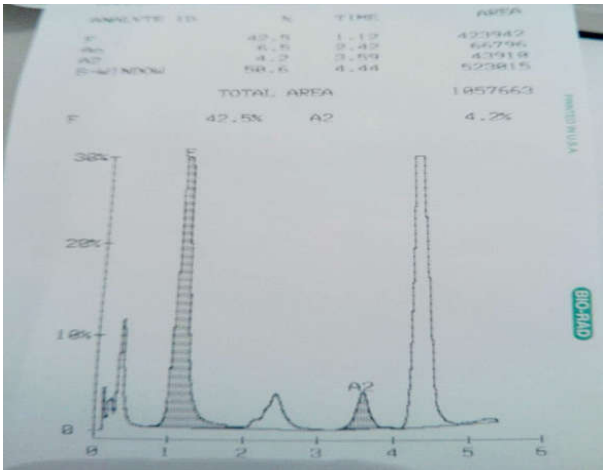

Fig. 1: Chromatogram of Sickle cell Trait sample. (Heterozygous)

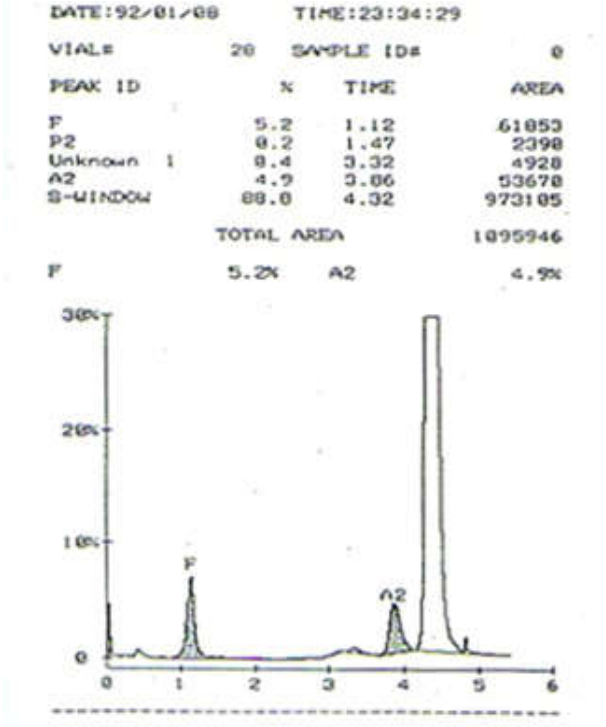

Fig. 2: Chromatogram of Sickle Cell Disease. (Homozygous)

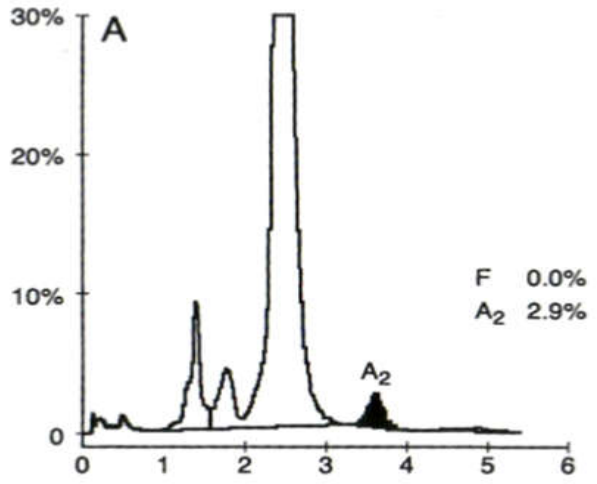

Fig. 3: Normal Chromatogram

On analysis of these chromatograms following observation were made.

Conclusion

We concluded that in our study in which total 1524 of tribal population was screened for sickle cell anemia by HPLC; 11% of tribal population was found to be affected from sickle cell trait & 0.5% of were suffering from sickle cell disease.

Discusion

When the samples of tribal population were analysed by using HPLC we found that about 11% of tribal population shared the burden of sickle cell trait while about 0.5% were suffering from sickle cell disease. Prepondrence of sickle cell gene in tribal

population was founded in number of studies which justified our selection of subjects in this study.Saha and Banerjee [27], Goud and Rao [28] while reviewing the incidence of sickle cell trait in Indian populations concluded that the HbS gene is mostly present in scheduled tribes and scheduled caste and very rarely in caste groups. The frequency distribution of HbS allele among various Indian populations groups has been reviewed and summarized by Bhasin *et al* [29]. One of the reasons for occurring in such a high incidences could be attributed to the practice of consanguinity among most of them and the similar findings were reported by Mukherjee and Das [30]. But it is surprising to note that why the other neighboring populations who live in the similar ecological niche do not exhibit similar trend for the SCT? In some of the tribal groups the HbS gene is completely absent [31].

Mukherjee and Das [30] are of the view that the highest gene frequency of HbS occur in region where malaria is highly endemic. HbS was largely absent in the areas of Horn of Africa and south of the Zambezi. Piel et al [32] shown a similar pattern of distribution of HbS frequency in malaria-free, hypoendemic ares of Africa.Some of the studies conducted on tribal population of Maharashtra vidharb region also found prephderance of sickle cell gene in their tribal subjects but prevalence was much higher than what we concluded in our study. The tribal groups with a high prevalence of HbS (20-35 %) include the *Bhils*, *Madias*, *Pawaras*, *Pardhans* and *Otkars*. It has also been estimated that Gadchiroli, Chandrapur, Nagpur, Bhandara, Yoetmal and Nandurbar districts would have more than 5000 cases of sickle cell anaemia [33]. Similar findings were shared by other studies on tribal population of states other than Maharashtra. The entire tribal population of 1,25,000 individuals in the Wayanad district of

Kerala was screened, followed by genetic counselling where carriers of HbS were advised not to marry carriers [34]. A very high prevalence of HbS is seen in these tribes (18.2 to 34.1 %) [35].

In Gujarat, the *Dhodia*, *Dubla*, *Gamit*, and *Naika* tribes have a high prevalence of HbS (13-31 %)10. More recently very extensive population surveys have been done by the Indian Red Cross Society, Gujarat State Branch where 1,68,498 tribals from 22 districts were screened and the overall prevalence of sickle cell carriers was 11.37 per cent.

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Original Article

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Study of Hormone and Lipid Profile in Polycystic Ovarian Syndrome Women between the Age 18 to 30 Years

Ramchandra K. Padalkar[1], Sangita M. Patil[1], Dhananjay V. Andure[1], Sonali S. Bhagat[1], Anita M. Raut[1]

Author Affiliation: [1]Department of Biochemistry, PDVVPF's Medical College, Ahmednagar, (MS), India.

Abstract

Background: Polycystic Ovarian Sydrome (PCOS) is an intense problem which manifiests later as infertility, obesity, insulin resistance, dyslipidemia, endothelial dysfunction & overt diabetes mellitus. PCOS is often associated with abnormalities of other endocrine glands. The study was undertaken to assess hormone levels and lipid profile in polycystic ovarian disease patients and compare it with age & gender matched healthy females. *Material and Method*: Total 90subjects were enrolled in the present study, out of these 45 were PCOS patients between age group 18 to 30 years, 45 healthy age & sex matched individuals without any evidence of PCOS as per clinical examination was taken as control subjects. Serum Low density lipoprotein – cholesterol (LDL-C), High density lipoprotein cholesterol(HDL-C) Total cholesterol, Triglycerides (TG), Thyroid stimulating hormone, Total T3 &T4 levels were determined. *Results:* The results of present study showsthat, women with PCOS have altered lipid & hormone profiles. In current study serum total cholesterol & LDL -C were higher while HDL- cholesterol was lower as compared with healthy women ($p<0.01$) The TSH levels were significantly ($p<0.001$) increased in PCOS patients compared with healthy controls while total T3 & total T4 levels were normal in both groups. *Conclusion:* In present study, polycystic ovarian patients had subclinical hypothyroidism & this is may be due to unopposed esterogen which stimulates autoimmune reaction like generation of Thyroid peroxidase antibodies. So Analysis of thyroid hormone profile may help in proper treatment. As lipid parameters were associated with the presence of PCOS which mayused for assessment of cardiovascular risk in women with PCOS

Keywords: Polycystic Ovarian Syndrome; Thyroid Hormone; Lipid Profile.

Introduction

According to National Institutes of Health, Polycystic Ovarian Syndrome (PCOS) is defined as ovulatory dysfunction with clinical evidence of hyperandrogenism and hyperandrogenemia in the absence of adrenal or thyroid disease including cushing's syndrome, cancer and congenital adrenal hyperplasia [1]. It is the most important endocrine disease of women with prevalence 4% to 12% [2]. Itis complex disorders in which hormone regulating the egg development of ovaries with other metabolic pathways are affected [3]. So other condition like dyslipidemia, hypertension, cardiovascular disease, type 2 diabetes, cancer of breast, ovarian and endometrial have been associated in PCOS [1].

Alternations of leutinizing hormone (LH) and insulin resistance are common signs in PCOD. Patient with hyperinsulinemia and insulin resistant has been associated with dyslipidimia [4]. Women with PCOS may have an underactive thyroid gland and mild hypothyroidism complicates PCOS problems especially if women are obese in spite of regular diet and exercise. Thyroid hormones acts as

Reprint Request: Ramchandra K. Padalkar, Professor and HOD, Dept. of Biochemistry, PDVVPF's Medical College Ahmednagar, Maharashtra 414111, India.
E-mail: dr.padalkar@rediffmail.com

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metabolic thermostat and if this metabolic thermostat is set at low level, it is difficult to lose weight and avoid sluggishes. If hypothyroidism is diagnosed and treated as early as possible, some of PCOS symptoms may diminish [5].

According to Rotterdam consensus workshop, PCOS is a syndrome of ovarian dysfunction with polycystic ovary morphology and hyperandrogenism. Thus no single criteria is sufficient for it [6]. Cristiah –Loan et.al have shown that the women with polycystic ovary syndrome altered lipid profile with higher cholesterol levels (both total and Low density lipoprotein) and lower high density lipoprotein- cholesterol compared with healthy women [7].

Thus, PCOS can be associated with reproductive and metabolic abnormalities whose diagnosis is based on anthropometric, biochemical and radiological abnormalities. In view of above information and several risk of complication, it is precious to study the hormonal and lipid profile in women with PCOS. Very few studies have been reported for correlation of hormonal and lipid profile testing and its application in Indian context. The aim of present study was one such attempt to measure lipid parameters, thyroid hormone and evaluate its clinical performance in PCOS women between the age 18 to 30 years.

Material and Method

The present study was conducted at Department of Biochemistry Medical College collaboration with Department of OBST and GYNAE.The study has approved by InstitutionalEthics Committee with all participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Study Design

Present study wasanalyticalcase controlstudy. Total 90 subjects were enrolled in the present study. Sampling was done by using simple random sampling type. In present study, population was not universe. Study was carried on available individual's that was accessible population.

Sample Size Calculation

Present study will be quantitative study thus the sample size calculated by using the following formula.

Sample size $\mathbf{n = 4\times\sigma^2/E^2}$

n=sample size, σ=Standard deviation in population E= Allowable error

Control Group

45 healthy age and sex matched individuals without any evidence of PCOS as per clinical examinations were taken as control subjects.

Patients Group

The study included total 45 patients between age group 18 to 30 years of PCOS. They were taken from outpatients attending the Obstetrics and Gynecology department of same hospitals.

Inclusion Criteria

Subject with oligo/amenorrhea, transvaginal sonography suggesting polycystic ovary and features of hyperandrogenemia, nonpregnant, not taking medication like oral contraceptive or any other medicine for last six month which alter thyroidhormone level was included.

Exclusion Criteria

Subject with pregnancy, systemic disease like liver, kidney, heart etc. associated other endocrine disorder i.e. hypothyroidism, hyperprolactinemia was excluded. As well as above subject taken with above mentioned medication also excluded from the study.

Collection of Sample

4ml blood sample was collected in plain vaccutainer from patients and controls between 9.00 to 11.00 am after fasting from 10.00 pm from previous day by using 20G disposable needle fromcubital vein with asceptic precaution. The separated serum was collected in polythene tube with cork and stored at 20°C (precaution was taken to avoid the hemolysis) and was used for analysis of respective parameters.

Estimation of Thyroid Hormone Level and Lipid Profile

Various biochemical parameters like serum T3, T4, Thyroid Stimulating Hormone were estimated. Hormone profile was measured by using enzyme linked immunosorbantassay (ELISA) method [8,9]. Serum total cholesterol and HDL-C were determined by CHOD-PAP method. Serum triglyceride (TG) was measured enzymatic GPO-PAP method end point

assay (using kit manufactured by span diagnostic Ltd) using semi-autoanalyser. LDL-C calculated by using friedewald formula (LDL-C= total cholesterol-TG/5- HDL-C) [10,11].

Statistical Analysis

The statistical analysis was carried out by using the SYSTAT software version 12. The results were expressed in Mean± Standard Deviation (Mean±SD). To test the significance between the study group and the control groups, data was were analysed by student's t-test. p value p<0.001 and P< 0.01 were considered to be statistically highly significant.

Results

Table No.1 showed that baselines characters and lipid profile in PCOS and control groups. In current study, serum total cholesterol, LDL-C, level were significantly increased (p<0.01) and HDL-C was decreased in PCOS group when compared with healthy control group. Table 2 illustrated that, the comparison of thyroid hormone levels in PCOS and control groups. In this study, serum level thyroid stimulating hormone was significantly higher (p<0.001) in PCOS women as compared with healthy control women.

Table 1: Baselinecharacteristics & lipid profile in PCOS& control groups

Parameters	PCOS Group n=45	Control Group n=45	P-value
Age (years)	30.1 ± 2.8	29.3± 6.2	--
BMI(Kg /m2)	36.1± 4.3	30.1 ± 4.0	--
Total cholesterol (mg/dl)	225.8 ± 60.1	168.4± 36.7	<0.01
HDL-c (mg/dl)	42.8 ± 9.4	50.8 ±15.1 .0.02	<0.01
LDL_C (mg/dl)	131.7 ± 45.3	90.2 ± 32.2 <0.00	<0.01
TG (mg/dl)	110.3 ±66.5	120.6 ± 81.1 0.14	0.14

Table 2: Comparison of thyroid hormone levels in PCOS group and control group

Parameters	PCOS Group N=45	Control Group n=45	P value
T3 (ng/ml)	1.20 ± 0.33	1.38 ± 0.5	>0.05
T4 (mcq/dl)	7.7 ± 0.89	6.42 ± 1.65	>0.05
TSH (µIU/ml)	8.33 ± 0.59	1.2 ± 0.11	<0.001

Discussion

The polycystic ovary syndrome is considered the most common gynecologicalendocrinopathy characterised by Hyperandrogenism [1]. It is a heterogeneous collection of sign and symptoms that gathered together form a spectrum of a disorder with a mild presentation in some and in others a severe disturbance of reproductive, endocrine and metabolic function [12]. Adequate levels of circulating thyroid hormone are of primary importance for normal reproductionfunction. Any impairment may develop the disturbances of female reproductive function [13]. Women suffering from PCOS are considered to be at high risk for dyslipidemia due to elevated androgen level and frequent association of this syndrome with obesity [7].

In current study, no statistical difference in serum level of T3 and T4 was seen in both groups. The serum TSH level was increased significantly in PCOS group when compared with control group. Result suggests the hypothyroidism in the PCOS. Our results were strongly supported to the previous research. According to ArchanaShirsath et al. higher the serum TSH level in PCOS,which may be due to unopposed estrogen which stimulates autoimmue reaction like generation of thyroid peroxidase antibodies which may lead to subclinical hypothyroidism in PCOS [5]. Janseen et al , Abalovich M et al have reported that a threefold higher prevalence of hypothyroidism in patients with PCOS [14,15]. Ravi B.V. and SadanaRoshni Gokaldas tried to find out correlation of thyroid stimulating hormone and insulin resistance in women with PCOS. According to them, no any significant correlation between serum TSH level, serum insulin and body mass index in PCOS. There was no statistically significant increase in TSH level in PCOS compared with control which was exactly controversial our results [16].

In present study, there were high level of total cholesterol, LDL-C and low level of HDL-C was observed in women with PCOS when compared with healthy women. Our results were matched with previous study. Cristaiian Joan IUMAAS et al have demonstrated that women with PCOS have alterd lipid profile [7]. Sidhwan s et al have studied the association with atherogenic changes in lipoprotein particles number and size independent of the body

weight. They demonstrated that independent of body weight, PCOS was associated with changes in lipoprotein profile that increases risk of cardiovascular disease. They also reported that increase LDL particle number and decrease LDL size which suggest that androgen may play important role inpathogenesisof lipid abnormalities in PCOS [17]. Sarama Saha et al have investigated on correlation between serum lipid profile and carotid intima media thickness in polycystic ovarian syndrome. According to their study, in polycystic ovary syndrome women carotid intima- media thickness was positively correlated with serum total cholesterol, TG and LDL- cholesterol and negatively correlated with serum HDL-C which suggests that even young polycystic ovary syndrome women are prone to atherosclerosis from early age [18]. CihanInan and CinanKaradag have reported that the group without polycystic ovary morphology had higher risk than the other groups in terms of increased insulin resistance, dyslipidemia and cardiovascular disease due to effects of hyperandrogenism [19]. Contrary to our results, Abdulazin A- Mulhim et al have showed that there were no differences in the blood sugar level, cholesterol, TG and LDL-C and HDL- C seen in PCOS. Lipid profile was not altered in their study which may be due to the fact that women included their study were relatively young [20].

Conclusion

Finding of present study concluded that, polycystic ovarian patients had subclinical hypothyroidism & this is may be due to unopposed estrogen which stimulates autoimmune reaction like generation of Thyroid peroxidase antibodies. So Analysis of thyroid hormone profile may help in proper treatment. As lipid parameters were associated with the presence of PCOS due to effect of hyperandrogenism and it might useful for assessment of cardiovascular risk in women with PCOS.

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Original Article

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Study of Oxidative Stress in Smokers by Estimation of Serum Malondialdehyde Uric Acid and Bilirubin

Raviraj Naik[1], Savita Deshmukh[1], Sarita Dakhure[2]

Author Affiliation: [1]Assistant Professor, Department of Biochemistry, Indian Institute of Medical Science and Research (IIMSR), Warudi, Jalna, 431202, Maharashtra, India. [2]Assistant Professor, Department of Pathology, GMC, Aurangabad, 431001, Maharashtra, India.

Abstract

Background: Study of Malondialdehyde Uric Acid and Bilirubin smokers provides opportunity to explain the correlation between cigarette smoking activity and oxidative stress. Cigarette smoking is the second cause of death in the world. cause oxidative stress, which overwhelm natural radical blocking or scavenging mechanisms. Cigarette smoke may promote atherogenesis by producing oxygen-derived free radicals that damage lipids. Cigarette smoking is associated with impaired endothelium-dependent vasodilatation and cardiovascular disease (CVD). As the current report concerns solely to the study oxidative stress in smokers, the results of this study can be correlated with other biochemical, physiological and clinical aspects. *Aim:* The Aim of our study was to determine oxidative stress in smokers by estimation of serum Malondialdehyde , Uric Acid and Bilirubin in smokers as compared to non-smokers. *Method:* A Total No. of 100 subjects were selected, out of which 50 were healthy individual and 50 were smokers less than 50 years of age. Uric acid and Bilirubin were analyzed using kits on automated biochemistry analyzer while MDA was estimated spectrophotometrically using Thiobarbituric acid. *Result:* The levels of Malondialdehyde were significantly higher while the levels of Uric Acid and Bilirubin was significantly lower in smokers as compared to their levels in non smokers.

Keywords: Malondialdehyde Uric Acid and Bilirubin; Smokers; Non-Smokers.

Introduction

Cigarette smoke contains oxygen radicals and causes formation of new radicals in the body.

The smoke is formed by dispersing of the products as a consequence of melting and distillation in hot medium at gas or droplet state [1]. If the smoke is passed through Cambridge glass fiber, 99.9 percent of the particles larger than 0.1 μm remain in the filter. The part which passesthrough the filter makes gas phase, and the remaining part makes the tar phase [2,3]. Cigarette-smoking is a well-known risk factor for atherosclerosis development and its complications including cerebral and cardiovascular diseases (CVD) [4,5] through vascular endothelial damage [6] that possibly occurs through oxygen free radicals production as superoxide radicals, hydrogen peroxide and hydroxyl radicals [7,8]. Several enzymes capable of producing oxygen free radicals including xanthine oxidase, NADPH oxidase, myeloperoxidase, and endotoxin [7,8]. As cigarette smoke contains superoxide and reactive nitrogen species that readily react with various biomolecules [9]. It has been hypothesized that some of the adverse effects of smoking may result from oxidative damage to endothelial cells, which results in nitric oxide (NO) shortage [10,11]. Nitric oxide (NO) shortage regulates vascular tone that accelerates insufficiency of coronary artery and vasoconstriction in many different tissues [12].Therefore imbalance

Reprint Request: Raviraj Rajan Naik, IB 66, OM Bungalow, Behind Nath Seeds, Paithan Road, Aurangabad – 431005 Maharashtra, India.
E-mail: raviraj_40@yahoo.com

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between oxidants and antioxidants may play an important role in the susceptible smoker [13,14]. In addition cigarette smokers have increased inflammatory responses that further enhance their oxidative stress [8,9]. Since in humans, uric acid and bilirubin are considerably the most abundant aqueous antioxidant, accounting for up to 60% of serum free radical scavenging capacity [15] and is an important intracellular free radical scavenger during metabolic stress including smoking [16,17], therefore,measurement of its serum level reflects the antioxidant capacity [15]. It was estimated that a single cigarette puff contains approximately 10 [14] free radicals in tar phase and 10 [15] radicals in the gas phase, which are capable of causing an increase in the generation of various reactive oxygen species (ROS) like superoxide ($O^{-}2$), hydrogen peroxide (H_2O_2), hydroxyl (OH•) and peroxide (ROO•) radicals. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation [18,19]. Cigarette smokers have an increased risk of cardiovascular diseases (CVD), possibly mediated by elevated levels of oxidized macromolecules owing to heightened ROS production. Smokers are exposed to significant quantities of ROS in both gas and tar phase. Further ROS production mediated through inflammatory processes may exacerbate those produced through direct exposure [20]. Blood of cigarette smokers routinely displays decreased antioxidant capacity and increased oxidized lipids compared to non-smokers [20]. This chapter describes the malondialdehyde (MDA) as index of lipid peroxidation. The determination of malondialdehyde (MDA) has attracted widespread interest, because it appears to offer a facile means of assessing lipid peroxidation in biological materials [21]. The primary form of bilirubin circulating in healthy individuals, is also a powerful antioxidant [22] at levels within the normal reference range. Thus, while seemingly counterintuitive, bilirubin has been inversely associated with risk of a number of disorders,associated with oxidative stress [23]. The concordance between the negative health consequences of smoking, including those recently highlighted by the Surgeon General and those associated with lower bilirubin concentrations, is striking [24].

Aim & Objectives

1. Present study has been taken up for estimation of serum Malondialdehyde ; Uric Acid and Bilirubin in smokers.
2. To have an estimate of oxidative stress in smokers.
3. To create awareness among smoker population in order to prevent occurrence of different diseases as a result of oxidative stress in smokers.

Material & Methods

Study Design

The current Prospective study was undertaken from Aurangabad ; Maharashtra.

Study Period

Dcember 2016 to December 2017.

Ethical Approval

The study was approved by the Institutional Ethical Committee of IIMSR Medical College Jalna.

Inclusion

We labeled smoker as the one who smokes atleast 10 cigarettes or Bidi per day.

The study composed of 70 male smokers and 30 nonsmokers between the age group of 20 to 50 years. All the subjects were consuming both vegetarian diet and non vegetarian diet, and belonging to different walks of community.

The subjects were volunteer participants in the study and gave informed consent.

Exclusion

Individuals who have any systemic illness or who were taking any medication or antioxidant for prophylaxis were not included to this study.

Site of Sample Collection

Samples were collected Medicine and TB Chest OPD of IIMSR Jalna.

Site of Sample Study

Central Clinical laboratory(Biochemistry section), IIMSR Jalna.

Study Subjects

Group I Non Smokers (Control) n = 30

Group II Cigarette Smokers n=70

Method

5ml blood sample was collected by standard venipuncture technique in plain bulb.Blood sample was centrifuged to obtain a clear serum sample. Serum was divided in two plain tubes i.e 2ml for uric acid and bilirubin estimation and remaining serum sample for MDA estimation.Uric acid and bilirubin estimation was done on fully automated transasia analyzer in central clinical laboratory while MDA estimation was done in biochemistry laboratory by Thiobarbituric method. MDA level of the plasma was measured by the following procedure according to Tomotsu et al. 0.5 plasma was shaken with 2.5 ml of 20% trichloroacetic acid (TCA) in a 10 ml centrifuge tube. 1ml of 0.6 % TBA was added to the mixture, shaken, and warmed for 30 min in a boiling water bath followed by rapid cooling. Then it was shaken into a 4 ml of nbutyl-alcohol layer in a separation tube and MDA content in the plasma was determined from the absorbance at 535 and 520 nm by spectrophotometer against butanol. The standards of 5, 10, 20 nmol/ml TEP were used. The results were expressed as nmol/ml plasma [25]. Statistical analysis was done by using Chi square test by calculating p value with the help of SPSS software. Difference between the parameters of two groups was considered significant if $p < 0.001$

Results

The levels of Malondialdehyde were significantly higher while the levels of Uric Acid and Bilirubin was significantly lower in smokers as compared to their levels in non-smokers.

Table 1:

Sr. No	Parameters(mg/dl)	Reference Range	Non-Smokers (Mean+/- SD)	Smokers. (Mean+/- SD)	p value
1.	Uric Acid	3.5-7.5 mg/dl	6.15+/- 0.65	3.74+/-0.93	<0.001
2.	Bilirubin	0.1-1.0 mg/dl	37.86 +/-4.93	29.06 +/-2.98	<0.001
3.	Malondialdehyde(MDA)	2.59+/-0.24µm/l	2.74 +/-0.36	3.18 +/-0.36	<0.001

Discussion

In this study we found that levels of Uric Acid in smokers were significantly lower than that in non-smokers. This finding are in agreement with other studies that showed low serum uric acid in regular smokers [26] and reduction of antioxidants including uric acid in smokers [27,28] indicating that oxidative stress increases everytime a cigarette is smoked [26]. It even proved that administration of uric acid raises circulating antioxidant defenses and allows restoration of endothelium-dependent vasodilation [29,30].

Therefore, high serum uric acid concentrations might be protective in situations characterized by increased cardiovascular risk and oxidative stress as smoking [29], and by reducing its level it increases susceptibility to oxidative damage and accounts for the excessive free radical production [31].

We also evaluated bilirubin in smokers that bilirubin levels are significantly decreased in them as compared to their non-smoking counterparts. Similar findings were also shared by some other studies like those done by Madhavan et al., 1997; Merz, Seiberling, & Thomann, 1998; Van Hoydonck, Temme, & Schouten, 2001; Zucker et al., 2004) [32-35]. The possibility that smoking leads to reductions in bilirubin, which in turn may contribute to smoking-related disease though diminished availability of this endogenous antioxidant, is intriguing. One possible mechanism for bilirubin reduction among smokers that has been suggested (van der Bol et al., 2007) [36], but not proven (Zevin & Benowitz, 1999) [37], is that of induction of UGT 1A1 by nicotine and/or other constituents of tobacco smoke. UGT 1A1 is the uridine diphosphate glucuronosyltransferase isoform, which catalyzes conjugation of bilirubin, the major metabolic pathway responsible for its disposition.

Lastly we focused our study on oxidative stress due to lipid peroxidation by estimating last product and indicator of lipid peroxidation process i.e Malondialdehyde(MDA) and we found that MDA were significantly increased in smokers as to their non-smoking counterparts. These results are in accordance with the earlier studies, showing elevated lipid peroxidation ~ 96 ~ The Pharma Innovation Journal among smoker subjects [38-40]. Chole et al [41] reported association of lipid peroxidation with the habit of either chewing betel nut or betel leaf or tobacco or smoking in the control subjects. In another study, significantly elevated MDA levels were reported in smokers than nonsmokers in patients with lung cancer [42].

Conclusion

After exclusion of other factors affecting uric acid level, the significant low serum uric acid level in smokers was attributed to reduce endogenous production as a result of chronic exposure to cigarette smoke that is a significant source of oxidative stress. As this reduction is proportionate with smoking status and predisposes to cardiovascular disease, it is recommended for smokers to stop or reduce smoking and introduce serum uric acid estimation as routine test since its cheap and simple to reflect their antioxidant level.

From the results of MDA obtained, we conclude that oxidative stress as indicated by serum lipid peroxidation is more intense in smoker subjects as compared to non-smoker subjects. There is a strong association between increased lipid peroxidation and cigarette consumption in smoker subjects. Evaluating the serum MDA levels might serve as a valuable biomarker to identify the high risk population, which may deserve further investigation for early diagnosis and treatment.

Lastly significant decrease in bilirubin also warrants danger signals as lower bilirubin levels has been found in concordance with incidence of different carcinomas.

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Original Article

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Surface Plasmon Resonance Mediated Evaluation of Cartilage Oligomeric Matrix Protein in serum of Elderly Patients with Knee Osteoarthritis: An Indian Perspective

Gunjan Deepak[1], Gill Kamaldeep[2], Rai Nitish[2], Soneja Manish[1], Bhalla Ashu S.[1], Kumar Uma[1], Dey Sharmistha[2], Dey Aparajit B.[3]

Author Affiliation: [1]Department of Medicine, All India Institute of Medical Sciences, New Delhi, India-110029. [2]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India-110029. [3]Department of Geriatric Medicine, All India Institute of Medical Sciences, New Delhi, India-110029.

Abstract

*The kn*ee osteoarthritis is the most common arthritis in elderly and diagnosed clinically and radiologically. New biomarkers are being tried to diagnose knee osteoarthritis. This work aims to explore the relationship between serum Cartilage Oligomeric Matrix Protein (COMP) and knee osteoarthritis in Indian patients. We recruited 72 elderly patients of knee osteoarthritis (diagnosed according to American College of Rheumatology criteria) and 23 asymptomatic healthy elderly controls from geriatric and medicine OPD. Radiographic severity was determined by K/L grade. The serum COMP level was determined by surface plasmon resonance (SPR) analysis using polyclonal rabbit antibody against COMP. Patients had significantly high level of serum COMP as compared to controls (1.75±.37ng/mL vs. 0.82±.16ng/mL; $p<0.0001$). The serum COMP significantly decreased as the duration of disease increased ($p<0.0001$). Its level was appreciably higher in patients with radiographically proven osteoarthritis ($p=0.045$). There was no significant difference in the level correlated with gender, body mass index, visual analogue scale, age and laterality of knee osteoarthritis. It was demonstrated that the serum COMP can differentiate between knee osteoarthritis elderly patients and the healthy subjects. It characterizes disease severity and early duration of osteoarthritis.

Keywords: Knee Osteoarthritis; Serum; Surface Plasmon Resonance; Cartilage Oligomeric Matrix Protein; Indian Elderly Population.

Introduction

Osteoarthritis is a painful, progressive, degenerative joint disease and characterized by loss of articular cartilage [1,2]. Knee osteoarthritis is the most common arthritis among the elderly people, and is a common cause of disability [3]. The conventional method of diagnosing knee osteoarthritis are clinical and plain radiographs of knee joint which neither capture early stages of osteoarthritis nor helps in monitoring the efficacy of treatment as well as the early progression of disease. The plain radiograph is also a subject of inter-observer variability.

There is an urgent need to identify osteoarthiritis using enhanced techniques in the early stage. Recently, several studies have been done to evaluate the use of reliable biomarkers in serum for early detection, gauge severity of knee osteoarthritis and predict the progression of disease [4]. One such potential biomarker is COMP. It is a non-collagenous, extracellular, pentameric glycoprotein of 524 kDa belonging to thrombospondin family [5,6]. It is primarily identified in cartilage, but is also found in tendon and synovium [7,8]. Its biological function is still debated but it has been suggested that COMP interacts with collagen and may be involved in regulating fibril formation and maintaining integrity of collagen network [6,9]. It has been suggested that

Reprint Request: Sharmistha Dey, Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110029, India.
E-mail: sharmistha_d@hotmail.com

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COMP may increase the ability of articular cartilage to resist mechanical wear [10]. In various studies, it has been demonstrated using ELISA that the serum COMP levels increases in patients with knee osteoarthritis and predicts the progression of disease [11-15]. This study first time reports the correlation of serum COMP and knee osteoarthritis in Indian patients by label free real time surface plasmon resonance (SPR) technology.

Methods

- *Selection of Patients*

In the study, 500 elderly patients were screened on the basis of musculoskeletal symptoms (like-joint pain, joint swelling, and restriction of mobility of joints, crepitus and morning stiffness) and finally 72 knee osteoarthritis patients were selected and diagnosed according to the American College of Rheumatology Criteria (ACR) [2]. The patients were recruited from the medicine and geriatric OPD of All India Institute of Medical Sciences (AIIMS), New Delhi, India. The Ethics Committee of AIIMS approved the study protocol (A-9/25.07.2007) and informed consent was obtained from each subject. The study was performed compliant to the rules and regulations of the Ethics Committee, all subjects gave written informed consent.

The patients above 60 years of age were included in the study. The patients having any inflammatory arthritis, systematic inflammatory disease, severe/ critical illness like- chronic kidney disease, congestive heart failure, hepatic failure etc., traumatic osteoarthritis, neoplasm and those on steroid were excluded. Twenty three age, sex and ethnicity matched healthy subjects were included.

The bilateral antero-posterior, weight bearing plain radiograph of knee was taken for all participants with the subject standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. The X-ray beam was aimed at the lower pole of the patella and kept parallel to the joint surface. The target–film distance was 36 inches [16]. The severity of knee osteoarthritis was graded by an experienced radiologist (ASB) who was blinded of the patient's profile. Severity grading of knee radiographs was done with the Kellgren/Lawrence (K/L) grading scale [17]: grade 1 was doubtful narrowing of joint space and possible osteophytic lipping; grade 2 was definite osteophytes and possible narrowing of joint space; grade 3 was moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4 was large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. The grade used for analysis was the higher of the two knees. We followed the good clinical practice guidelines according to Helsinki guidelines.

BMI (body mass index) was defined according to World Health organization criteria for Asian population [18]. The groups composing of disease duration were arbitrarily divided into four sets. Patients with radiographic knee OA were defined as having radiographic knee OA of K/L grade ≥2 in at least 1 knee.

- *Separation of Sera from Blood*

Five milliliter of venous blood was collected from anticubital fossa under aseptic conditions in morning hours after 30 minutes of rest. It was allowed to settle down for 1 hr at room temperature. The buffy coat was removed from the blood and centrifuged at 3000 rpm for 20 min. The serum was collected and stored at -70^{0}C in multiple aliquots.

- *Estimation of COMP Level in Serum by SPR*

The level of serum COMP was determined using Biomolecular Interaction Analysis (BIA) in BIAcore 2000 (Pharmacia, Biosensor AB, Sweden) machine that examines and characterizes bio-molecular interactions in real time and is based on SPR principle [19]. For the evaluation of serum COMP levels, the IgG-COMP of human origin (abcam®, Cambridge, USA), was immobilized on the surface of CM5 sensor chip by amine coupling. For this, equal volumes (115 µl) of N-hydroxyl succinimide (NHS) (2.3 mg in 200 µl of water) and N-ethyl-N'-(3-dimethyl-aminopropyl) carbodiimide (EDC) (15mg in 200 µl of water) obtained from Pharmacia were mixed and 75 µl of this solution was passed at the flow rate of 5 µl/min across the CM5 sensor chips to activate the carboxy methylated dextran surface. After this 0.1 µl (50 ng) of COMP antibody in 10 mM sodium acetate (209.9 µl, pH 3.9) was passed at the flow rate of 5 µl/min across the activated surface and the un-reacted groups were blocked by ethanolamine (50 µl).

To prepare the standard curve of COMP protein, seven different concentrations (0.38, 0.80, 1.60, 4.80, 8.00, 11.20 and 14.40 ng/ml) of the commercial recombinant COMP (Immunodiagnostic AG, Bensheim) were passed over the immobilized antibody and the corresponding RUs (Resonance Units) were obtained. A standard curve of RU vs.

concentration of COMP was plotted. After this 40 ìl of 1:99 dilutions (in HBS-EP buffer) of serum for each sample was passed over the immobilized COMP antibody on the sensor chip at a flow rate of 10 µl/ min. The RU for each sample was recorded and the concentration of COMP was derived from the standard curve.

The On and Off values of the COMP protein were also calculated by passing six different concentrations (0.64, 1.28, 3.84, 6.40, 8.96, and 11.52 ng/ml) of protein over the immobilized anti-COMP antibody.

- *Statistical Analysis*

The statistical analysis was done by GraphPad Prism Instat 3 statistical software package (GraphPad Software, Inc. California, USA). The continuous variables were summarized as mean ± SD, and categorical variables as proportions, n(%). Comparison between groups was done by unpaired student's t-test. The association between serum COMP and various variables of knee osteoarthritis patients was accessed by one way analysis of variance (ANOVA). The p value of <0.05 was considered statistically significant.

Results

- *Clinical Data of Knee OA Patients*

500 elderly patients were screened and 98 (19.6%) patients had knee osteoarthritis. But only 72 (14.4%) patients were selected for final serum COMP analysis. 26(5.2%) patients were excluded as they were having neoplasm 10(2%), severe systemic illness 6(1.2%), traumatic arthritis 4(0.8%), on steroids 3(0.6%), refused 3(0.6%).

Mean age group for patients and controls were 65.2±5.5 and 62.9±5.86 years (Mean±SD), respectively. The patients group had 26 males and 46 females whereas the control group comprised of 14 males and 9 females. The mean BMI of patients and controls were 24.5±4.8 kg/m^2 and 23.4±4.1 kg/ m^2, respectively. The patients and controls were

Table 1: Serum COMP level in different clinical parameters of knee OA patients (n=72)

Clinical Parameters	Number of Patients	Serum COMP Level (mg/ml)	p value
Subjects			
Patients	72	1.75±0.37	<0.0001
Controls	23	0.77±0.09	
Age (years)			
60-64.9	33	1.78±0.41	0.581
65-65.9	22	1.75±0.26	
≥70	17	1.66±0.43	
Sex			
Male	26	1.73±0.36	0.739
Female	46	1.76±0.39	
Duration of Disease (months)			
3-23.9	15	2.14±0.30	<0.0001
24-47.9	32	1.81±0.29	
48-71.9	15	1.45±0.20	
≥72	15	1.39±0.26	
BMI (kg/m^2)*			
<25	40	1.68±0.31	0.07
≥25	32	1.84±0.43	
Severity of OA (X-ray grading)			
<2	12	1.55±0.36	0.045
≥2	60	1.79±0.36	
Laterality of knee OA			
Unilateral	07	1.47±0.29	0.148
Bilateral	65	1.72±0.38	
VAS (Visual Analogue Scale)			
<5	50	1.72±0.41	0.37
≥5	22	1.81±0.29	

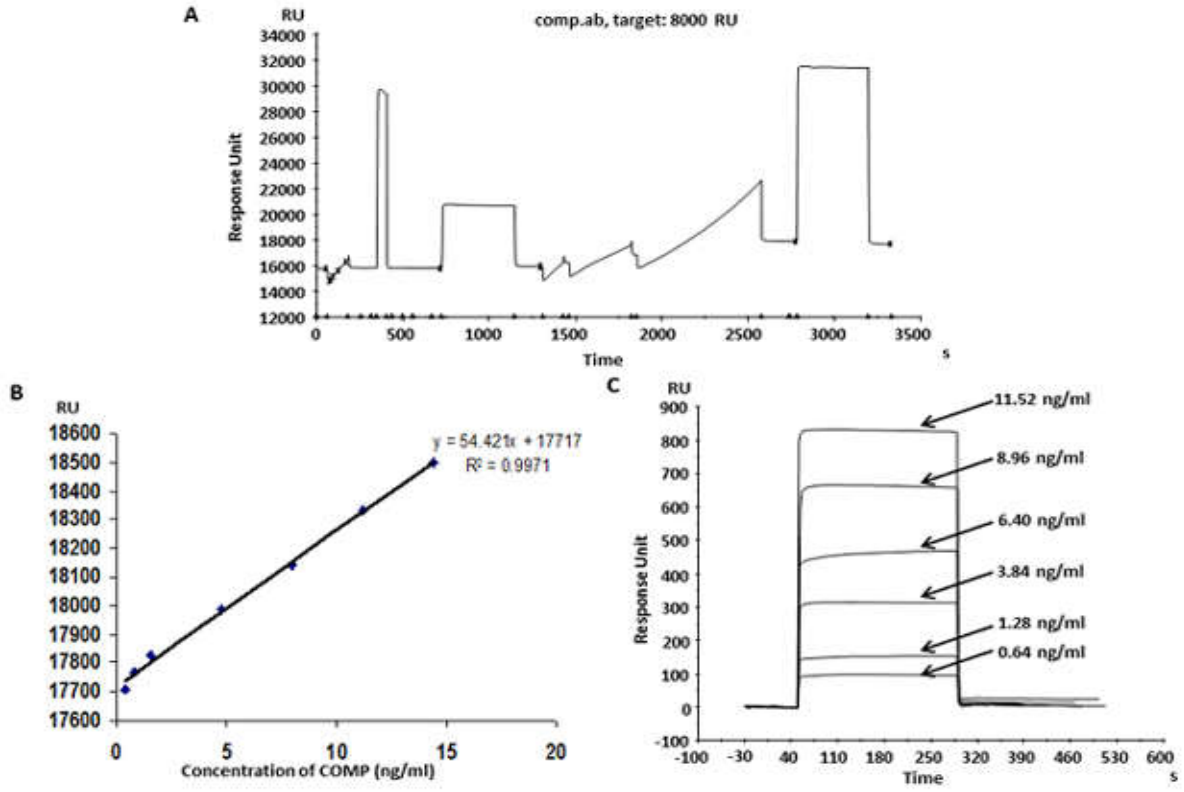

Fig. 1: Assessment of COMP using SPR. (A) Immobilization profile of anti-COMP antibody on the CM5 chip, (B) A standard graph was plotted between different concentrations of COMP and their respective RU, (C) Six different concentrations of COMP passed over the immobilized antibody to calculate the on and off values.

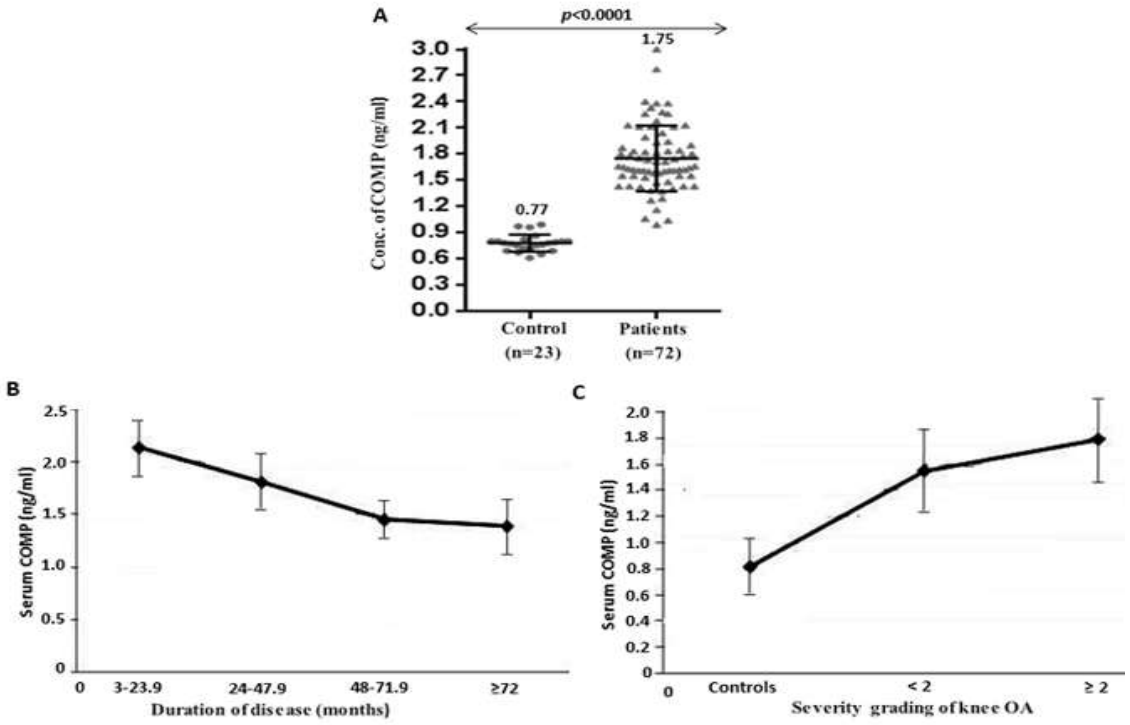

Fig. 2: Evaluation of COMP. (A) Scatter diagram exhibiting the comparison of mean COMP values in control and patients, (B) A comparison of mean COMP levels in patients according to the duration of the disease, (C) A comparison of mean COMP concentrations according to the grading of the knee in OA patients. Error bar shows the standard deviation

matched for age and BMI.

- *COMP Assessment by SPR*

The SPR signal for immobilization of antibody of COMP was found to be 17671.6 RU (Fig. 1A). Five different concentrations of recombinant COMP in HBS-EP buffer were passed over the immobilized COMP antibody and the RUs obtained were (17709.6, 17768.6, 17825.9, 17987.2 18140.9, 18330.2 and18500.0). The standard curve was plotted between RU obtained from the sensorgram and respective concentrations of COMP protein as mentioned above (Figure 1B). The binding of the COMP as ligand was linear and was shown in the Figure 1B The RU was increasing linearly as the concentration of COMP increases. The On (Ka) and Off (Kd) values calculated for the COMP were 1.74 x 10^{12} M and 5.73x10^{-13} M, respectively (Figure 1C).

- *Correlation of COMP with Clinical Parameters*

The correlation of expression levels of COMP with clinical parameters in knee OA patients are shown in Table 1. As shown in the Table 1, significant correlations were found between patients (1.75±0.37ng/ml) and control group (0.77±0.09ng/ml; p<0.0001), severity of the diseases on K/L scale (p=0.045) and duration of disease (p<0.0001). However, there was no significant association with other clinical parameters like age, gender, BMI, laterality of knee OA and VAS. The levels of serum COMP of patients and controls ranged from 0.86 to 3.00 ng/ml, and 0.61 to 1.40 ng/ml, respectively. The mean serum COMP level decreased progressively as the duration of disease increased (p<0.0001). The mean serum COMP level was significantly elevated in patients with knee OA of K/L grade ≥2 (p=0.045) (Figure 2). There was a decreasing trend in the mean serum COMP level with the advancement of age, but it did not reach significance level (p=0.581). The level did not vary differently in both the sexes, although mean serum COMP was slightly higher in females (p=0.739). The patients having higher BMI had higher mean serum COMP level but was not statistically significant (p=0.07). Same trends were seen in unilateral vs. bilateral knee OA (p=0.148) and low and high VAS scale (p=0.37).

Discussion

Our study is different in many aspects from earlier studies. The relationship between serum COMP and knee OA has been investigated previously in several studies in different population, however, to the best of our knowledge, this is the first study in which serum COMP level has been measured using BIA core which utilizes the natural phenomenon of SPR to deliver high quality data in labels free real time. SPR biosensor offers better sensitivity than traditional antibody based methods such as ELISA and Western blotting.

The serum COMP level was significantly higher in patients (1.75±0.37 ng/ml) as compared to asymptomatic healthy controls (0.77±0.09 ng/ml; p<0.0001) in Indian populations. In this study, the mean serum COMP level significantly decreased as the duration of knee OA advanced (p<0.0001). It has been demonstrated that there is loss of cartilage volume, as disease progressed [20,21]. It has been postulated that level of serum COMP may be related to the volume of cartilage [22]. So, it could be possible that longer duration of disease is associated with less volume of cartilage and decreases in level of serum COMP.

The mean serum COMP was higher in radiographically defined knee OA (K/L grade ≥2) than less severe grade (K/L grade <2) {1.79±0.36 vs. 1.55±0.36; p=0.045}. It has been suggested that COMP up-regulates and is responsible for degradation of cartilage in osteoarthritis patients [23].

There was no significant elevation of serum COMP level in various age groups and different gender. Clark et al. showed the increasing level of serum COMP with the advancement of age [12], which was not observed in the present study and in Brazilian population [24]. Serum COMP level did not vary significantly with gender in some earlier studies [12,24] and in the present study; but another study by Jorden et al had shown that serum COMP varies with sex in Caucasian population [25]. It may be due to the ethnic variability, shown in other studies [25,26]. Study done by Valdes et al. had reported that there is a role of genetic polymorphism in various ethnic groups and both the genders, which may be one of the reasons for variable serum COMP levels in different ethnic groups and genders. In this study, serum COMP level was higher in patients having higher BMI, but did not reach the significant level as shown by other studies [12,24]. The different cutoff values for classifying BMI were applied in Asian population.

There was increase in serum COMP in bilateral knee OA, but did not reach significant level as shown by Clark et al. The involvement of OA joints lead to increase in serum COMP level [12]. In the present study, the number of patients having bilateral OA knee was higher as compared to the unilateral knee OA, hence, was not statistically significant (p=0.148).

The association of serum COMP level with different VAS scale in patients was not observed, which was reported in Brazilian and Egyptian population [24,27]. It was probably due to the fact that symptoms were not well correlated with severity of disease; and the perception of pain was subjective one.

Conclusion

It can be concluded that the serum COMP is higher in knee OA patients compared with healthy controls and can be used as a biomarker for symptomatic radiographic knee OA in Indian patients. It is related with severity of knee OA and early duration of disease. We need to follow up the study for further characterizing the role of COMP in knee OA with treatment.

Key Messages

- The level of serum COMP was found to be significantly higher in osteoarthritis patients as compared to controls (1.75±.37ng/mL vs. 0.82±.16ng/mL; $p<0.0001$).
- The serum COMP significantly decreased as the duration of disease increased ($p<0.0001$).
- It was demonstrated that the serum COMP can differentiate between knee osteoarthritis elderly patients and the healthy subjects.

Acknowledgement

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Conflict of Interest

There was no conflict of interest.

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Original Article

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Study of Lipid Profile in Young Smokers and Non-Smokers

Raviraj Naik[1], Savita Deshmukh[1], Sarita Dakhure[2]

Author Affiliation: [1]Assistant Professor, Department of Biochemistry, Indian Institute of Medical Science and Research (IIMSR), Warudi, Jalna, 431202, Maharashtra, India. [2]Assistant Professor, Department of Pathology, GMC, Aurangabad, 431001, Maharashtra, India.

Abstract

Background: Study of lipid profile in young smokers provides opportunity to explain the physiological consequences of the cigarette smoking activity. Cigarette smoking is the second cause of death in the world. The addictive liability and pharmacological effects of smoking are primarily mediated by the major tobacco alkaloid nicotine. Cigarette smoke may promote atherogenesis by producing oxygen-derived free radicals that damage lipids. Cigarette smoking is associated with impaired endothelium-dependent vasodilatation and cardiovascular disease (CVD). As the current report concerns solely to the study of lipid profile in normal healthy controls and smoking male subjects, the results of this study can be correlated with other biochemical, physiological and clinical aspects. *Aim:* The Aim of our study was to determine and compare lipid profile in smokers and non-smokers. *Method:* A Total No. of 100 subjects were selected, out of which 50 were healthy individual and 50 were young smokers less than 35 years of age. The lipid parameter were analyzed using kits on automated biochemistry analyzer. *Result:* The levels of total cholesterol, Triglyceride, LDL-C and VLDL-C were significantly higher while the levels of HDL was significantly lower in smokers as compared to their levels in non smokers.

Keywords: Lipid Profile; Smokers; Non-Smokers.

Introduction

Smoking may be explained as habit of inhalation of smoke arising from the burning of tobacco in a pipe or in the form of a cigar or cigarette. Nowadays the cigarette smoking is fashion but it causes many illnesses. Smoking is the major preventable cause of death in many areas of the world today. The World Health organization estimates that worldwide 5 million deaths are cause prematurely by smoking every year [1].

In India around, 5,500 adolescents start smoking cigarettes every day, by joining the 4 million young people, under the age of 15, who already smoke regularly. Like other developing countries, the most susceptible time for initiate smoking in India is during adolescence and early adulthood i.e 15 – 24 years [2].

A large number of risk factor which predispose to atherosclerosis and Coronary Artery Disease (CAD) have been identified. These include modifiable ones like hypertension, dyslipidemia, smoking, diabetes mellitus, changing life style and non modifiable ones like age and sex. As the number of risk factor in an individual increases, so does the risk of developing atherosclerosis and its complication mainly coronary artery disease (CAD). In subject with more than one of these risk factor the risk is more additive [3]. Although smoking has been established as an independent risk factor for Coronary Heart Disease [4], the mechanism by which it increases the risk of coronary heart diseases is unclear. Some

Reprint Request: Raviraj Rajan Naik, IB 66, OM Bungalow, Behind Nath Seeds, Paithan Road, Aurangabad – 431005 Maharashtra.
E-mail: raviraj_40@yahoo.com

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explanations have been postulated: the increased carbon monoxide in the blood of cigarette smokers may damage the endothelium and accelerate the entry of cholesterol into the wall of the artery [5]. The formation of carboxyhemoglobin creates relative anoxemia in the tissue, including the myocardium [6].

Smoking enhances platelets aggregation, and the nicotine absorbed from cigarette smoke may induce cardiac arrhythmia through its pharmacologic action. Cigarette smoke contains numerous compounds, many of which are oxidants and prooxidants, capable of producing free radicals and enhancing the oxidative stress [7].

Cigarette smoke is a complex mixture of over 7000 chemical compounds [8,9]. Cigarette smoking predisposes the individual to several different clinical to several different atherosclerotic syndromes, including stable angina, acute coronary syndromes, asthma [9], sudden death and stroke.

It is associated with average 70 % increase in risk of death from coronary artery disease [10]. An additional mechanism has been recently suggested that smoking adversely affect the concentration of the plasma lipids and lipoproteins. However, studies to date have revealed incomplete, inconclusive or conflicting results about the association of smoking on the plasma lipid and lipoprotein levels. In some studies, smokers had increased plasma cholesterol in other plasma cholesterol level have actually been lower only a few studies have specifically examined the plasma lipoprotein according to smoking status or number of cigarettes smoked (dosage) Smokers are reported to have higher low density lipoprotein (LDL) and lower high density cholesterol levels than non smokers [11].

Aim & Objectives

1. Present study has been taken up to find out the alteration of serum lipid profile between young smokers & non smokers & also to see any dose related changes in serum lipid among the smoking population.
2. To create awareness among young smoker population as well as young non-smoker population decrease the burden of adverse effects of smoking inn society.

Material & Methods

Study Design: The current Prospective study was undertaken from Aurangabad; Maharashtra.

Study Period: Dcember 2016 to December 2017.

Ethical Approval: The study was approved by the Institutional Ethical Committee of IIMSR Medical College Jalna.

Inclusion

We labeled smoker as the one who smokes atleast 10 cigarettes per day [12]. The study composed of 100 male smokers and nonsmokers between the age group of 20 to 35 years. All the subjects were consuming both vegetarian diet and non vegetarian diet, and belonging to different walks of community. The subjects were volunteer participants in the study and gave informed consent.

Exclusion

Subjects having diseases, which are known to influence the blood lipids or patients on lipid lowering drugs or a diet restriction for any reason and persons chewing tobacco, ex- smokers, obese persons, alcoholics and having risk factors like Hypertension, Diabetes Mellitus were excluded from the present study.

Site of Sample Collection

Samples were collected Medicine and TB Chest OPD of IIMSR Jalna.

Site of Sample Study: Central Clinical laboratory (Biochemistry section), IIMSR Jalna.

Study Subjects

Group I Non Smokers (Control) n = 50

Group II Cigarette Smokers n=75.

Method

In order to ensure accurate and reproducible results overnight 12 hours fasting blood samples were collected from these subjects. Serum was separated by centrifugation at 3600 rpm for six minutes. The clear serum sample were employed on fully automated biochemistry analyzer for estimation of LDL , VLDL , Cholesterol , Triglycerides & HDL. Statistical analysis was done by using Chi square test by calculating p value with the help of SPSS software. Difference between the parameters of two groups was considered significant if $p < 0.001$.

Table 1:

Sr. No.	Parameters (mg/dl)	Normal Range	Non-Smokers (Mean+/- SD)	Smokers. (Mean+/- SD)	P value
1.	Mean Total Cholesterol	150-250 mg/dl	194.11+/-2.97	212.37+/-33.00	<0.001
2.	Mean HDL	40-60 mg/dl	37.86 +/-4.93	29.06 +/-2.98	<0.001
3.	Mean LDL	90-140 mg/dl	126.74 +/-14.54	153.54+/- 30.16	<0.001
4.	Mean VLDL	0-40 mg/dl	22.09+/-5.06	30.17 +/-7.13	<0.001
5.	Mean Triglycerides	60-170 mg/dl	116.37+/-29.02	142.14 +/-36.11	<0.001

Results

The levels of total cholesterol, Triglyceride, LDL-C and VLDL-C were significantly higher while the levels of HDL was significantly lower in smokers as compared to their levels in non smokers.

Discussion

In this study we found that total cholesterol and triglycerides levels in smokers were significantly higher than that in non-smokers. Similar findings were shared by some of the studies [13]. However in some other studies no significant changes were observed in cholesterol levels in smokers and non smokers [14].

The reason for increased serum total cholesterol and Triglyceride levels in smokers are increased catecholamine secretion leading to lipolysis , which in Turn elevates hepatic output of Triglycerides and VLDL [15], hyperinsulinemia leads to decreased activity of lipoprotein lipase [16], increased activity of hepatic lipase and decreased lecithin Cholesterol acyl transferase (LCAT) activity [17]. The mean levels of LDL–C and VLDL – C in this study among smokers were higher than non smokers. These findings were similar to other studies [13,18].

The mechanism of increased LDL- C and VLDL- C levels in chronic smokers are due to hyperinsulinemia leading to decreased activity of lipoprotein lipase [16] and increased activity of cholestryl ester transfer protein (CETP) and phospholipid transfer protein. Decrease in HDL- C level in smokers compared to non smokers was also significant.

This findings is similar to me other study findings as well[13] However some other studies have reported no significant difference in HDL – C level between smokers and non smokers. The explanations cited for low levels of HDL – C in smokers are due to low levels of estrogen leading to fall in HDL-C level and increased activity of cholestryl ester transfer protein (CETP) [19].

Conclusion

Finding of this study concludes that compare with non smokers the young smokers developed Dyslipidemia and are thus at higher risk of cardiovascular diseases although some more cardiovascular parameters needs to be studies and compared to confirm increased CVD risk in young smokers.

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Review Article

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Handshake of DNA and Protein: A Perspective

Anju Singh

Author Affiliation: Nucleic Acids Research Lab, Department of Chemistry, University of Delhi, Delhi-110007, India.

Abstract

DNA is a passive molecule and it can not exist on its own in cellular environment. It exist alongwith proteins and their interaction play a pivotal role in various biological processes such as replication, transcription, recombination, and repair etc. and via their interaction they regulate and modulate gene expression. Association of DNA-protein has an utmost importance in biological processes. There are plethora of biochemical and biophysical techniques to study the interaction of DNA-protein. Present article is an effort to give a glimpse of DNA-Protein interaction.

Keywords: DNA-Binding Proteins; Reporter Assay; DNA Foot Printing; CD; Fluorescence Spectroscopy.

Introduction

DNA, a fundamental unit of life, is polymorphic and adopts a variety of unique secondary and tertiary structures that may play functional role in gene regulation. It has been one of the most important sources not only for the understanding of the fundamental basis of human life but also for the development of a novel group of therapeutics modeled on its endogenous structure. The DNA has the potential to adopt numerous conformations depending on the primary structures, hydration, ions, proteins, drugs and spherical stress. Its primary structure dependence is far from being fully understood but in addition to the most common forms of DNA namely the duplex several other forms such as hairpins, cruciform three stranded structures such as triplexes, four stranded structures such as G-quadruplexes and C- quadruplexes (i-motif) and even polyads have also been discovered.

Reprint Request: Anju Singh, Nucleic Acids Research Lab, Department of Chemistry, University of Delhi, Delhi-110007, India.
E-mail: anju11278@gmail.com

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Evidences shows the flexibility in the DNA topology and this distinctive flexibility are detectable for instance in the growing number of unusual DNA structure and conformations as a result of the intricate interplay between the various factors [1]. Although enormous progress have been made in elucidating the DNA dynamic behavior but still our knowledge is limited to explore DNA characteristics. The structural and conformational variability shown by DNA have been found biologically very important [2,3]. Therefore, it is intriguing to explore DNA and its interaction with various ligands; proteins etc.

It is widely known that various intra/inter molecular interactions perform crucial roles in cellular processes in particular ways. Proteins- DNA interactions are a common feature in cellular processes such as mismatched DNA repair [4]and gene regulation [5] etc. The main function of nucleic acids is the storage and faultless transmission of genetic information to progeny and transmission and coding of this information into proteins whereas proteins are concerned with the execution of biological processes. The function of nucleic acids is controlled and made possible by their interactions with specific proteins thus the interactions between the proteins and nucleic acids play a very

fundamental role in living cells, by controlling every step of the entire mechanism of information, translation, repressor mechanisms and presumably differentiation. For instances, proteins involved in (a) repair and replication is-ligases and repair proteins, nucleases and excision enzymes, gyrases, DNA polymerases. (b) transcription-RNA polymerase, cyclic AMP receptor protein, repressors, rho factors which terminate transcription (c) nucleases-restriction endonucleases, exo- and endonucleases (d) recombination rec A protein and (e) package- histones, protamines, virus condensation proteins.

Specificity in DNA-Protein interactions comes from protein recognition of the linear order of base pairs through hydrogen bonds and salt bridge contacts through the major and minor grooves. Proteins can also recognize particular regions of DNA through an indirect readout mechanism in which contacts are not made with the bases in the major and minor grooves, but with phosphate groups and sugar residues. Proteins involved in package and repair are less specific to the base sequence. The coulombic interactions between the phosphate groups and the basic amino acids residues of the proteins are assumed to be the major source of binding in these cases. In contrast, proteins which facilitate and regulate the transcription are very specific to the nucleotide sequence. Great advances have been made in the detection of protein-DNA interactions which would have a wide applicability in the rapidly developing biological research field. However, we find that today our knowledge of interactions between proteins and nucleic acid is still rather limited. The main problem is that these systems are all rather complex and require simultaneous observation of two associated macromolecules. In order to circumvent the difficulties inherent in these systems, model compounds involving nucleic acid and protein constituents have been studied.Many diseases arises owe to the miscommunication of these two regulatory elements i. e. DNA and protein. Overexpression of transcription factors are usually observed in oncogenes [6]. For this reason extensive studies were done on transcription factors till date. DNA being polymorphic also exists in various canonical and non-canonical structures and various proteins bind specifically to DNA structures. DNA bases are planar and aromatic moieties and aromatic amino acids specifically interact with DNA via base stacking to DNA bases and intercalation mode of binding. DNA contains negatively charged sugar-phosphate backbone so proteins containing positively charged amino acids such as lysine, arginine interact via binding to the DNA backbone. So, basically DNA-protein interacts via specific as well as non-specific binding.The latter interactions can also be specific due to sequence dependent formation of DNA structure. Protein might produce drastic conformational changes in DNA which is essential and responsible for their efficient function. Thus, in order to understand the DNA-protein complex function clearly, various techniques are required and used to investigate binding between DNA-protein. For better understanding we should not rely on a single technique, so we tried to summarize a few of the techniques in this review.

Characterization of DNA-Binding Protein

There are various biochemical and biophysical methods which are currently used for investigation of DNA-protein interaction and these are summarized in Figure 1. Here, in present article a few techniques are discussed.

Nitrocellulose Filter Binding Assay

Nitrocellulose method is now obsolete but earlier it was used very frequently to investigate DNA-protein interaction. This method is very rapid and simple to perform. This process is based on the principle that any protein can bind to the nitrocellulose without losing their capacity to bind DNA. In this method labeled DNA with protein are set to incubate and allow enough time to attain equilibrium [7,8]. Mixture is made to filter through a filter disk composed of nitrocellulose. Protein can bind effectively to nitrocellulose while DNA is unable to bind. If any DNA bind to protein that can be retained on the filter due to complex formation. Now dry the filter and count it. The demerit of this method is that the exact location of binding site can not be recognized as well as if mixture of protein used for incubation it was difficult to explain about the protein binding affinity of different proteins. One more demerit is also worth to discuss here that sometimes single-stranded nucleic acids are also retained at nitrocellulose filters under various conditions which put a question mark on the authentication of this technique.

Foot Printing Assay

Foot printing assay is specifically used in order to investigate about the binding of protein to DNA and precise binding of a protein to a particular sequence of DNA. The DNA region which is subjected to study is end-labelled and allowed to interact with a protein either crude extract or in pure form. Further, the DNA-

protein complex is treated with enzymatic or chemical agent who specifically cleaves DNA, to see the effect of these on protein bound DNA. These reagents do not affect protein bound DNA because protein protect DNA from cleavage. These complexes are then run on denaturating PAGE and subjected to autoradiography. DNA ladder is used for comparing the protein bound DNA and DNA alone (Figure 2). The protected region is observed as gap in the continuous bands of the digestion products. The DNase I was used as the first reagent for DNA foot printing experiment [9,10]. Due to its large size DNA is readily prevented by the attack of DNase owe to the steric hindrance. DNase I reagent is used under mild conditions so that it does not perturb the DNA-protein complex at larger extent [11]. With advantages there are some disadvantages of DNase I as foot printing agent as well. Due to its size it is difficult to cutting it immediately in vicinity of protein bound DNA complex.Along with this a large amount of protein is required to ensure that DNA-binding site is saturated. So, this technique is not suitable where small amount of protein is available. The cleavage of DNA by DNase I is variable i. e. some sequences cleaved very rapidly whereas some sequences are not digested even after long time of incubation. Thus, an uneven ladder of digestion product is observed in electrophoresis.

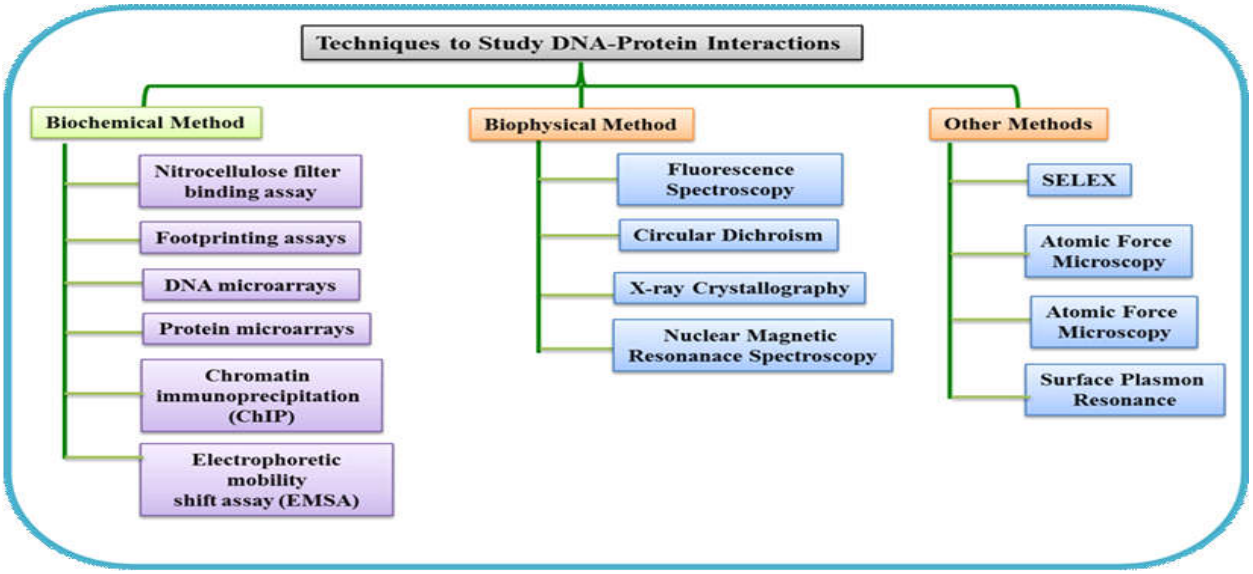

Fig. 1: Schematic representation of techniques used to study DNA-protein interaction.

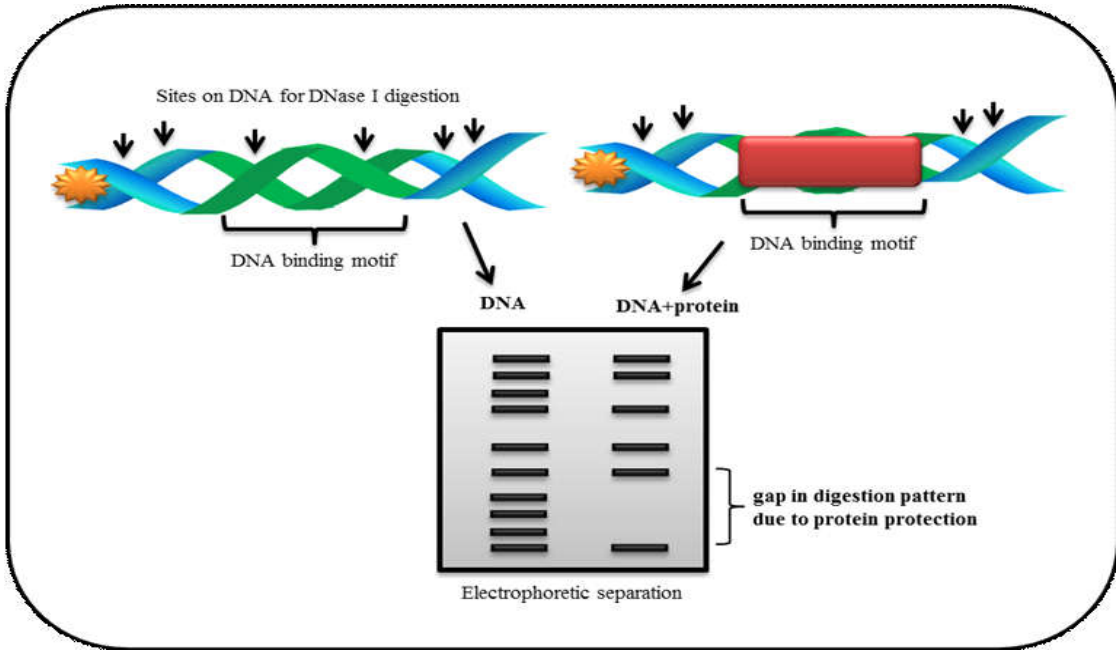

Fig. 2: Schematic representation of DNase I Foot printing

Characterization by Electrophoretic Mobility Shift Assay

EMSA is also known as gel retardation assay in which a radiolabeled duplex DNA or a labelled restriction fragment which consists of one or more binding sites for a specific DNA-binding protein motif. The restriction fragment can be labeled at both ends or at one end and it can give a single band in gel [12].Then incubation is set up for fragment either in absence of protein and increasing concentration of protein. These are allowed to incubate for some time to facilitate binding. Then these samples are loaded in polyacrylamide or agarose gel. The gel buffer consists of low salt concentration for the stabilization of DNA-protein complex. Distribution of radioactivity can be visualized by foot printing method. The DNA-protein complex migratesslowly in comparison to the DNA without any protein. So, this is the reason why we call it gel retardation assay.

Biophysical Techniques

Biophysical techniques include spectroscopic methods to investigate the DNA-protein complex formation. All the biophysical techniques which are used for structure study of DNA and protein alone can also be applied for the binding interaction. The most sensitive and valuable techniques are fluorescence and circular dichroism spectroscopy. Other spectroscopic techniques such as nuclear magnetic resonanceand X-ray techniques require large sample amount as well as more time for data analysis. These techniques need very expensive instruments as well.

- *Fluorescence Spectroscopy*

Fluorescence spectroscopy is a very sensitive techniqueused to elucidate DNA-protein interaction. Protein can produce intrinsic emission spectra due to presence of tryptophan and tyrosine amino acid in its structure. Often quenching of intrinsic emission spectrum and/or shift in wavelength maxima is observed on protein binding to DNA. So, by comparing the change observed in free protein spectra after addition of DNA is considered for calculation of binding constant and other binding parameters [13]. Fluorescence anisotropy is also used for the investigation of DNA-binding curve when molecular size of Protein-DNA complex is sufficiently different from the fluorescing component which is specifically in all cases is protein. Sometimes an extrinsic fluorophore can also be attached to DNA single strand or duplex at 5′ ends to increase the sensitivity and investigation of DNA-protein interaction [14].

- *Circular Dichroism*

CD is also a very informative technique which is used to investigate DNA-Protein interaction. Generally it is not used for calculation of binding parameters and binding constants rather it is applied for analyzing structural changes in DNA-protein interaction [15]. The far UV-CD (170-250nm) is exploited for the secondary structure of proteins and investigation is carried out to see the effect of DNA binding to protein in this region whereas near UV-CD (250-300nm) signal is specifically arise due to DNA component and study is done to reveal conformational changes in the DNA structures when protein binds to it. A large distortion is observed when aromatic amino acids of protein intercalate between the DNA bases [16]. CD is found to be less sensitive technique to study DNA-protein complex.

Conclusion

DNA-protein association and interaction has an utmost importance in the biological system. Several biochemical and biophysical techniques are available to study and interpret the binding interaction of DNA-protein. Every technique is self-efficient in one or other aspect of elucidation of DNA-protein interaction. Many topics have been left here owing to the restriction of space but all the techniques should combined to study these interactions and give insight about the exact mechanism involve in DNA-protein binding.

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Review Article

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Heat Shock Proteins and Their Therapeutic Applications: An Overview

Jasleen Saini[1], Jaspreet Kaur Boparai[2], Ramanpreet Kaur[2], Pushpender Kumar Sharma[3]

Author Affiliation: [1]M.Tech Biotechnology, [2]PhD Scholar, [3]Assistant Professor, Department of Biotechnology, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab-140406, India.

Abstract

Heat shock proteins (HSPs) are a class of proteins that play important role in protein folding, maintaining homeostasis, and suppressing the aggregation of mis-folded proteins. The synthesis of these proteins in the cell is highly regulated, and is induced under various stress conditions that include the pH, temperature, and starvation, UV and chemical exposure and the oxidative stresses; however few Hsps also expresses constitutively. The major classes of HSPs include the HSP60, HSP70 and HSP90 and the small heat shock proteins that ranges from 12-40 kDa in size. The small heat shock proteins like Hsps 18 are well known to facilitate the refolding of substrate proteins and maintaining its biological activity, for which this protein has been explored as an efficient delivery system for the vaccines development. This review will discuss various HSPs and their close relatives involved in folding, assembly, regulation, and degradation of other proteins. The review will further highlight the various approaches by virtue of which the Hsps can be employed in therapeutic interventions.

Keywords: Molecular Chaperons; Folding; Therapeutics; Cancer; Stress; Heat Shock Protein.

Introduction

During stress conditions like high or low temperature, pH, osmotic stun, starvation, UV and chemical exposure, oxidative stress, almost all organisms expresses heat shock proteins that help these organisms to survive and perform their proper functions under these conditions [1-2]. These proteins function as molecular chaperons, due to their assisting role in proper folding of the partially or mis-folded proteins, it also suppress the aggregation of mis-folded proteins [3]. During this process they require ATP, and are assisted by their co-chaperons for efficient functioning. Synthesis of Heat shock proteins should be transitory even in sustained stress conditions, as their continuous synthesis would unfavorably affect the protein homeostasis as well as various cellular functions. A mechanism regulating the synthesis of Heat shock proteins involve binding of Hsp70 to transactivation domain of HSF1, thus repressing transcription of heat shock gene [4] Figure 1. Another mechanism leading to inhibition of heat shock protein synthesis involves binding of heat shock protein binding factor 1 (HSBP1) to HSF1 trimer and Hsp70, preventing HSF1 binding to DNA [5]. Heat shock response in prokaryotes is regulated by sigma factor, which is encoded by *rpoH* gene. This sigma factor binds to RNA polymerase and helps in transcription of the heat shock genes [6-7-8]. In *E. coli* various genes, whose transcription involve ó-32 factor, have been identified that include e.g. *htpY, dnaK, rpoD, grpE, groES, groEL, clpB* etc. Regulation of heat shock protein synthesis in prokaryotes is shut down by the feedback inhibition mechanism that is induced by *DnaK* chaperone machine. *DnaK* chaperone machine

Reprint Request: Pushpender Kumar Sharma, Assistant Professor, Department of Biotechnology, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab-140406, India.

E-mail:pushpg_78@rediffmail.com, psnp7819@gmail.com

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includes *DnaK, DnaJ* and *Grp E* proteins. These three proteins help in down regulation by repressing the translation of *rpoH* mRNA. Repression of mRNA translation lead to degradation of σ-32 and repression of its activity [9]. In eukaryotes, Heat Shock Factors (HSF), carry out the process of transcription [10]. Cells without stress have HSF bound to HSPs in the cytoplasm , whereas under stress conditions, HSF separates from the HSPs and form a trimer. HSF trimer enters into the nucleus and its phosphorylation occurs which are pre-requirements for binding of HSF to heat Shock Elements that carry the transcription process [11].

This review endeavors to offer an overview of heat shock proteins and their role in as autoimmunity, cancer and other vascular diseases. Various Hsps and their close relatives that are known to be involved in folding, assembly, regulation, and degradation of other proteins are also discussed in this article. It further highlights the mechanisms underlying these functions that provide insights into the approaches by which heat shock proteins can be used as therapeutic interventions. Primary objective of this review article is to glimpse the structure and functioning of various classes of HSPs and to further provide insights about their role in therapeutics.

Heat Shock Proteins as Molecular Chaperons

Molecular chaperones help in stabilizing unstable proteins by binding to them and assisting the processes like folding, assembly and regulation of denatured conformations of proteins [12]. Principal categories of chaperones that perform these functions are the small heat shock proteins, proteins of Hsp90 family [13], Hsp 100 family [14], Hsp 70 and Hsp 60 family [15]. These chaperones are sometime assisted by co-chaperonin that helps these chaperones in the folding process. For example, *Gro ES* is a co-chaperone that assist chaperone *Gro EL* in folding of proteins. Similarly, *Dna J* is a co-chaperone that assists *dnak*. During the folding process, chaperones require energy which it attain by hydrolyzing ATP into ADP. During ATP hydrolysis, conformational changes take place in the heat shock proteins [16]. The table below depicts members and functions of Heat shock proteins.

Major Families of Heat Shock Proteins

Hsp90 Family

HSP90 is an ATP-dependent molecular chaperone, which is known to stabilize the substrate proteins during the formation of steroid receptor complexes [31]. Hsp90 maintain the active or inactive conformation of client proteins and inhibit the aggregation process [32]. Structurally, It is a dimeric protein comprises of four domains that include the N-domain, charged linker, middle domain and C-domain. Middle domain consists of ATPase site, and is responsible for ATP hydrolysis and binding of substrate proteins [33]. Interestingly, Hsp90's, ATP-binding region has a lid that generally exists in an open conformation during ADP-bound structure, but changes to a closed conformation when ATP bound [34]. C- Terminal domain comprises of three stranded β-sheet and α-helix coil [35]. The middle domain of Hsp90 has three regions- two α–β–α domain (large and small) and a helical coil [36]. Charged linker region comprises of repeats of charged amino acids which are highly conserved and are connected with N terminal domain of *Hsp90.* ATP bound conformation of *Hsp90* was depicted along with *p*[NH] *ppA* (adenosine 5_-[β,γ - imido] triphosphate; 'AMP-PNP') and p23/Sba1 co- chaperone [37]. *Hop/ Sti1* (*Hsp70-Hsp90* organizing protein) is a co-chaperone which associates *Hsp70* chaperone system with Hsp90 chaperone machinery and also inhibits ATPase activity of *Hsp90* [38-39]. Notably, inhibition of ATPase activity is associated with another co-chaperone *Cdc37/p50* [40].

Genes of *Hsp90* family from various dissimilar organisms like fruit flies, yeasts, chickens, Mammals, trypanosomes, and bacteria has shown very much identity in these sequences. All the proteins of *Hsp90* family in eukaryotes and bacteria *E. coli* contains a region of high negative-charge at the carboxyl end, which is usually observed to be different, except four amino acid residues, *glu-glu-val-asp,* that is observed to be similar among *Hsp90* of eukaryotes. Earlier studies have demonstrated that proteins of the *Hsp90* family are present abundantly even under normal temperatures conditions, and are induced by heat. In *Drosophila melanogaster*, only one gene of this family i.e. *Hsp83* is known. In yeast *S. cerevisiae*, two genes of this family are known – *Hsc83* and *Hsp83*, while *Hsc83* is reported to be constitutively expressed and induced moderately by heat, in contrary, the *Hsp83 i*s constitutively expressed at a minor level and is strongly induced by heat [41]. In *Arabidopsis thaliana,* truncated cDNA of *Hsp83* was isolated, sequenced and cloned to full length by primer extension methods. The study revealed that the level of homologous transcripts of this cDNA increases upon induction at high temperature [42]. The protein of the endoplasmic reticulum also contains the sequence *glu-glu-val-asp* at the same position in the protein, but not at the C-terminal position. The ER

protein *GRP94* is glucose regulated protein as it is induced by glucose starvation and the cytosolic one is induced by glucose restoration [43].

Hsp70 Family

The 70 kDa family of proteins is the most abundant family of heat shock proteins. Hsp70 play a role in folding of the denatured proteins into their native state and further holds the unfolded polypeptides [15]. The structure of Hsp70 consists of an ATPase domain, a β- sandwich sub domain at the Carboxy end which is its substrate binding domain and an α-helical subdomain. The activity of Hsp70 takes place when hydrophobic peptides of the proteins interact with substrate binding domain of Hsp70 in an ATP-dependent manner. Hsp40 is a co-chaperone that helps Hsp70 in folding process, and is essential to activate the ATPase activity of Hsp70 that results in Hsp70-ADP complex. The release of ADP is aided by a nucleotide-exchange factor for the opening of nucleotide binding cleft. When ATP binds the ATPase domain of Hsp70, a conformational change in the substrate binding domain takes place and substrates that are bound gets released which completes the ATPase cycle [44]. All Hsp40 comprises of a domain known as J-domain which necessarily help in cellular activity via interacting with it. The J-domain has a conserved sequence of tripeptides - Histidine-Proline-Aspartic acid (HPD) [45]. Hsp40s are classified into three classes on the basis of the functional domains contained in them. Type I Hsp40s are highly conserved and consist of a glycine-phenylalanine (G/F) and cysteine rich region that consists of four motifs of CXXCXGXG which is a glycine/methionine rich region. A carboxy terminal peptide binding domain and a dimerization domain. Type II Hsp40s consist of the J domain and the G/F rich region, along with the peptide-binding region at the C-terminus. Type III Hsp40s possess only the conserved J-domain that can exist anywhere on its sequence [46]. Apart from folding, members of 70 kDa heat shock proteins appear to play necessary roles in Clathrin-dissociation activity from vesicles coated with bovine and depict high ATPase activity too [47]. Hsp70kDa family is also required for the synthesis of protein, its translocation as well as storage [48]. Various cell organelles like cytoplasm, nucleus, mitochondria and endoplasmic reticulum found to have hsp70 and other members of its family [49]. Various proteins associated with Hsp70 family are inducible only under stressful conditions, whereas, few are constitutive in nature and expresses under normal conditions too [49-50].

Hsp60 Family

Hsp60 (GroEL) is a chaperonin that belongs to molecular chaperone family and assist protein folding of denatured proteins [18-51]. The structure of GroEL is in the form of a cage consisting of two seven-membered rings of 57kDa each within the central cavity [25]. The detail investigation of the structure GroEL structure has revealed the presence of three domains – Equatorial domain, Apical domain and a middle Hinge domain. The equatorial domain is an ATP-binding region and the apical domain is a substrate-binding region consisting of hydrophobic residues. The third domain middle hinge is a connecting domain between the other two domains [52-53]. Co-chaperonin named as GroES (Hsp10) assists GroEL in the protein folding process which binds upon the open-cavity of GroEL as a lid (Figure3). Binding of GroES causes rotation of the subunits of Hsp60 in a way that protein is released from the hydrophobic site into hydrophilic part which facilitates its folding. Interestingly, energy in the form of ATP is required for the same. Denatured protein binds to the hydrophobic region of apical domain on the inner side of GroEL. Consequently, ATP binding leads to a conformational change which facilitates release of substrate protein and binding of GroES. Furthermore, hydrolysis of ATP causes release of GroES lid and liberates the substrate protein. Complete folding of the client protein is achieved after manifold of cycles [53-54].

Contrary to GroEL-GroES chaperone system, TRiC is member of Hsp60 Family present in the eukaryotic cytosol which is constitutively expressed [51]. Large sized proteins that could not undergo folding via GroES-GroEL chaperone machinery undergo folding *via* binding to TRiC. TRiC binds to emerging polypeptides from the ribosomes which are brought to TRiC through a chaperone GimC or through DnaK-DnaJ chaperone machinery [26-54]. Hsp60 activity is regulated by several post-translational modifications that include acetylation [55], glycosylation [56] and ubiquitination [57].

Small Heat Shock Protein Family

Small Heat Shock Protein Family comprises of members of molecular size ranging from 12kDa to 40kDa. These are unique in having an 'alpha-crystallin domain' which remains conserved throughout. Besides this, sHsps also consists of a C-domain and N-domain, as revealed by X-ray crystallography studies [58-59]. Small heat shock proteins display chaperone activity. sHSP 18 act as molecular chaperone same as that of alpha-crystallin. Both sHSP 18 and alpha-crystallin are effective in preventing inactivation of restriction enzyme from

heat [45]. Hsp18 play a role in maintaining the biological activity of the proteins as depicted in one study, where authors have showed the role of sHsp18.1 preventing the denaturation of enzymes NdeI and SmaI from thermal inactivation [60]. Another study also confirm that Hsp18 bound to a heat denatured luciferase enzyme could re activate it in the presence of wheat germ extracts or rabbit reticulocyte and thus shows that this small heat shock protein can help in refolding of substrate proteins [61-62]. Due to its chaperone activity in maintaining the protein stability, sHsp18 is also used as an efficient delivery system for the vaccines [60].

HSP20/α-crystallin, another small heat shock proteins categorized as a molecular chaperone Members of the sHsp 20 family have a common structure that consists of α -crystallin core structure that is found in the C-terminal position . Any alteration in these chaperones are reported to be associated with different diseases such as prion disease, cystic fibrosis, cataracts, or neurodegenerative diseasesincluding Huntington's disease, Parkinson's disease and Alzheimer's diseasedue to the aggregation of a protein because of partial unfolding and exposure of hydrophobic surfaces of proteins [63].

Hsp27(HspB1), a Class1 Heat shock protein is known to repress senescence [64] and block apoptosis in cancer by inhibiting the mitochondrial release of apoptotic proteins. Hsp27 facilitates apoptosis by binding to cytochrome c, inhibiting the formation of apoptosome and thus preventing the activation of Caspase-3 [30]. Another study related to sHsp30 proposed that induction of this protein is known to down regulate the stress stimulation of H^+-ATPase activity. It provides various tolerances to several stresses like heat shock, osmotic stress, glucose starvation, organic acid stress etc. These stress tolerances are not affected by the loss of hsp30 in cells rather the time required for adaptation to these stress conditions is extended [65].

Heat Shock Proteins As Therapeutic Targets

Heat Shock proteins are important in maintaining the homeostasis in cells. One of their main functions includes transport of peptides among the components of the cell. Due to this approach, stress proteins are targeted for the regulation of immune system. Foreign antigenic peptides induce the expression of heat shock proteins and generate immune response in the body. These proteins also help in presenting the foreign peptides to the immune cells. HSPs can also be used in the anti-cancer vaccines, as these help in presenting the foreign peptides from the cytosol to the MHC-1 complex in the endoplasmic reticulum. MHC-1 and antigen complex after binding with the CD4 receptors of the T-cytotoxic cells induces an immune response against the antigenic peptide. MHC-1 complex becomes active by the cytokines released by T-helper cells which are associated with MHC-2 complex to generate an immune response. This strategy helps in designing an approach for the vaccination in which antigenic peptides and chaperone complex can be introduced into the tumor cells to generate an immune response [66].

HSP Based Anticancer Vaccines

Heat shock proteins are important due to their functional role in preventing the accumulation of degraded or misfolded proteins. Hence, *Hsps* play a major role in preventing body tissue degradation and aging process [67]. Moreover, the level of expression of these chaperone elevate in cancer [68-69]. *HspC2* was found to have high expression level in breast cancer cells [70], while *HspB1* increased expression is associated with prostate cancer, liver cancer, pancreatic cancer as well as gastric cancer [71-72]. Overexpression of heat shock proteins has been known to be problematic in various anticancer therapies, and can cause resistance to these therapies *via* refolding of proteins and preventing the apoptosis [71-73]. Hence, down regulation of over-expressed *Hsps* can be an effective way to overcome this problem. Hsp90, one of the most abundant proteins among Heat shock proteins have been targeted in a variety of cancers, such as Breast cancer, Colon cancer, Solid Neoplasm, Gastric Carcinoma. Targeting and inhibiting Hsp90 resulted in degradation of oncogenic proteins [74]. Various clinical trials are recruiting related to Hsp90 inhibitors as mentioned in table below. The first inhibitor of Hsp90 as 17-AAG (17 Allylamino-17-Demethoxygeldanamycin) [75]. Nonetheless, more research is essential to state a clear and thorough mechanisms of Hsps in allograft rejection. Immunogenic properties of the Hsps is well known [76]. Mechanisms lying behind Hsps mediated immunity involve the binding of these Hsps to antigenic peptides and their presentation to Antigen Presenting Cells (APCs) and cross priming of Cytotoxic T-lymphocytes [77-78]. Thus, immunotherapy can be used to deploy Hsp vaccines to the patient. An example illustrating the use of Hsps as vaccines involves *Gp96* vaccine in the treatment of melanoma and carcinoma is in clinical trials [79-80]. Hsps belonging to large molecular weight chaperone families including Hsp110 and Grp170

are important targets in the development of anticancer vaccines as they are known to bind to the antigens Trp2 and Gp100 in case melanoma. In addition to this, studies revealed that both Hsp110 and Grp170 increased the immunogenic peptide effects greatly [81-82]. Some Hsps are known to be expressed on surface tumor cells but are absent on the surface of normal cells. Thus, natural killer cells recognize these tumor cells due to the responsiveness of Hsps expressed on their surface [83-84]. Hsp based vaccines have the potential to treat various kinds of cancer as mentioned in the table depicting interventions used in clinical trials.

Heat Shock Proteins in Neurodegenerative Diseases

Neurodegenerative diseases occur due to generation of neurotoxicity through misfolding and aggregation of proteins. These include Huntington's Disease, Parkinson's Disease, Sclerosis, Muscular Atrophy, Ataxias etc. [85]. Among neurodegenerative diseases, Alzheimer's is a type of dementia which is characterized by the formation of amyloid plaques leading to brain cell death. In the diseased state, a protein named Amyloid Precursor Protein (APP) is cleaved by β and γ secretase to generate $A\beta_{42}$ which is a neurotoxic fragment. The aggregation and oligomerization of this neurotoxic fragment leads to the plaque formation; hence causing neuronal cell death [86- 87]. Heat shock proteins have been observed to be linked with the protein aggregates. HspB proteins have been found in plaques and amyloids [88] and HspA1 proteins have been associated with Aβpeptides [89].

Parkinson's disease is another neurodegenerative disease which is characterized by the presence of mutations in α-synucleic gene. Various studies suggest that several members of Hsps have been found to be associated with the treatment of Parkinson's disease. An example includes small heat shock protein Hsp27 which inhibits the toxic effects caused due to mutations in α-synuclein [90]. Moreover, Hsp27 and α-B crystallins have been found successful in the treatment of neurodegenerative diseases by preventing the fibril formation through their binding to Aβ peptides [91].These studies suggest that Heat shock proteins have been implicated in the treatment of neurodegenerative diseases.

Other Studies Related to Hsp Vaccines

Several investigations have associated heat shock proteins to autoimmune diseases. Evidences suggested that Hsp60 is implicated in rheumatoid arthritis [92] and Type 1 diabetes [93]. Hsp60 also facilitates the secretion of various cytokines, thus can be a potential target for inflammatory diseases [94]. A study has demonstrated the use of therapeutic agents which are competitive inhibitors of Hsp70 against Gaucher disease, lysosomal storage diseases and β-galactosidase deficiency disorders in order to stabilize the damaged proteins [95]. Another therapeutic agent, Salvianolate has shown to treat ischemia in a rat model [96]. Small Heat shock proteins like Hsp27, Hsp22 and HspB5 are characterized by the presence of α-crystallin domain that is correlated with inflammation, apoptosis and tumorogenesis [97-98-99]. Heat shock proteins find

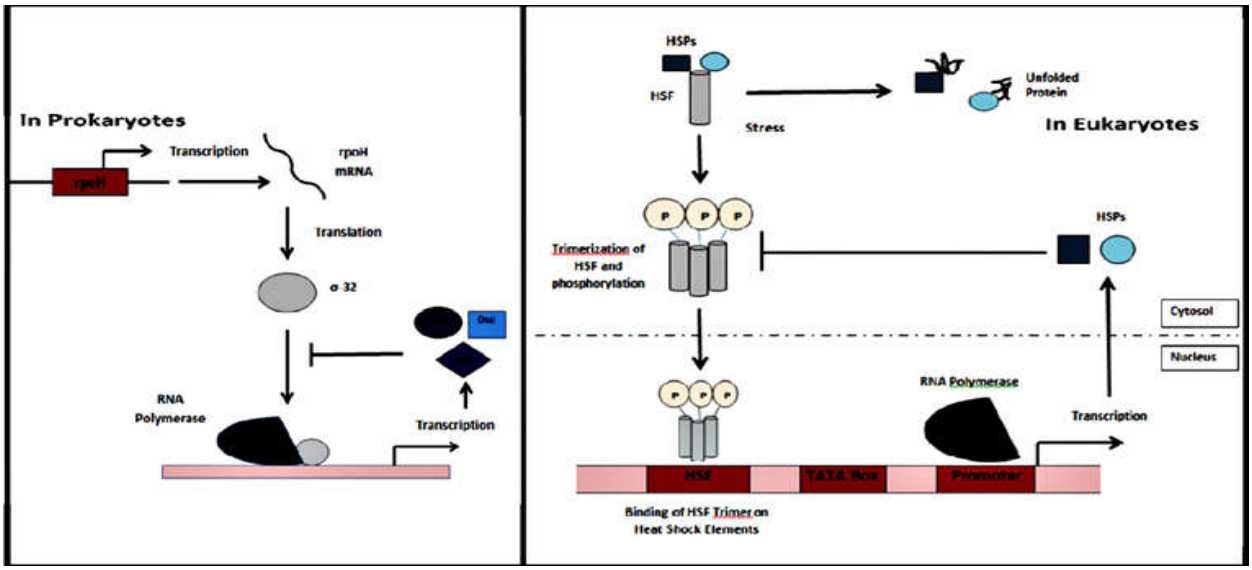

Fig. 1: Regulation of Heat Shock Proteins in **(a)**Prokaryotes **(b)**Eukatyotes

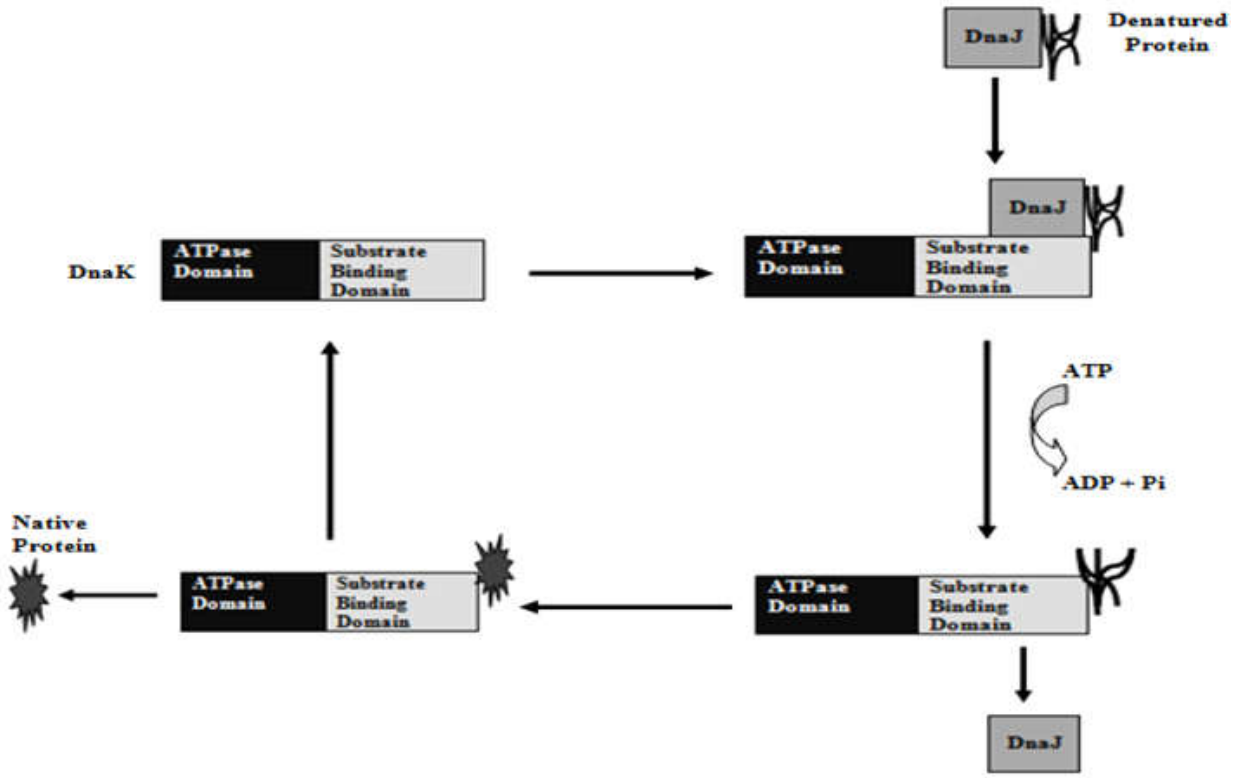

Fig. 2: Schematic Representation of DnaK-DnaJ Chaperone Machinery

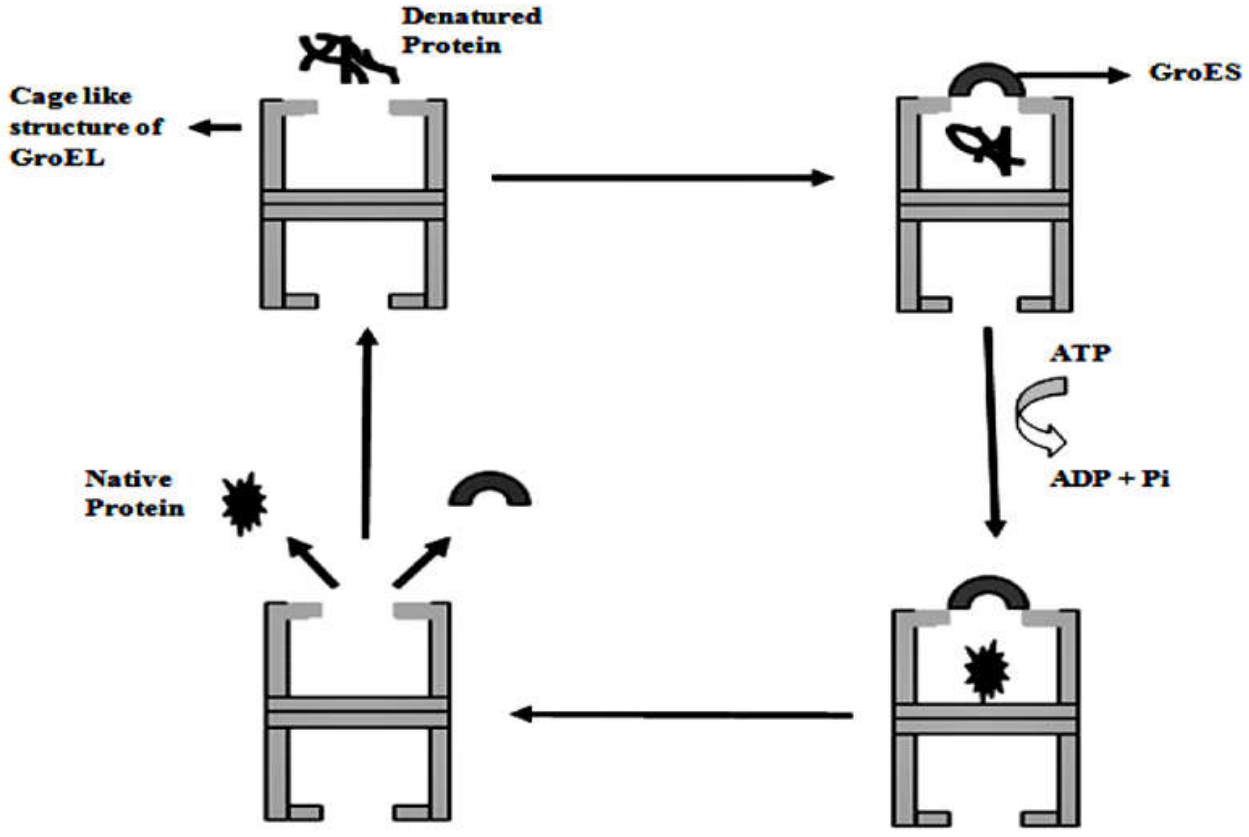

Fig. 3: Protein Folding *via* GroEL-GroES chaperone system

Table 1: The table below depicts members and functions of Heat shock proteins.

S. no.	Hsp Family	Members	Functions	Reference
1.	Hsp100	Class I proteins - Hsp104, bacterial ClpB and their distant relatives ClpA, ClpC); Class II - ClpX and HslU.	Protein disaggregation in association with Hsp70 chaperone system; Proteolytic degradation of proteins; Remodeling of client proteins	[14, 17, 18]
2.	Hsp90	HtpG in the bacterial cytosol; Grp94/gp96 in the endoplasmic reticulum of eukaryotes; Hsp75/TRAP1 in the mitochondrial matrix and; Hsp90 in eukaryotic cytosol (Hsp83 in *Drosophila* Hsc82 and Hsp82 in yeast, Hsp90α and Hsp90β in humans, Hsp86 and Hsp84 in mice).	Protein folding; Regulation of the stability and active state of substrate proteins; Contribution in a ide range of cellular processes like signal transduction, intracellular transport, and protein degradation.	[19, 20, 21]
3.	Hsp70	DnaK, HscA or Hsc66 and HscC or Hsc62 in Prokaryotes; Hsc70 in Cytosol, Hsp70 and its paralogs HSPA1A, HSPA1B, and HSPA1L in Eukaryotes ; Binding immunoglobulin protein (BiP or Grp78) in endoplasmic reticulum and; mtHsp70 or Grp75 in mitochondria.	Folding and refolding of client proteins; Proteolytic degradation of unfolded proteins; Transmembrane transport of proteins	[22, 23]
4.	Hsp60	Group I proteins - GroEL in bacterial cytosol, Hsp60 in mitochondria and Rubisco binding protein (RuBisCoBP) in chloroplasts; Group II- Thermosome/TF55 in archaea and TRiC/CCT in the eukaryotic cytosol.	Binding to the substrate protein and enabling its folding	[24, 25, 26, 27]
5.	Small Heat Shock Proteins	Class I sHsps - Hsp27 (HspB1), αB-crystallin (HspB5), Hsp20 (HspB6) and Hsp22 (HspB8); Class II sHsps - HspB2, HspB3,HspB4 (αA-crystallin), HspB7, HspB9 and HspB10.	Prevents aggregation of denatured proteins; Increase the stability of microfilaments, intermediate filaments and microtubules; Blockage of apoptosis (e.g., Hsp27)	[28, 29, 30]

Table 2: Table below shows Interventions involving Heat Shock Proteins in Clinical Trials

S. No.	Heat Shock Protein involved	Intervention	Disease	Phase of trial	Source
1.	Heat Shock Protein Peptide Complex-96 (HSPPC-96)	Biological: **Heat Shock Protein Peptide Complex-96 (HSPPC-96)**; Procedure: Tumor Resection; Radiation: Radiation	Glioblastoma Multiforme; Astrocytoma, Grade III; Anaplastic Ependymoma; Clear Cell Ependymoma; Ependymoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02722512?term=heat+shock+proteins&rank=1
2.	Hsp70	Biological: **Heat Shock Protein 70-peptide complexes (HSP70)**	Breast Neoplasms	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT00027131?term=heat+shock+proteins&rank=2
3.	Heat Shock Protein gp96	Biological: **HSPPC-96**; Procedure: conventional surgery	Brain and Central Nervous System Tumors	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT00293423?term=heat+shock+proteins&rank=4
4.	Heat Shock Protein gp96	Biological: **gp96**	Glioma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02122822?term=heat+shock+proteins&rank=6
5.	Hsp70	Biological: **Heat Shock Protein 70 HSP70**	Leukemia,Myeloid, Chronic; Leukemia,Myeloid,Philadelphia-Positive	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00027144?term=heat+shock+proteins&rank=7

6.	Heat Shock Protein Peptide Complex-96	Drug: **HSPPC-96 or Oncophage**	Malignant Melanoma	Phase 3	https://clinicaltrials.gov/ct2/show/NCT00039000?term=heat+shock+proteins&rank=8
7.	Heat Shock Protein gp96	Biological: **autologous gp96 vaccination**	Liver Cancer; Pancreatic Adenocarcinoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02133079?term=heat+shock+proteins&rank=12
8.	Heat Shock Protein gp96	Biological: **autologous gp96 vaccination**; Drug: Oxaliplatin+S-1	Gastric Carcinoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02317471?term=heat+shock+proteins&rank=13
9.	Heat Shock Protein Peptide Complex-96	Drug: ipilimumab; Drug: **HSPPC-96**	Melanoma	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02452281?term=heat+shock+proteins&rank=14
10.	Hsp90	Drug: CDKI AT7519; Drug: **Hsp90 Inhibitor AT13387**	Adult Solid Neoplasm	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02503709?term=heat+shock+proteins&rank=19
11.	Heat Shock Protein Peptide Complex-96	Drug: **autologous human tumor-derived HSPPC-96**	Lymphoma, Follicular; Lymphoma, Small Lymphocytic	Phase 2	https://clinicaltrials.gov/ct2/show/NCT00081809?term=heat+shock+proteins&rank=21
12.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**; Drug: bevacizumab	Recurrent Glioblastoma; Recurrent Adult Brain Tumor; Gliosarcoma	Phase 2	https://clinicaltrials.gov/ct2/show/NCT01814813?term=heat+shock+proteins&rank=22
13.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**	Brain and Central Nervous System Tumors	Phase 2	https://clinicaltrials.gov/ct2/show/NCT00905060?term=heat+shock+proteins&rank=27
14.	Hsp70	Biological: **recombinant 70-kD heat-shock protein**	Leukemia	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00030303?term=heat+shock+proteins&rank=28
15.	Hsp70	Biological: OVA BiP peptide; Biological: gp209-2M antigen; Biological**: recombinant 70-kD heat-shock protein**; Biological: tyrosinase peptide	Melanoma (Skin)	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00005633?term=heat+shock+proteins&rank=29
16.	Heat Shock Protein Peptide Complex-96	Biological: **HSPPC-96**	Renal Cell Carcinoma	Phase 2	https://clinicaltrials.gov/ct2/show/NCT01147536?term=heat+shock+proteins&rank=38
17.	Hsp 90	Drug: Erlotinib Hydrochloride; Drug: **Hsp90 Inhibitor AT13387**; Other: Laboratory Biomarker Analysis; Other: Pharmacological Study	Recurrent Non-Small Cell Lung Carcinoma; Stage IV Non-Small Cell Lung Cancer	Phase 1 / Phase 2	https://clinicaltrials.gov/ct2/show/NCT02535338?term=heat+shock+proteins&rank=55
18.	Hsp 90	Drug: Dabrafenib; Drug**: Hsp90 Inhibitor AT13387**; Other: Laboratory Biomarker Analysis; Other: Pharmacological Study; Drug: Trametinib	Recurrent Melanoma; Solid Neoplasm; Stage IIIA Skin Melanoma; Stage IIIB Skin Melanoma; Stage IIIC Skin Melanoma; Stage IV Skin Melanoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02097225?term=heat+shock+proteins&rank=57
19.	Hsp 90	Drug: **Hsp90 Inhibitor AT13387**;	Estrogen Receptor Negative; HER2/Neu Negative;	Phase 1	https://clinicaltrials.gov/ct2/show/NCT02474174

		Other: Laboratory Biomarker Analysis; Drug: Paclitaxel; Other: Pharmacological Study	Progesterone Receptor Negative; Recurrent Breast Carcinoma; Stage IIIA Breast Cancer; Stage IIIB Breast Cancer; Stage IIIC Breast Cancer; Stage IV Breast Cancer; Triple-Negative Breast Carcinoma		3?term=heat+shock+proteins&rank=61
20.	Hsp90	Drug: **MPC-3100 (an Hsp90 inhibitor)**	Cancer	Phase 1	https://clinicaltrials.gov/ct2/show/NCT00920205?term=heat+shock+proteins&rank=67
21.	Hsp110-gp100	Biological: Recombinant Human **Hsp110-gp100 Chaperone** Complex Vaccine; Other: Laboratory Biomarker Analysis	Recurrent Melanoma; Stage IIIB Skin Melanoma; Stage IIIC Skin Melanoma; Stage IV Skin Melanoma	Phase 1	https://clinicaltrials.gov/ct2/show/NCT01744171?term=molecular+chaperone&rank=1
22.	Hsp70	Biological: **CN54gp140 glycoprotein-hsp70 conjugate vaccine**	HIV Infections	Phase 1	https://clinicaltrials.gov/ct2/show/NCT01285141?term=hsp&rank=16

their immense applications in the transplantation processes, various Hsps like Hsp60 has been correlated with allograft rejection and autoimmunity [100].Clinical and experimental studies with Heat shock proteins have provided various opportunities to the researchers for treating the fatal diseases that remain uncured previously. Table 2 below present information resources towards the Heat Shock Proteins in Clinical Trials.

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Review Article

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Cholesterol Reducing Drugs and its Effect on the Level of CoQ_{10}

Sachin C. Narwadiya

Author Affiliation: Scientist C., Vigyan Prasar, A50, Institutional Area, Sector 62,Noida-201309.

Abstract

The cholesterol lowering drugs are becoming popular among the society. As the society is suffering from obesity due to disturbed life style and increasing fast food habits. The Co-enzyme Q10 also termed as CoQ10 is located in the mitochondria of eukaryotic cells. It has the property of solubility in oil and it's like vitamin. The CoQ10 plays and important role in cellular respiration specifically in electron transport chain. The CoQ10 reactions results into the generation of Adenosine Tri-Phosphate (ATP). The maximum amount of energy of the body needs generated through this way. The organs having more energy requirements like heart, liver, kidneys have the elevated levels of CoQ10. It is evident that the cholesterol level reducing drugs also critically reduces the CoQ_{10}. The reduction of the CoQ_{10} level further shows adverse effects. The biosynthesis of cholesterol and CoQ_{10} has same point during the pathway. The therapy needs more in-depth research. The consideration and recognition of a drug as cholesterol level reducing drug will have more critical trials. The society will be really benefited if the drug is safe and have minimum or no side effects.

Keywords: Adenosine Triphosphate-ATP; Co-Enzyme Q10; Cholesterol Lowering Drugs; Obesity.

Co-enzyme Q_{10}

The Co-enzyme Q_{10} abbreviated as CoQ_{10} is present in mitochondria of eukaryotic cells. The co-enzyme is having the property of solubility in oil and it's like vitamin. The CoQ_{10} plays and important role in cellular respiration specifically in electron transport chain. The resultant of its participation leads to generation of Adenosine Tri-Phosphate (ATP). The maximum amount of energy of the body needs generated through this way. The organs having more energy requirements like heart, liver, kidneys have the elevated levels of CoQ_{10}. (Ernster L;Dallner G,1995)(Dutton P.L *et al*, 2000)

Beef mitochondrial CoQ_{10} was isolated during 1957. It is observed that in heart muscles its level is more due to high energy requirements. (Crane *et al* 1957)

The CoQ_{10} is available in 3 states i.e fully oxidized state called ubiquinone, semiquinone, ubiquinol. In human being the biosynthesis is the major source of CoQ_{10}. Deficiency of CoQ_{10} in humans may result because of reduced biosynthesis and increased utilization in body. Some chronic disease conditions like cancers, heart diseases also thought to be the reason behind reduction in the CoQ_{10} levels. The intake of 1200 mg/day is observed as safe level. The plasma CoQ_{10} level is assessable and it indicates the dietary intake status rather than the tissue level.

The mechanism of action of CoQ_{10} has enhancement in cardiac bio-energy, free radical hunter, anti-oxidant effect, improved endothelial effects and prevention of myocardial sodium-potassium-ATPase activity. (Greenberg & Frishman, 1990)

The biosynthetic pathways of CoQ_{10} and cholesterol are same at some points. The mevalonate,

Reprint Request: Sachin C. Narwadiya, Scientist C., Vigyan Prasar, A50, Institutional Area, Sector 62,Noida-201309.
E-mail: snarwadiya@gmail.com

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which is the intermediary precursor of CoQ_{10} and suppressed by the cholesterol lowering drugs like statins.

CoQ_{10} biosynthesis occurs through steps like formation of benzoquinone, formation of isoprenoids side chain and joining of the two.

The patients on the treatment regimen of CoQ_{10} may develop the symptoms like nausea, Vomiting, Apetite loss and inflammation in stomach.

Carine Cleren *et al,* 2008 showed that the CoQ_{10} results in significant neuroprotective effects.

The physical state of CoQ_{10} is crystalline powder which is insoluble in water. The absorption of CoQ_{10} facilitates through micelle formation.

Cholesterol and its Biosynthesis

The free cholesterol and cholesteryl ester are the two forms of free cholesterol in tissues and plasma. Both forms in plasma transported in lipoproteins. In human beings the fats are absorbed as fatty acids from diet. These lipids then get transported across various tissue and organs for utilization and storage. The lipids were not soluble in water. Abnormalities in the metabolism of lipoprotein may cause hypo or hyper lipoproteinemias. The percent wise distribution of lipids in plasma is as under

Lipid	Percentage
Triacylglycerol	16%
Phospholipids	40%
Cholesterol	14%
Cholesteryl esters	36%
Long chain fatty acids	04%

The fats are less dense than the water and hence the density of lipoproteins reduces with increase of lipid proportion. The four major groups of the lipoproteins chylomicron, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high-density lipoproteins.

The cholesterol biosynthesis took place in the various tissues through acetyl-CoA which also act as precursor for various steroids like corticosteroids, sex hormones, bile acids and vitamin D.

The Low Density Lipoprotein (LDL) is important for the uptake of the cholesterol and cholesterol esters into many tissues. The High Density Lipoproteins HDL removes the free cholesterol from tissues transported to liver where it is eliminated from body. The removal of cholesterol may be as it is or after conversion to bile acids. Cholesterol is the main constituent in gallstones and plays a chief role in progression of atherosclerosis.

The cholesterol synthesized in body is about half of the body need (about 700mg/dl) and rest of the cholesterol is provided by the average diet.

The biosynthesis of the cholesterol took place through 5 steps.

1. Synthesis of mevalonate from acetyl CoA
2. Mevalonate losses CO_2 to form the Isoprenoids
3. Condensation of six Isoprenoids to form squalene
4. Cycalization of Squalene end up the product as the lanosterol
5. Cholesterol finally formed from the lanosterol (Robert K.Murray *et al.* 2003)

The CoQ_{10} and cholesterol biosynthesis share some point during pathway. The cholesterol level reducing drugs also adversely affect the CoQ_{10} level. This is the reason behind the generation of side effects of therapeutic use of the cholesterol level reducing drugs. Since the human being have the natural ability of biosynthesis of CoQ_{10} but deficiency may arise due to error in the biosynthetic pathways. At such event the supply of CoQ_{10} via medication may help for improvement.

Cholesterol Lowering Drugs

Statins and fibrates are the cholesterol level reducing drugs. Statins inhibit the Rac, a type of a small G protein. (K. Pahan, 2006)

The statins along with reducing of cholesterol level also deplete CoQ_{10} level. This will further results in weakening of heart muscles and immune system. The reports suggest that the CoQ_{10} protects the LDL from lipid peroxidation more effectively as cpared with the alpha-tocopherol. (Yamaonto Y *et al*, 1991, Stocker R *et al*, 1991) the cholesterol limiting drugs also known as Hypolipedemic drugs. There are many drugs that lower the cholesterol level. These drugs also show side effects. The drugs include Statin, Berberine etc.

Weijia Kong *et al* in 2004 in the study compared the drugs Berberine (BBR) and Statin. They observed that the Berberine have unique mechanism distinct from Statins. When the Berberine was administered orally to 32 patients suffered from high level of cholesterol for 3 months, the results observed that 29% showed reduced serum cholesterol level, 35% showed the reduced triglycerides and 25% showed the reduced LDL cholesterol level. In case of study with hamsters along with reduced serum cholesterol

level there were 3.5 fold increases in hepatic Low Density Lipoprotein Ribosomal mRNA and 2.6 fold increases in the Low Density Lipoprotein Ribosomal protein.

Teemu J. Murtola *et al* (2007) re-confirms the carcinogenicity of statins while study on Cholesterol-Lowering Drugs along with their effects as Prostate Cancer Risk. They found that the statin users were at more risk for development of prostate cancers as compared to other users.

Squalestetin I is a major hindering agent of cholesterol synthesis. The Squalestetin I is obtained from the fungi and it inhibits the squalene synthetase, an enzyme of mevalonate pathway without affecting the ubiquinone and dolichol. (Baxter A. *et al* 1992, Thelin A. *et al* 1992)

Ezemtmibe drug acts by block of cholesterol absorption. It is generally given with the statin. The drug Bile Acids Binding Resigns were used for the patients with high cholesterol level except triglycerides (TGs). The Nicotinic Acid shows its effects by slight increase in HDL level with decreasing the LDL, TGs and total cholesterol levels. Gemfibrozil and other fibrates reduce the triglycerides level by 20-30 %. Astrovastatin is widely used HMG-CoA reductase inhibitor. Niacin is a vitamin B and used as one of the cholesterol lowering drugs. It is observe that the niacin reduces the levels of both triglycerides and total cholesterol with increase in level of HDL.

Newman TB and Hulley SB in their study published in 1996 the fibrates includes Benzafibrates, Ciprofibrate, Gemfibrozil, Fenofibrate and statin includes Atorvastatin, Lovastatin, Pitavastatin, Rosuvastatin, Fluvastatin, Mevastatin, Pravastatin, Simvastatin, Simvastatin+Ezetimibe, Lovastatin+ Niacin, Simvastatin+Niacin. They also observed that during 1985 – 1995 the prescription of the lipid-lowering drugs increased more that 10 times due to aggressively promotion by their manufacturers. The time required for the approval of the Lipid-lowering drugs may be 30 years+ by the FDA as the drugs were approved clinical trial dependent. During the trials the millions of asymptomatic people were given the medications. Cholesterol lowering drugs may increase non-cardiovascular mortality. The classes of the lipid lowering drugs like fibrates and the statins cause cancer in rodents.

Six year duration study of lipid influencing drugs as part of project among male survivors of myocardial infarction in was studied during 1975 as the Coronary Drug Project. (Coronary Drug Project, 1975) The projects concluded with the conclusion that the three lipid-influencing regimens out of the five lipid-influencing regimens studied the will need to be discontinued early because of adverse effects. Treatment with the clofibrate and niacin did not show any adverse effects studied.

The drugs studied were low dose and high dose estrogen, aspirin, clofibrate and niacin.

Bile Acid Sequestrants or the Resins

Bile acids are the breakdown product of cholesterol excreted into the intestine by the liver as bile. Out of the total bile acids the 90% will be reabsorbed from the intestine and used to make cholesterol in liver. Bile Acid Sequestrants or the resins hinder with this intestinal re-absorption by binding the bile acids in the gut and thus promoting their excretion from the body.

Cholesterol Absorption Inhibitors

Cholesterol absorption inhibitors act via obstruction of the assimilation of cholesterol at the brush border of the intestine and this will not affect the absorption of triglycerides or fat soluble vitamins.

These drugs have the benefit of not being absorbed and can help in lowering of cholesterol by about 20% on their own.

(www.cvtoolbox.com,2007)

Fibric Acid Derivatives

The fibric acid derivatives or Fibrates are the drugs like Gemfibrozil (Lopid), Fenofibrate (Lipidil micro, Lipidil Supra, Lipidil EZ), and Bezafibrate (Bezalip). They can reduce the triglycerides (35-50%) levels; increase the high density lipoproteins (HDL) levels (15-25%).

(www.cvtoolbox.com)

The drugs explained above have some benefits and some side effects too. The therapeutic use of any cholesterol level reducing drug is the method of choice as per the need of the patient. The physician may take proper care of the patient and prescribe as per the status of the patient.

Sources of CoQ_{10}

Sources of CoQ_{10} include meat and fish and in some amount found in nuts also. There is little or none is in grains, vegetables or fruits. The body has natural ability to synthesize CoQ_{10} hence when dietary source is not adequate it can be synthesize by body. Dietary intake through food is about 2-5 mg per day.

Benefits of CoQ_{10} as drug

The use of $CoQ_{10\ as}$ drug is beneficial in many diseases especially metabolic disorders. In case of diabetes it helps to improve function of endothelial cell lining of blood vessels. In case of high blood pressure it helps to lower blood pressure in some patients. In case of migraine headache CoQ_{10} proved more beneficial over the use of riboflavin. (http://www.coq10coenzymeq10.com)

The currently used lipid lowering drugs have effects as well as side effects also. The time required to establish a compound to be lipid lowering drug is more than three decades.

Conclusion and Discussion

It is evident that the cholesterol level reducing drugs also critically reduces the CoQ_{10}. The reduction of the CoQ_{10} level further shows adverse effects. The biosynthesis of cholesterol and CoQ_{10} has same point during the pathway. The therapy needs more in-depth research. The consideration and recognition of a drug as cholesterol level reducing drug will have more critical trials. The society will be really benefited if the drug is safe and have minimum or no side effects. The cholesterol level reducing drugs need to be taken with proper prescription only. The CoQ_{10} levels need to be monitored while on the cholesterol level reducing drug medication. Berberine drug need to be more deep investigation for side effects, depletion of CoQ_{10} level apart from other benefits. Combination of various cholesterol levels reducing drug as part of combination therapy also advantageous if implemented after serious study and clinical trials. Squalestetin I may also become useful as its do not have side effect in the form of CoQ_{10} level depletion. The best therapy out of the above describe drugs may serve better and can save more lives. At present the countries are facing the death due to hyper cholesterol level and its assimilation in arteries which supplies blood to heart muscles. The heart attack is caused due to low supply of blood to heart muscles. The world level organizations like World Health Organization W.H.O may come forward in this regard to formulate a good therapy for saving lives losses due to hyper cholesterol levels.

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Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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