Corresponding address: **Red Flower Publication Pvt. Ltd,** 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110091(India), Phone: 91-11-22754205, 65270068, Fax: 91-11-22754205, E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com, Web:www.rfppl.com.

**Printed at:** Saujanya Printing Press, D-47, Okhla Industrial Area Phase-I, New Delhi - 110 020.

**Indian Journal of Pathology: Research and Practice (IJPRP)** is peer-reviewed journal that provides most recent developments in all fields of pathology. The review articles focus on recent progress in pathology, while Commentaries covers interesting current problems and at hypotheses for future developments in the entire field of pathology. Original articles focus on novel findings on all aspects of general, anatomic and molecular pathology. IJPRP publishes clinical studies to encourage readers on preliminary findings that may be helpful for their further studies.

**Subscription Information**

*India*

Individual (1 year): Rs.1000

Life Subscription (Valid for 10 Years): Rs.5000

Institutional (1 year): Rs.19000

*Rest of the World*

Individual (1 year) USD150

Insitutional (1 year) USD795

*Payment methods*

*Bank draft / cashier s order / check / cheque / demand draft / money order* should be in the name of **Red Flower Publication Pvt. Ltd.** payable at **Delhi**.

*International Bank transfer / bank wire / electronic funds transfer / money remittance / money wire / telegraphic transfer / telex*

1. **Complete Bank Account No.** 604320110000467
2. **Beneficiary Name (As per Bank Pass Book):** Red Flower Publication Pvt. Ltd.
3. **Address:** 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India)
4. **Bank & Branch Name:** Bank of India; Mayur Vihar
5. **Bank Address & Phone Number:** 13/14, Sri Balaji Shop,Pocket II, Mayur Vihar Phase- I, New Delhi - 110091 (India); Tel: 22750372, 22753401. **Email:** mayurvihar.newdelhi@bankofindia.co.in
6. **MICR Code:** 110013045
7. **Branch Code:** 6043
8. **IFSC Code:** BKID0006043 (used for RTGS and NEFT transactions)
9. **Swift Code:** BKIDINBBDOS
10. **Beneficiary Contact No. & E-mail ID:** 91-11-22754205, E-mail: redflowerppl@vsnl.net

***Online*** You can now renew online using our RFPPL renewal website. Visit www.rfppl.com and enter the required information and than you will be able to pay online using your credit card, debit card, or though net banking.

Send all Orders to: **Red Flower Publication Pvt. Ltd.,** 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India). Phone: 91-11-22754205, 65270068, Fax: 91-11-22754205, E-mail: redflowerppl@vsnl.net, Website: www.rfppl.com.

# Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors.

### Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Original articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

### Online Submission of the Manuscripts

Articles can also be submitted online from http://www.rfppl.com (currently send your articles through e-mail attachments)

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the figures/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: **Red Flower Publication Pvt. Ltd., 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, Fax: 91-11-22754205, E-mail: redflowerppl@vsnl.net.**

### Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

### Title Page

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)
2) The title of the article, which should be concise, but informative;
3) Running title or short title not more than 50 characters;
4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;
5) The name of the department(s) and institution(s) to which the work should be attributed;
6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript;
7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);
8) Source(s) of support in the form of grants, equipment, drugs, or all of these;
9) Acknowledgement, if any; and
l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

### Abstract Page

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Material, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

### Introduction

State the background of the study and purpose of thestudy and summarize the rationale for the study or observation.

### Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http://www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l 7-c_e.html).

**Results**

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

**Discussion**

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, what this study adds to the available evidence, effects on patient care and health policy, possible mechanisms); Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

**References**

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_requirements.html) for more examples.

**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.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc).

**Tables**

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Number tables, in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

**Illustrations (Figures)**

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the figure instead of to the side.

Original color figures can be printed in color at the editor's and publisher's discretion provided the author agrees to pay

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

**Sending a revised manuscript**

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point to point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

**Reprints**

Journal provides no free printed reprints, however a author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

**Copyrights**

The whole of the literary matter in the journal is copyright and cannot be reproduced without the written permission.

**Declaration**

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name (s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of the source is made.

**Abbreviations**

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

**Checklist**

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned Source of funding mentioned
- Conflicts of interest disclosed

**Authors**

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions
- Identity not revealed in paper except title page (e.g. name of the institute in Methods, citing previous study as 'our study')

**Presentation and Format**

- Double spacing

- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Key words provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS). References cited in square brackets
- References according to the journal's instructions

**Language and grammar**

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

**Tables and figures**

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Figures necessary and of good quality (color)
- Table and figure numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Figure legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed figures/tables provided
- Manuscript provided on a CDROM (with double spacing)

**Submitting the Manuscript**

- Is the journal editor's contact information current?
- Is a cover letter included with the manuscript? Does the letter

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article?

Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)

**Indian Journal of Pathology: Research and Practice**

VOLUME 1 NUMBER 3 PAGES 109-156 2012

## Contents

*Revised Rates for 2013 (Institutional)*

| Title | Freequency | Rate (Rs): India | Rate ($):ROW |
|---|---|---|---|
| Indian Journal of Agricultural and Forest Meteorology | 3 | 21000 | 800 |
| Indian Journal of Agriculture Business | 3 | 11500 | 600 |
| Indian Journal of Agriculture, Ecosystems and Environment | 3 | 18000 | 800 |
| Indian Journal of Anatomy | 2 | 3000 | 260 |
| Indian Journal of Ancient Medicine and Yoga | 4 | 6600 | 330 |
| Indian Journal of Anesthesia and Analgesia | 2 | 4000 | 600 |
| Indian Journal of Animal Feed Science and Technology | 3 | 22000 | 850 |
| Indian Journal of Animal Reproduction Science | 3 | 19000 | 700 |
| Indian Journal of Cancer Education and Research | 2 | 4500 | 500 |
| Indian Journal of Dental Education | 4 | 3000 | 288 |
| Indian Journal of Emergency Pediatrics | 4 | 6000 | 302 |
| Indian Journal of Food Additives and Contaminants | 3 | 28000 | 900 |
| Indian Journal of Food and Chemical Toxicology | 3 | 22000 | 800 |
| Indian Journal of Food Chemistry | 3 | 37000 | 1100 |
| Indian Journal of Food Engineering | 3 | 25000 | 800 |
| Indian Journal of Forensic Medicine and Pathology | 4 | 12000 | 576 |
| Indian Journal of Forensic Odontology | 4 | 3000 | 288 |
| Indian Journal of Genetics and Molecular Research | 2 | 4800 | 262 |
| Indian Journal of Library and Information Science | 3 | 7200 | 600 |
| Indian Journal of Nutrition & Food Sciences | 3 | 38000 | 900 |
| Indian Journal of Obstetrics and Gynecology | 2 | 1500 | 200 |
| Indian Journal of Pathology: Research and Practice | 3 | 22000 | 915 |
| Indian Journal of Pediatric Education | 4 | 3000 | 150 |
| Indian Journal of Plant and Soil | 3 | 51000 | 1700 |
| Indian Journal of Preventive Medicine | 2 | 3000 | 270 |
| Indian Journal of Soil Science | 3 | 34000 | 1000 |
| Indian Journal of Surgical Nursing | 3 | 1450 | 70 |
| International Journal of Neurology and Neurosurgery | 2 | 7200 | 276 |
| Journal of Human Nutrition and Dietetics | 2 | 3000 | 270 |
| Journal of Psychiatric Nursing | 3 | 1450 | 70 |
| Journal of Social Welfare and Management | 4 | 6600 | 276 |
| Meat Science International | 3 | 20000 | 800 |
| New Indian Journal of Surgery | 4 | 6300 | 360 |
| Physiotherapy and Occupational Therapy Journal | 4 | 6600 | 360 |

1. Advance payment required by Demand Draft payable to Red Flower Publicaion Pvt. Ltd. payable at Delhi.
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 10% discount.
4. Claim must be made within six months from issue date.

**Order from**

**Red Flower Publication Pvt. Ltd.**, 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, P.O. Box No. 9108, Delhi - 110 091 (India), Tel: 91-11-22754205, 65270068, Fax: 91-11-22754205. E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com, Website: www.rfppl.com

*Original article*

**Indian Journal of Pathology: Research and Practice**

# Prestorage Leuco Reduction of Blood Components

**Arun Bhattathiri***

**Karuna Rameshkumar****

*Postgraduate, St. John's Medical College Hospital, Bangalore - 560034

**Professor, Clinical Pathology, St. John's Medical College Hospital, Bangalore - 560034

Accepted on 15.08.2012

**Abstract**

**Introduction:** Non haemolytic febrile transfusion reactions can be prevented by leucodepletion. In spite of effective leucodepletion, presence of platelet derived cytokines will limit the lifespan of platelets.

**Objective:** To analyse the effect of pre storage leuco reduction by using clinical and laboratory parameters on packed cells and platelets.

**Materials and methods:** 250 units of packed cells and 250 units of platelet concentrates which were prepared by TACE (quadruple bags were included for the study. Pre leucocyte count was done by automated cell counter and neubauer chamber. After separation of packed cells and platelets, post leukocyte count was done. As the number of cells was too low in platelet concentrate, Nagotte chamber was used for counting cells. After transfusion, occurrence of NHTFR was watched for and increment of haemoglobin was noted whenever possible.

**Results:** The leucoreduction was in the range of 45% to 80% in packed cell preparation and 98-99.7% in platelet preparation. The reduction in platelet was achieved irrespective of the level of reduction in the packed cell concentrate. However no incidence of NHTFR was noted in n 500 transfusions up followed up in both packed cells and platelet concentrate.

**Conclusions:** Reduction of residual leucocytes is important in the preparation of blood components. Utilisation of new generation filters or leuco depletion processes with better performance characteristics may help to reduce specific leukocyte subsets as well as activation of inflammatory system such as cytokines, which will improve the quality of the component prepared.

**Keywords:** Packed cells; Platelet concentrate; Leucoreduction; Blood components.

## Introduction

The improvement of transfusion medicine technology is an ongoing process primarily directed at increasing the safety of allogeneic blood component transfusions for recipients[1]. Febrile non-haemolytic transfusion reactions due to leukoagglutinins are frequently seen in patients who have been given multiple blood transfusions[2]. Multiple blood transfusions may lead to the production of leukocyte antibody which in many cases is responsible for non-haemolytic febrile transfusion reaction. The severity of these reactions depends on the number of leukocytes present in the transfused blood[3]. Leukocytes have the ability to distinguish between self-cells (body's own cells) and foreign (allogeneic) cells on the basis of human leukocyte antigen(HLA) proteins that are present on cell membrane and are effectively unique to a person. During allogeneic

**Corresponding Author:**

Dr Karuna Rameshkumar, Professor of Clinical Pathology, St. John's Medical College Hospital, Bangalore - 560034.

E-mail : karunark@yahoo.com

blood transfusion person receives a large number of allogeneic donor leukocytes and these are recognized as foreign cells by recipient immune system which leads to several adverse reactions. Leukocyte depleted blood transfusion is recommended to avoid such leukocyte mediated adverse reactions[4].

Leukocytes can be separated on the basis of their size, dielectric properties, affinity separation, freeze thawing and centrifugation, but all these methods are time consuming and costly. Filtration is another method for leukocyte depletion, which is comparatively less expensive and more efficient as it gives more than 90% of leukodepletion of blood along with minimal loss of cells. However, present filtration procedures also have some limitations as they work efficiently with blood components but not with whole blood and show nonspecific adhesion of large number of platelets and red blood cells along with leukocytes. With this background, we evaluated the effect of pre-storage leukoreduction by using clinical and laboratory parameters on packed red cells and platelets, which are the most common components used.

## Material and methods

The study was prospective in nature and was done during the period from December 1 2009 to November 30, 2010 at St.John's Medical College Hospital, Bangalore.

*Leukodepletion*

At the time of component preparation leuko reduction was done using T-ACE automatic component separator. The samples were collected before and after leuko reduction to assess the level of leuko reduction.

After collection of samples the following parameters were evaluated.

1. Haemoglobin by automated cell counter, SLS-Haemoglobin method.
2. Total WBC count by using Neubauer counting chamber for leuko reduced packed RBCs.
3. Total WBC count using Negotte counting chamber for leuko reduced platelets.

After the issue of packed red cells and platelets which were assessed for leuko reduction, the patients to whom the units were issued were followed up.

The clinical parameters included

1. The demographic details of the patient transfused
2. Haemoglobin increment.
3. Presence or absence of any febrile non-haemolytic reactions and time of onset. Follow up was done for 48 hours after transfusion.

The clinical and laboratory parameters thus obtained were correlated to study the level and effect of leuko reduction.

## Results

A total of 500 units (250 units of Packed cells and 250 units of platelets) randomly selected during the study period were included. All units were screened for HIV, Hepatitis B and C, Malaria and for syphilis by VDRL as per the standard protocols followed in the blood bank.

*Packed red cell concentrates*

Leukocyte depletion of the 250 packed cells was in the range of 45% to 80%. After calculating the level of depletion from the samples, the units were categorized into 4 types to see the efficacy of the process.

1. Bags with a leukoreduction of < 1000 cells/ micro litre
2. Bags with a leukoreduction > 1000 but < 2000 cells/ micro litre
3. Bags with a leukoreduction of >2000 but <4000 cells/micro litre
4. Bags with a leukoreduction of > 4000 Cells/ micro litre

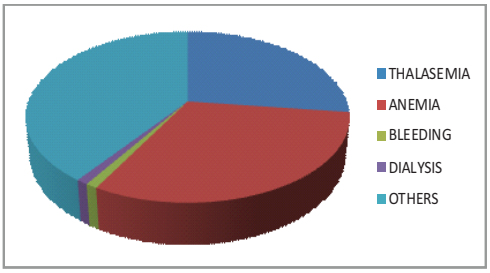
There were 29 bags in the first category, 77 bags in the second category, 95 bags in the third

category and 51 bags in the fourth category. This indicated only in 20% of units, the maximum level was achieved. This was probably due to the some retained buffy coat sticking to the primary bag and in the tube after the first spin which will get mixed with packed cell concentrate.

The maximum utilization of packed cells was from the OPD, where children with thalassemia received the units. The next ones which utilized maximum were Paediatric ICU (22 units) and Obstetric ICU (25units). A total of 100 units were issued to all other wards combined including surgery department and operation Theatre. The other wards include cardio thoracic ward, paediatric intensive care, urology, emergency medicine, orthopaedics, gynaecology, neonatal wards, oncology, emergency, and those which are released to outside the hospital. The indications given is represented as pie chart (Figure 1) As mentioned earlier, the maximum utilization was in the OPD for thalassemia children followed by obstetrics and female medical ward for anaemia.

The Haemoglobin increment was followed up whenever possible. An increment of 0.9 g/dl to 1.0 g/dl of haemoglobin increment was

**Figure 1: Indications for utilisation**




The indications are represented as a pie chart. Anaemia ranked 1st followed by others.

observed in this study with an average of 0.7g/dl increment. When the weight of the bag was correlated with haemoglobin increment, the units which have weight more than 270mg showed an increment of 1.0g/dl of haemoglobin probably because they had a higher haematocrit, whereas it decreased in units less than 255mg with an average increment of 0.7 to 0.8g/dl. Post transfusion haemolytic or non haemolytic febrile transfusion in patients who has been transfused with these units was also observed in this study. No such reactions were reported during this study.

In platelet concentrates the leuko reduction was divided into 2 categories- Units with a total leukocyte count of less than 25 cells /cu mm and units with total leukocyte count of greater than 25 cells /cu mm. In 25% of the units, the maximum reduction was achieved. As the numbers of cells were very less, Nagotte chamber was used. As the platelet concentrates were given as two to five units per patient, the increment could not be calculated per bag. The leuko reduced platelet concentrates were also followed for any reaction in the patient, but no adverse reactions were observed.

## Discussion

The reasons of reduced efficiency of leuko reduction in packed red cell concentrates may be several. The study was done in quadruple blood units only, so that both packed red cells and platelets can be prepared from same unit. All the bags which used for blood collection did not have an in built filter. TACE–I exerts pressure on the main bag to separate the components after centrifugation. The instrument pushes the bag from top to bottom which will allows the flow of plasma first followed by buffy coat to the buffy coat bag. The amount of buffy coat collected is dependent on the weight of buffy coat bag. There are no optical sensors for the buffy coat bag. During separation time, some of the buffy coat sticks to the primary bag and some amount of buffy coat layer get retained in the tubing and in the primary bag. After plasma separation, when the residual plasma in the platelet bag is allowed to flow into the primary bag, this may gets mixed up with the retained buffy coat layers in the tubing from the primary bag.

In contrast a higher level of leuko depletion was obtained in platelet concentrates. The platelet concentrates were prepared by buffy coat method. In this method the buffy coat bag was allowed to hang for a at least one hour before centrifugation. The low spin centrifugation of the buffy coat bag resulted in settling of WBCs leaving behind plasma rich in platelets and thus a higher amount of leukodepletion is achieved.

*Factors influencing the occurrence of reactions*

Irrespective of the level of leukoreduction, no reactions were observed during the study. The practice of use of anti histamine drugs as pre medication to the recipient before transfusion may be one of the reasons for non occurrence of such reactions. The sample size of the study was only 250 units of packed red cell concentrates and 250 units of platelet concentrates. Since this sample size is only a small fraction of the total number of transfusions per year in the hospital, the probability of getting a transfusion reaction also is lower.

The largest prospective randomized study of leukoreduction which enrolled 2780 patients documented no differences in the outcome measures studied, including in-hospital mortality, hospital length of stay, intensive care unit length of stay, and postoperative length of stay, antibiotic usage, and readmission rate. Subgroup analyses based on age, sex, amount of blood transfused, and category of surgical procedure showed no effect of leukocyte reduction. The patients who received leukocyte- reduced blood exhibited a lower incidence of febrile reactions ($p =< 0.06$)[5].

*Pathogen inactivation*

The concept of pathogen inactivation in blood components is to reduce the residual risk of known pathogens and to effectively eliminate new, yet unknown pathogens. However, the different approaches advocated such as use of toxic or mutagenic chemicals increase the blood safety without compromising the product efficacy or causing adverse effects, The choice of a pathogen reduction approach depends on whether it is used to treat components for transfusion such as RBC, PLT and plasma, or for products manufactured from the plasma. Two distinct methods, methylene blue (MB) and solvent-detergent (SD) are currently employed for the treatment of plasma intended for transfusion[6]. MB is a phenothiazine colorant that inactivates most viruses and bacteria after exposure to visible light. While it has the advantage of being useful for single plasma units, its ineffectiveness against intracellular pathogens and probable interaction with coagulation factors considerably reduce its efficacy. The SD approach acts by disrupting the envelope proteins of targeted pathogens, thus compromising the integrity of the pathogen and rendering it non infectious. This approach is used on small pools of plasma. The limitation of this technique is that it is not active against non-enveloped pathogens, and that levels of coagulation factors such as protein S may be decreased significantly by some of the SD treatment methods.

All the units were screened for transfusion transmitted diseases as per protocol and none of the units had been positive for any of them. Finally, other than the reduction of incidence of adverse reactions, utilisation of new generation filters or leuko reduction processes with better performance characteristics may help to reduce specific leukocyte subsets as well as activation of inflammatory system which will improve the quality of the component prepared.

**References**

1. Bassuni WY, Blajchman MA, Al-Moshary MA. Why implement universal leukoreduction. *Hematol oncol stem cell ther*. 2008; 1(2):106-23.
2. Rock G, Baxter A, Grey E. Leucocyte depleted blood, a comparison of available preparations.

*Canadian medical association journal*. 1984; 130: 1566 – 1568.

3. Macnamara E, Clarke S, Sr. Mccann. Provision of Leucocyte poor blood at the bed side. *Journal of Clinical pathology*. 1984; 669–672.
4. Sharma RR, Neelam M. Leuco reduced blood components: Advantages and strategies for its implementation in developing countries. *Asian Journal of transfusion science*. 2010; 4: 3-8.
5. Bilgin YM and Menitov AB. Transfusion-related immunomodulation: a second hit in an inflammatory cascade? *Vox Sanguinis*. 2008; 95: 261–271.
6. Jeffrey McCullough. Pathogen Inactivation A New Paradigm for Preventing Transfusion-Transmitted Infections. *Am J Clin Pathol*. 2007; 128: 945-955.

## Subscription Form

I want to renew/subscribe to international class journal **"Indian Journal of Pathology: Research and Practice"** of Red Flower Publication Pvt. Ltd.

**Subscription Rates:**
• India: Institutional: Rs.19000, Individual: Rs.1000, Life membership (10 years only for individulas) Rs.5000.
• All other countries: $795

Name and complete address (in capitals):

*Payment detail:*
Demand Draft No.
Date of DD
Amount paid Rs./USD

1. Advance payment required by Demand Draft payable to Red Flower Publicaion Pvt. Ltd. payable at Delhi.
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 10% discount.
4. Claim must be made within six months from issue date.

*Mail all orders to*
**Red Flower Publication Pvt. Ltd.**
41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India)
Tel: 91-11-22754205, Fax: 91-11-22754205
E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com
Website: www.rfppl.com

*Original article*

**Indian Journal of Pathology: Research and Practice**

# Role of Pleural Fluid ADA & the Combination Tests in Tubercular Pleural Effusion

**Akshata J.S.***

**Manjunath K.N.****

*M.D, Asst. Prof. Dept. of Pulmonary Medicine, SDSTBRC & RGICD, Bengaluru, Karnataka, INDIA

**M.S., D.N.B., M.ch, Asst. Prof. Dept. of Plastic & Reconstructive Surgery, MSRMC, Bengaluru, Karnataka, INDIA

Accepted on 11.07.2012

**Abstract**

Tuberculosis is an oldest and common infectious disease. Detected as far back as 10,000 BC it still remains a major public health problem world wide The use of biological markers in the diagnosis of tuberculous pleural effusion (TPE) is a breakthrough Adenosine deaminase(ADA) has been proposed to be a useful surrogate marker for TB in pleural, peritoneal and pericardial fluids. Although sensitive it is not a specific diagnostic tool, but combined with other tests the sensitivity can be increased. Materials&methods; 100 patients of suspected TPE were studied .Sensitivity and specificity of pleural fluid ADA alone and in combination with Mantoux test, pleural fluid cytology studied. Conclusion; pleural fluid ADA (>63U/L) alone was essentially diagnostic of TPE, between 40-63U/L it is highly suspicious of TPE along with >50% lymphocytes in pleural fluid confirms the diagnosis.

**Keywords**: Tubercular pleural effusion; ADA; Mantoux test; Pleural fluid cytology.

## Introduction

Tuberculosis is an ancient disease which continues to haunt us even into the new millennium. It is one of the oldest and commonest infectious diseases. Detected as far back as 10,000 BC it still remains a major public health problem world wide.

Tuberculosis commonly affects the lung but extrapulmonary tuberculosis is not uncommon. Tuberculous pleuritis is one of the two most common extrapulmonary manifestations of TB, the other being lymphatic involvement. Tuberculosis remains the most common cause of pleural effusion in countries where it is highly endemic and with the increase of HIV infection, the incidence of pleural effusion in tuberculosis patients is on rise. Tuberculous pleural effusion (TPE) traditionally affected adolescents and young adults between 28-40 yrs.

Diagnosis of pulmonary TB is confirmed mainly by sputum examination for AFB. However, the diagnosis of tuberculous pleural effusion requires special investigations like pleural fluid biochemistry and cytology,as Pleural fluid staining for AFB is usually negative. The use of biological markers in the diagnosis of tuberculous pleural effusion is a breakthrough Adenosine deaminase (ADA) has been proposed to be a useful surrogate marker for TB in pleural, peritoneal and pericardial fluids. Many studies in different

**Corresponding Author:**

Dr Manujunath K.N., M.S., D.N.B., M.ch, Asst. Prof. Dept. of Plastic & Reconstructive Surgery, MSRMC, Bengaluru, Karnataka, INDIA. Mob: 09535971056

E-mail : drknmanjunath@gmail.com

parts of the world have confirmed high sensitivity (77-100%) and specificity (86%) at a predetermined cut off value for early diagnosis of TPE. Although sensitive it is not a specific diagnostic tool, but combined with other tests the sensitivity can be increased.

Hence this study is being undertaken to evaluate the role of combined use of pleural fluid ADA, cytology and Mantoux test (MT) in diagnosis of TPE.

## Materials & Methods

100 patients both out patients and inpatients suspected to be a tuberculosis were studied. A detailed history was taken and a thorough physical examination was done. Following investigations was performed on all:

1. Chest radiograph, PA view (lateral or decubitus)- site noted and size of pleural effusion was graded (Minimal-fluid<1/3 rd of hemithorax, Moderate-1/3 rd - 2/3rd of hemithorax, Massive- >2/3 rd of the hemithorax)
2. Where ever indicated three samples sputum smear for acid fast bacilli by Ziehl-Neelson staining.
3. Mantoux test using 5TU (intermediate strength)
4. Pleuralfluid

ADA level estimated by spectrophotometer (Galanti and Guisti)

- Cytology: totalcount, differential count, any atypical cells.
- Pleura fluid for AFB culture using Lowenstein Jensen medium whenever feasible.

All the cases were finally grouped as tuberculous or non tuberculous.

*Tuberculous*

*Absolute criteria:*

1. Pleural fluid/sputum smear revealed Mycobacterium tuberculosis on Ziehl-Neelsen staining.
2. Pleural fluid/pleural biopsy specimen/ sputum grew Mycobacterium tuberculosis on Lowenstein-Jensen culture.
3. Histopathological evidence suggestive of tuberculosis in pleural biopsy specimen/ palpable lymph nodes or other tissue sites.

*Suggestive criteria*

1. History of fever, pleuritic chest pain, malaise, anorexia, weight loss and features of toxemia consistent with the clinical presentation of TPE.
2. Good response to antituberculosis treatment.

TPE was diagnosed if any one of the absolute criteria or both of the suggestive criteria were present.

*Non tuberculous*

1. Malignant pleural effusion- Cytological or histopathologically proven cases.
2. Parapneumonic-with suggestive clinical symptoms and good response to antibiotic course.
3. Transudates due to congestive cardiac failure and relieved with diuretics.

Patients under tuberculous group were put on anti tubercular drugs either DOTS CAT I or III accordingly. Follow-up was done at 15 days, 1, 2 months and clinical and radiological improvement noted. Those with suspected parapneumonic were put on antibiotics.

## Results

In our study, Tuberculosis was the most common cause of pleural effusions encountered (74%), followed by malignancy (18%). However, malignant effusion was the most common among non tuberculous cases. (table 1)

74.32% of the tuberculous pleural effusion cases were observed below the age of 50 years.

**Table 1: Distribution of Etiology of Pleural Effusion in the Study Population**

| Parameters | No. of Cases | % |
|---|---|---|
| Tuberculous (TB) | 74 | 74 |
| Non Tuberculous (NTB) | 26 | 26 |
| Malignancy (M) | 18 | 18 |
| Synpneumonic (SP) | 6 | 6 |
| Transudate (T) | 2 | 2 |

**Table 2: Clinical Symptoms in Study Population**

| Symptoms | TB | | NTB | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Cough Dry | 37 | 50.00% | 5 | 19.23% |
| Productive | 35 | 47.30% | 6 | 23.08% |
| Chest pain | 47 | 63.51% | 15 | 57.69% |
| Dyspnea | 30 | 40.54% | 20 | 76.92% |
| Fever | 64 | 86.49% | 14 | 53.85% |
| Anorexia | 58 | 78.38% | 18 | 69.23% |
| Weight loss | 17 | 22.97% | 13 | 50.00% |
| Hemoptysis | 9 | 12.16% | 4 | 15.38% |

**Table 3: Result of Pleural Fluid ADA at 63 U/L Cutoff Values**

| | TB | NTB | Total |
|---|---|---|---|
| Positive | 50 | 0 | 50 |
| Negative | 24 | 26 | 50 |
| Total | 74 | 26 | 100 |

| | |
|---|---|
| Sensitivity | 67.57% |
| Specificity | 100% |
| Positive Predictive Value | 100% |
| Negative Predictive Value - | 52% |
| False Negatives | 0% |
| False Positives | 32.43% |

**Table 4: Result of Combination of ADA and Cytology in Diagnosis of Tuberculous Pleural Effusion** (ADA > 40U/L and Cytology >50% Lymphocytes)

| | TB | NTB | Total |
|---|---|---|---|
| Positive | 62 | 1 | 63 |
| Negative | 12 | 25 | 37 |
| Total | 74 | 26 | 100 |

| | |
|---|---|
| Sensitivity | 83.78% |
| Specificity | 96.15% |
| Positive Predictive Value - | 98.41% |
| Negative Predictive Value - | 67.57% |

**Table 5: Result of combining cytology and Mantoux test in Diagnosing TPE**
(Lymphocytes >50% and Mantoux test positive)

| | TB | NTB | Total |
|---|---|---|---|
| Positive | 35 | 3 | 38 |
| Negative | 39 | 23 | 62 |
| Total | 74 | 26 | 100 |

| | |
|---|---|
| Sensitivity | 47.30% |
| Specificity- | 88.46% |
| Positive Predictive Value - | 92.11% |
| Negative Predictive Value - | 37.10% |

**Table 6: Result of Combining ADA and Mantoux test in Diagnosing TPE**
(ADA >40 U/L and Mantoux test positive)

| | TB | NTB | Total |
|---|---|---|---|
| Positive | 35 | 2 | 37 |
| Negative | 39 | 24 | 63 |
| Total | 74 | 26 | 100 |

| | |
|---|---|
| Sensitivity | 47.30% |
| Specificity | 92.31% |
| Positive Predictive Value - | 94.59% |
| Negative Predictive Value - | 38.10% |

**Table 7: Combined Role of ADA, Cytology and Mantoux test in the diagnosis of TPE**
(ADA > 40 U/L + Cytology >50% lymphocytes + Mantoux test – positive)

| | TB | NTB | Total |
|---|---|---|---|
| Positive | 34 | 1 | 35 |
| Negative | 40 | 25 | 65 |
| Total | 74 | 26 | 100 |

| | |
|---|---|
| Sensitivity | 45.95% |
| Specificity | 96.15% |
| Positive Predictive Value | 97.14% |
| Negative Predictive Value | 38.46% |

**Table 8: Comparison of Various Tests and Their Combinations in the Diagnosis of TPE**

| Test/Criteria | Sensitivity | Specificity | PPV* | NPV** |
|---|---|---|---|---|
| ADA >40 U/L | 89.18% | 84.61% | 94.29% | 73.33% |
| ADA >63 U/L | 67.57% | 100% | 100% | 52% |
| Mantoux test | 51.43% | 76.92% | 85.71% | 37.03% |
| ADA >40 U/L+>50% lymphocytes | 83.78% | 96.15% | 98.41% | 67.57% |
| ADA >40 U/L+ positive Mantoux test | 47.30% | 92.31% | 94.59% | 38.10% |
| MT positive + >50% lymphocytes | 47.30% | 88.46% | 92.11% | 37.10% |
| ADA >40 U/L, positive Mantoux test & >50% lymphocytes | 45.95% | 96.15% | 97.14% | 38.46% |

*PPV- positive predictive value; **NPV- negative predictive value

Majority (44.59%) were found in the age group of 21-30 yrs. Mean age was 39.45yrs (Range 15-75).Majority of cases among malignant etiology (66.67%) were found above the age of 50 years with a mean age of 58 years. Male preponderance was observed in the study both among tuberculous and non tuberculous group. In both the groups' majority of patients hailed from a rural locality. In the study population, 65% were either smokers or ex-smokers. 24% were either alcoholic or ex-alcoholics. 27% of them were indulges in both smoking and alcoholism. Among the tuberculous group cough was the commonest symptom (97.3%) followed by fever and anorexia (86.49%), chest pain (63.51%).In malignant cases, dyspnea was the most common (76.92%) followed by chest pain (57.69%) and weight loss (50%).Hemoptysis was little more common in malignant cases (15.38%) than the Tuberculosis group (12.16%). (table 2)

18 patients (24.32%) of tuberculous pleural effusion presented with associated parenchymal lesions in the chest x-ray.Majority (20.27%) showed minimal infiltration shadows. Consolidation was the major associated finding (34.62%) in the non-tuberculous group. Majority (58.11%) of the tuberculous pleural effusion were of moderate size occupying $1/3^{rd}$ to $2/3^{rd}$ of the hemi thorax. All the 7 (26.92%) massive pleural effusion among non-tuberculous group were proved to be of malignant etiology. Among the tuberculous pleural effusion cases 51.42% gave positive result while only 23.07% were Mantoux test positive among the non tuberculous group. The mean total WBC count in the tuberculous group was 1761.82cells/$mm^3$of which majority (73.32%) were lymphocytes. Lymphocyte predominance was also seen in malignant pleural effusion. All synpneumonic effusions showed neutrophil predominance (77.17%). 70 of 74 i.e. 94.59% of tuberculous cases and 14 of 18 i.e. 77.78% of malignant cases showed lymphocyte predominance (>50%) in pleural fluid cytology. A typical cells suggestive of malignant origin were found in 17 of 18 (94.4%) cases of malignant pleural effusion.

The pleural fluid ADA level was in the range of (13-157 U/L) among the tuberculous cases with a mean value of 76.29±30.91 U/L.The mean value of ADA among non tuberculous cases was 26.77±19.09 U/L. A definitive diagnosis of tuberculous pleural effusion can be made at ADA level above 63U/L, as Specificity of 100% was obtained at a cut off value of 63U/L, but the sensitivity of the test dropped to a low 67.57%. (table 3)

Results of various tests, and the individual sensitivity and specificity are given below. The combination of tests also compared with each other, after all tests Pleural fluid ADA with sensitivity of 89.18% and specificity of 84.61% emerged as a single best diagnostic test for tuberculous pleural effusion with sensitivity of 89.18% and specificity 84.61% at 40 U/L cut-off level.(table.4,5,6,7,).

Combined use of pleural fluid ADA and lymphocyte predominance was found to increase the specificity to 96.14% with 98.41% positive predictive value.(table.8)

## Discussion

Definitive diagnosis of TPE depends on the demonstration of tubercle bacilli in the sputum, pleural fluid or pleural biopsy specimen or the demonstration of granulomas in the pleural biopsy.Smears of pleural fluid for mycobacteria are usually negative and pleural fluid cultures are positive for Mycobacteria in fewer than 40%[1,2]. BACTEC culture provides higher yield and faster results (18 days; 3-40 days) when compared to conventional cultures (33.5days; 21-48 days) [3]. But, All these above tests are either time consuming or invasive. Hence was the need for a simple and sensitive test for the diagnosis of TPE.

*Mantoux test*

Ocana et al[4] of Spain in their study of 221 cases of pleural and peritoneal fluids of which 46 were TPE noted that 33(68.7%) were tuberculin positive.Epstein et al[5], found that of 23 TPE cases 11 of the 13 patients (73%) for whom MT result was avaible were positive. Seibert et al[6] from Alabama studied 70 patients of TPE and found that 93% were MT positive. The positivity of MT was 95% in patients associated with parenchymal lesions also and 91% in patients with isolated pleural effusion. Pedro et al[2] of Spain conducted a study on 254 patients of TPE and found that tuberculin skin test was positive in only 109(66.5%) of the cases. Maria Virjinia et al[7] in their study of 140 cases of pleural effusion noted that MT was positive in 50% confirmed and 70.6% probable TPE cases. It was also found that 35% of the patients with effusion due to non tuberculous etiology also gave a positive response to MT giving it a sensitivity of 50% and a specificity of 64.4% in the diagnosis of TPE.

All these studies used only MT as diagnostic tool and found that it had low sensitivity and specificity

*Pleural fluid cytology*

Pleural fluid analysis is useful in the diagnosis of tuberculous pleuritis. Grossly it's a clear, straw colored odourless non viscid fluid commonly and haemorrhagic at times. Invariably an exudate with protien level >5g/dl frequently with raised LDH and decreased glucose level [8]. If eosinophils are found in pleural fluid in significant numbers (>10%) one can virtually exclude the diagnosis of TPE, unless patient has a pneumothorax or has had a previous aspiration [8]. Studies have confirmed that patients with TPE rarely contain >5% mesothelial cells in their pleural fluid [910]. Unfortunately, the absence of mesothelial cells is not diagnostic of TB. Any condition in which the pleural surfaces are extensively involved by an inflammatory process, mesothelial cells are not found in the pleural fluid.

Pettersson et al[11] studied the diagnostic value of total and differential WBC counts in 140 pleural effusion patients. Total counts were higher in exudates. A lymphocyte predominance (>80% of all leukocytes) was seen in 29 of the 31 (93.5%) of TPE cases. However 18 of 24 (75%) fluids of malignant origin also showed lymphocyte predominance. None of the TPE cases had >10% eosinophils. Hence they concluded that lymphocyte predominance was not characteristic particularly of TPE but also in those of malignant etiology. So it is not disease specific. Epstein et al[5] in their study found that of 23 TPE cases only 54% had lymphocyte predominence which was much less as compared to previous studies.

Pedra et al[2] studied 254 cases of TPE and found that about 93% had >50% lymphocytes in their differential counts with mean value of 77%.In a much recent study at Peshawar, Anwar et al[12] performed pleural biopsy using Abraham's needle to establish the etiology of 74 patients with lymphocyte predominant exudative pleural effusion. In 71.1% of cases in which a definite histopathological diagnosis was established. 52.7% had tuberculous and 18.9% had a malignant etiology to their pleural effusions. They concluded that tuberculosis followed by malignancy were the most common causes of lymphocytic exudative pleural effusion.

*Pleural fuild ADA*

ADA level is ten times higher in lymphocytes than in erythrocytes and particularly in T-lymphocytes with variations according to cellular differentiation. ADA plays a part in the differentiation of lymphoid cells and the maturation of monocytes to macrophages [13]. For this reason ADA has been looked on as a marker of cell mediated immunity which encompasses the delayed hypersensitivity reaction.

Ocana et al[2,4] measured ADA in 221 patients of pleural and peritoneal effusions. All patients with a pleural fluid ADA level of >70 U/L had tuberculous etiology and no patient with ADA <40 U/L had TPE. In D.K. Gupta et al[14] study of 53 cases of pleural effusion found that the mean ADA level in those of tuberculous origin was 77.7 U/L in contrast to 14.5 U/L in cases of malignant effusion.Rajendra Prasad et al[15] studied 47 cases of pleural effusion and found that at 30 U/L cut off value the sensitivity and specificity of ADA for diagnosing TPE was 100%.L Valdes et al[40] in their study of 405 patients of pleural effusion of various etiology found the mean value of ADA in cases of TPE 107.5 U/L. Also a sensitivity of 100% and specificity of 95% at 47 U/L cut off value. In a recent study, Gaga et al[16] of Athens found that with calorimetric determination of ADA by Guisti, its mean value was noted as 94 U/L in tuberculous and 28 U/L in non tuberculous cases. The sensitivity and specificity for TPE was calculated to be 97% both.

Likewise several studies have been conducted by many since 1983. It has been suggested that an elevated pleural fluid ADA level predicts TPE with a sensitivity of 90-100% and a specificity of 89-100% when the Giusti method. The reported cut off value varies between 33-50 U/L[20]. In regions with a high prevalence of TB and in patient groups with a low risk of other causes of pleurisy the positive predictive value of this marker is increased. In lower incidence of TB the positive predictive value decreases and so there is higher likelihood that a test would give a false positive test.

The majority of ADA in TPE is ADA2 whereas ADA1 is found to be the predominent form in parapnuemonic and empyema cases[17]. Thus this separation of ADA activity into ADA1 and ADA2 will alleviate a major limitation in distinguishing TPE from parapnuemonic empyemas. The ADA1 to ADA ratio will slightly increase the sensitivity and specificity of ADA in diagnosing TPE but is found not to be cost effective in vast majority of cases. Nevertheless an ADA1 to total ADA ratio in fluid > 0.42 is a good indicator of TB [17, 18]. This test is still not widely available in the market. Studies have reported that pleural fluid ADA level didn't vary with the HIV status of the patient which is another plus point for countries gripped by the HIV and AIDS pandemic [19].

As noted though ADA has both good sensitivity and specificity, the specificity needs to be further increased. Hence use of another test in combination is being evaluated since a few years. The combination tests need to be simple, easily available and cost effective. As an answer to the search, many workers have been evaluating the use of cytology and cell counts in pleural fluid. It has been noted that in addition to ADA of >40 U/L if the diagnostic criteria for TPE also includes a pleural fluid lymphocyte to neurophil ratio >0.75, the specificity is increased [8].

Burgess L.J. et al[20] studied 303 cases of pleural effusion of which 143(58%) were TPE. The ADA in TPE at cut off value of 50 U/L showed a sensitivity of 91% and specificity of 81%. 6 non tuberculous cases were misclassified with ADA >50 U/L criteria alone. So when lymphocyte neutrophil ratio of > or equal to 0.75 was also considered, 17 patients of TPE were omitted. But 13 of these were also misclassified when only an ADA criterion was used. Though the sensitivity decreased from 91% to 88%, the specificity of the tests increased from 81% to a gross of 95%. Maria et al[7] of Columbia in their study of 140 pleural effusion cases, 42 were of confirmed tuberculous origin and 19 were probably TPE. The mean ADA value was higher in TPE with a sensitivity and specificity of 88% and 86% respectively at a cut off value of 47 U/L. They also noted that MT performed in the cases gave a sensitivity of 50% and specificity of 64.4%. When both

criteria of ADA >47 U/L and a positive response to MT was considered together, the sensitivity reduced to 46.9% but the specificity increased to a high 94.9%. These studies show that ADA with a high sensitivity and specificity is a good screening as well as a confirmative test in TPE. Its specificity can be further increased by combining pleural fluid cytology and/or MT thus decreasing the few false positive results.

In our present study of 100 cases of pleural effusions carried out in during 1 year period, the role of combined use of pleural fluid ADA, cytology and Mantoux test in the diagnosis of tuberculous pleural effusion was evaluated.

Mean ADA of pleural fluid was 76.29±30.91 U/L in TPE and 26.77±19.09 U/L in non-tuberculous cases. The difference in ADA values were statistically significant between TPE v/s Malignancy ($p<0.001$) and TPE v/s Synpneumonic ($p<0.01$).

In TPE, pleural fluid ADA at a cut off level of 40U/L showed a sensitivity, specificity, positive predictive value and negative predictive value of 89.18%, 84.61%, 94.29% and 73.33% respectively, specificity of 100% was obtained at a higher cut off value of 63U/L but the sensitivity decreased to 67.57%. On combining the criteria of ADA > 40U/L and lymphocyte predominance (>50%) in TPE cases, though the sensitivity decreased to 83.78%, the specificity increased to 96.15%. PPV was a high 98.41% and NPV was 67.57%. When the criteria of ADA > 40U/L and positive Mantoux test were used in combination in TPE, the sensitivity was a low 47.30% and specificity was found to be 92.31%. PPV and NPV were 94.59% and 38.10% respectively.

All the three criteria – ADA > 40U/L, lymphocyte predominance (>50%) and positive Mantoux test when used in combination resulted in sensitivity 45.95% and specificity of 96.15%. It shows that sensitivity further decreased without any increase in specificity. Thus combination of Mantoux test with pleural fluid ADA and cytology had no added value.

At the end of this study, pleural fluid ADA emerged as a single best sensitive test with fair specificity as well. It being a simple and less time consuming test is an added advantage. Tuberculosis still remains the leading cause of pleural effusion and ADA with good positive predictive value is a valuable time sparing diagnostic tool. But since it can still give false positive results caution is advised. The use of combination of pleural fluid ADA and differential cell counts will help in further increasing the specificity.

## Conclusion

Finally, it can be concluded from the present study that pleural fluid ADA, cytology and Mantoux test combination can be used in the differential diagnosis of pleural effusion as follows:

- In a clinically suspected case of tuberculous pleural effusion if the pleural fluid ADA is >63U/L, it is essentially diagnostic of TPE.
- However if ADA is between 40-63U/L it is highly suspicious of TPE, and then the cytology report will aid in confirming the diagnosis. A lymphocytic exudate (>50%) with high ADA value (>40U/L) is highly suggestive of tuberculous pleural effusion.
- Pleural fluid ADA >40U/L and a positive Mantoux test also increases the specificity of diagnosis.
- A lymphocytic exudate (>50%) with low ADA (<40U/L) is more in favour of non haematologic malignancies.

## References

1. Berger HW, Mejia E. Tuberculous pleurisy. *Chest.* 1973; 63: 88-92.
2. Pedro P, Valdes L, Alvarez D, San Jose E et al. Tuberculous pleurisy: a study of 254 patients. *Arch Intern Med*. 1998; 158: 2017-21.
3. Maartens G, Bateman ED. Tuberculous pleural effusions: increased culture yield with bedside

inoculation of pleural fluid and poor diagnostic value of ADA. *Thorax.* 1991; 46: 96-9.

4. Ocana JM, Martinz-Vazquez, Segura RM et al. Adenosine deaminase in pleural fluids, a test for diagnosis of TPE. *Chest.* 1983; 84: 51-53.
5. Epstein DM, LR Kline, Albelda SM et al. Tuberculous pleural effusion. *Chest.* 1987; 91: 106-109.
6. Seibert AF, Haynes J, Middleton R et al. Tuberculous pleural effusion-a twenty year experience. *Chest.* 1991; 99; 883-886.
7. Maria Virginia Villegan, Luz Angela Labrada, Nancy Saravia et al. Evaluation of polymerase chain reaction, adenosine deaminase and interferon gamma in pleural fluid for differential diagnosis of pleural tuberculosis. *Chest.* 2000; 118: 1355-1364.
8. Richard W Light. Pleural Diseases 4th edition, Tuberculous pleural effusion. 182-92.
9. Light RW, Erozan YS, Ball WC. Cells in pleural fluid:their value in differential diagnosis. *Arh Intern Medicine.* 1973; 132: 854-860.
10. Hurwitz S, Leiman G, Shapiro C. Mesothelial cells in pleural fluid:TB or not Tb? *S Afr Med J.* 1980; 57: 937-939.
11. Petterson T, Riska H. Diagnostic value of total and differential leukocyte counts in pleural effusions. *Acta Med Scand.* 1981; 210: 129-35.
12. Anwar R, Farooqi JI. Causes of lymphocytic exudative pleural effusion as revealed by percutaneous pleural biopsy: experience from Peshwar. *Pak J Med Sci.* 2003.
13. Jacobus P et al. Significance of ADA activity and its isoenzymes in tuberculous pleural effusion. *Chest.* 1994; 106.
14. Gupta DK, Suri JC, Goel A. Efficacy of Adenosine deaminase in the diagnosis of pleural effusions. *Ind J Chest Dis And All Sci.* 1990; 32: 205-208.
15. Rajendra Prasad, Tripathi RP, Mukerji PK et al. Adenosin deaminase activity in pleural fluid-a diagnostic test of tuberculous pleural effusion. *Ind J Chest And All Sci.* 1992; 34: 123-126.
16. Gaga M, Papamichalis G et al. Tuberculous Effusion: ADA activity correlates with CD4 cell numbers in the fluid Respiration 2005; 72: 160-162.
17. Perez-Rodriguez E, Castro DJ. The use of ADA and ADA isoenzymes in the diagnosis of tuberculous pleuritis. *Curr Opin.* 2000; 6: 259-266.
18. Valdes L, San Jose E, Alvarez D et al. ADA isoenzyme analysis in pleural effusions: Diagnostic role and relevance to the origin of increased ADA in tuberculous pleurisy. *Eur Respir J.* 1996; 9: 747-751.
19. Riantawan P, Chaowalit P et al. Diagnostic value of pleural fluid ADA in tuberculous pleuritis with reference to HIV coinfection and a Bayesian analysis. *Chest.* 1999; 116: 97-103.
20. Burgess LJ, Maritz FJ, Le Roux I et al. Combined use of pleural adenosine deaminase with lymphocyte / neutrophil ratio; increased specificity for the diagnosis of tuberculous pleuritis. *Chest.* 1996; 109: 414-419.

*Review article*

**Indian Journal of Pathology: Research and Practice**

# Immune Status of NHL Patents of Various Group

**Vandana Shrivastava***

**R.K. Bhatnagar****

**Rishi Kumar Saxena*****

**Menu Singh******

*Reader, Department of Microbiology, I.T.S. Centre for Dental Studies & Research, Delhi-Meerut Road, Murad Nagar, Ghaziabad

Accepted on 04.10.2012

**Abstract:**

Aim of Present Study, to show the immune status of Non-Hodgkin's lymphoma patients we have cauterized again them according to stage wise also to study immune status (cellular as well as Hum oral) of these patients. Our studies reveal the status of patients which is help full for there treatment as well as there improvement status.

**Key words:** NHL (Non Hodgkin's lymphoma); B cell (B lymphocyte); IgM (Immunoglobulin M); IgG (Immunoglobulin G); IgA (Immunoglobulin A); IgE (Immunoglobulin E); IgD (Immunoglobulin D).

## Introduction

Lymphoma is malignant tumor of immune system which develops in the lymphatic system of an individual originates from lymph nodes. Enlargement – of lymph node is painless, discrete and from in other symptoms, loss of weight, fever and sweating. In this certain type of lyrnph cell, being to divide and multiply abnormally in the lymph nods. There cells spend and disseminated to other part of body like lungs spleen, line and bone marrow.

Non Hodgkin's lymphomas appear to represent tumor of immune response. (lukes et al. 1975)

The hum oral immunity, which is also called as anti body mediated immunity, needs antibody to play important role in the protection from infections. These antibodies are gamma globulins. These are live classes of immunoglobulin ie IgG, IgM, IgA, IgE, IgD Synthesized by B cells. When B cells are stimulated, they divide and transform into plasma cells, which synthesize immunoglobulin generally in lymphomas cell mediated immunity suppressed but in certain cases of NHL which are B cell origin hum oral immunity is also suppress. The majority of NHL cases are B cell origin.

Antigenic ally Stimulated B Cells undergoes blast- transformation, becoming successively plasma blasts, intermediate transitional cells

**Corresponding Author:**

Dr. Vandana Shrivastava, 66 Kunj Vihar Bhind Road, Near Gola Mandir, Gwalior - 474005.

E-mail : Shvandana@rediffmail.com

and; plasma cells the mature plasma cell is the antibody secreting cell.

Plasma cell is end cells and has a short life span of two as three days. A plasma cell only makes an antibody of Single specificity of a single immunoglobulin class.

*Aims and objective*

Our aims of study are to identify the deficit part of the system (immune) by using different parameter. And objectives are to identify and isolate the abnormal cell which ore playing important role. Indifferent stage of disease.

*Review of literature*

Non Hodgkin's lymphoma is the disorders involving primarily the lymphoid tissue. NHL is originated from lymphoid tissues. They are of monoclonal origin. These disorders are lethal unless controlled or eradicated through therapy.

Data et al. (1971) reported elevated level of IgG in Non Hodgkin's lymphoma (NHL) which is an important component of humoral immunity.

Piessens, et al. (1973) reported that in NHL cases peripheral. blood lymphocyte carrying surface immunoglobulin (B cells) function was decreased or impaired.

Gajlpeczalska, et al. 1973, reported the decrease in number and functional impairment in surface immunoglobulin bearing B lymphocyte in NHL subject.

Alexanion (1975), reported that Non Hodgkin's lymphoma (NHL) of B cells may be associated with abnormal serum immunoglobulin and antibody deficiency. Same observation was reported by murray (1980).

Crispen, et al. (1976) reported a 74% reduction in different cells of all type of child cancer.

Jones, et al. Hancock, et al (1977) advani et.al (1980) reported impairment of delayed hypersensitivity and abnormalities of blood. T-Cell functions in NHL patients.

Bjorkholm, (1978) reported that cell mediated immunity is selectively impaired in virtually all entreated patients.

Lichtenstein and taylor (1980), reported that the incidence of quantitative immunoglobulin abnormalities in patients with T – cells lymphoma and B cells lymphoma.

Kumar and penney (1980), reported that in early stage of non Hodgkin's lymphoma abnormalities of immunological function are usually minimal but impairment of both antibody and cell mediated immunity is often noted in advanced disease.

Lindemalm, et al. (1983) concluded that functional abnormalities of both T and B lymphocyte in non Hodgkin's lymphoma are closely related to the presence of active disease.

Buchi et al. (1984) Studied the lymphocyte population in the blood of patient with leukaemic non Hodgkin's lymphoma. A statistically significant decrease in at both the T and B cells were detected in low grand-malignant lymphoma (LGML) as well as lymphocyte was not found to be altered in both high grade malignant lymphoma (HGML), the number of the circulating B Lymphocyte was not found to be altered in both HGML and LGML.

## Material and Methods

Study done to test the immune states of the patient of non Hodgkin's lymphoma. The patients were the diagnosed cases of NHL (cancer hospital & research Institute Gwalior) nub. Of cases were – 55.

Control – Same

We had taken all records of the patients. From there.

For the blood collection – we took 5ml of patient blood for. Serum isolation as well as for Heparinied Blood for other Parameters.

Parameters for study of Immune status of patients.

1. Estimation of total protein.
2. Estimation of Gamma globulin and A, G ruto
3. Estimation of Immunoglobulin IgG and Igm
4. Absolute lymphocyte count
5. Quantization of T and B cells.

We separated lymphocyte, after washing, we had taken 1 ml of RPMI Medium and add to there washed cells and put 10% fetal calf serum (FCS).

B cells Detection done by EAC Rosetting (1978).

Total protein determination done by lowry et al. (1957).

Total gamma globulin estimation by electrophoresis kohn J. et al. (1960) and gamma globulin by varley (1940).

Estimation of IgG by Mancini et al 1965.

And purified the IgG as per method of Mckinney and pertinson (1987) The semipurified IgG was further purified by Colum chromatography using sephadex G 200 in a glass column of 80x2.5 cm.

Quantization of IgG done by Mancini et.al (1965).

Estimation of IgM is done in two steps.

1. Purification of IgM as antigen (weir 1978) by using Colum chromatography using G 200 Sephadex Colum.
2. Quantization of IgM done by Mancini (1965) the values for unknown were derived from the standard prepared.

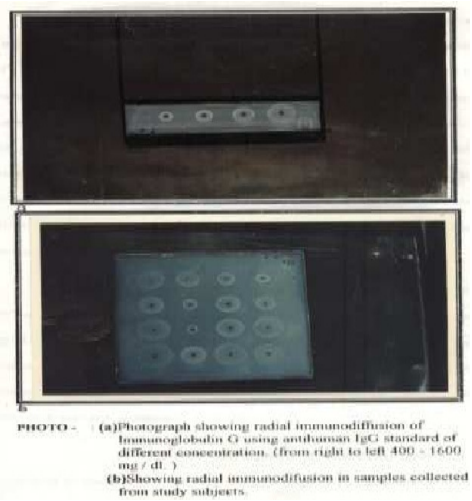


PHOTO - (a)Photograph showing radial immunodiffusion of Immunoglobulin G using antihuman IgG standard of different concentration. (from right to left 400 - 1600 mg / dl. )
(b)Showing radial immunodifusion in samples collected from study subjects.

*Statistical Analysis*

Data were analysed using various statistical formulas and expressed. As Meant se (X ± SE) and statistical significance was determined using "t" test.

**Results and discussion**

In NHL patients (Non Hodgkin's lymphomas) there is a malignant monoclonal proliferation of lymphoid cells usually identifiable as B cells. Occasionally T cells ate also affected. Majority of NHL ate of B cell origin.

Observation were made in 55 NHL patients by compassing the mean values of each parameter ie. Total serum protein IgM, IgG antibodies, and total leukocyte count.

In NHL patients significant decrease in total leukocyte count in stage II as compared to control (P <0.001)

| | | |
|---|---|---|
| Control | 7609±212 | |
| N = | 55 | |
| Stage II | NHL (20) | 5957.57±382 |

State III and stage IV not shares the significant decrease the leukocyte count as compare to control.

Pooled values indicated significant decreased percentage of E rosetting T cells in NHL patients. When with control. In theses patients, stage wise data of E rosetting cells decreased values nor visible in all the three stages II, III, and IV (P L 0.001).

Pooled data on EAC resetting B cells increased percentage was observed in NHL cases (P<.001).

Control = 55 20.30±0.57

NHL = 55 24.3 ± 1.15 stage III (25) 20.19±17 stage II NHL = (20) 28.35 ± 17

Pooled data on total serum protein content was also studied in NHL patients.

Control N = 556.8 ± 0.12

NHL N = 55 9.3 ± 0.29

Control (6.8 ± 0.12)

N = 55

Stage II NHL 20 8.8 ± 0.39

Stage III NHL 14 9.5 ± 0.52

Stage IV NHL 26 9.5 ± 0.54

Serum albumin globulin ratio was also one parameter studied in NHL cases. The value was quite high (P <0.01).

In stage use data of serum albumin globulin ration increased in NHL patients in all the stage (II, III, IV) results was marginal high (P< 0.05) in stage II and III but in stage IV results was highly increase (P< 0.001).

A/G ration control 1.59±0.06 (Pooled)

NHL (N = 55) 2.30 ± 0.16 (P< 0.001)

Control

N = 55 = 1.59±0.06

Stage II NHL (20) = 2.43±0.40

Stage III NHL (14) = 2.18 ±0.29

Stage IV NHL (21) = 2.27±0.15

(P<0.001)

Pooled value of Serum Immunoglobulin G.

Control

N = 55 1199.7±48.5

Serum Immunoglobulin IgM% is expensed as X ± SE NHL = 1451.6±58.7

P<0.01 = significant

Serum IgG level was increased in NHL cases of stage II, III patients, but in stage IV value was significantly high when compare to control (P<0.01).

Serum IgM level was raise in NHL patients but not significant.

Control 116.05±3.5

N = 55 Pooled data

NHL (55) 112.5±2.0

Serum Immunoglobulin (IgM)

Control 116.05±3.5

N = 55

Stage II (20)= NHL (20) 112.9±3.6

Stage III (14) = 114.4±4.4

Stage IV (21) = 11.25±3.02

Pooled values of total Gamma Globulin

Control N = 55 16.07 ± 0.80

NHL = 55 12.56±0.76

(Total Gamma Globulin % 15 expressed as (X - ±SE)

(P<0.01)

Stage use Total gamma Globulin

Control = (N 55) = 16.07±0.80

Stage II NHL (20) 13.09±1.45

Stage III NHL (14) 13.78±1.98

Sate IV NHL (21) 11.49±0.86

## Discussion

The present study was under taken of NHL patient stage wise as well as pooled data. The observation were mode according to stage of the patients. Control war. (Pooled data)

In NHL cares the Severity of immune deficiency may vary and is not necessarily relatable to extent of the disease. (lany and Kaplan 1974). The nature of immune defect and mechanism under lying the immunological dysfunction in partly exposed, some fact of immune deficiency seems to be altered with active disease.

T Cell indicated significant decrease in NHL patients. In the stage wise data also indicated significant decrease in T cells. The clinical manifestation of NHL might well dependant on degree of T cell immunocompetance.

Hancock, et al 1977, Jones et al. 1978 and advani, et al. (1980) reported impairment of delayed hypersensitivity and abnormality in T cells function of NHL patients. Cur results in agree meet with pieces et al. (1973) which indicate decrease in numbers and functional impairment in surface immunoglobulin bearing B lymphocyte in NHL patients which was also supported by Gajl – peczlska (1973) and simonsson, et al. (1983). In this study pooled data of T cells indicated significant decreases in Tells.

In this study Igm level remained same in pooled data of NHL when compared with control subject but IgG level was increased in these cases Datta et al (1971)

In stage uses data gradual increase in IgG level with advancement of studies showed maximum increase in IV stage. With hyper gamma globulinemia may be due to rise in globulin. Similar observations were reported by data et al. (1971) and Lichtenstein and taylor (1980). Other investigators reported NHL of B cells to be associated with abnormal serum immune globulin. A antibody deficiency (alexanian 1975 and marray, 1980) kumar and panny (1982) observed minimal abnormalities in immunological function but impairment of both antibody mediated and cell mediated immunity is recorded in advance stages of the the disease. However the exact mechanism for increase or decrease of there immunoglobulin is not yet clear. It very difficult to comment on it. It is also very difficult to rule out of the cause of infection, which is not apparent in these subjects causing the alteration in immunoglobulin.

Serum proteins indicated significant rise in pooled data of NHL patients. In stage wise data also almost show the same result in all stages, Total serum proteins showed significant rise then control.

## Summary and Conclusion

In NHL patients T cells decreased significantly in all stages as well as in pooled data B cells are also increased in NHL patients of all stages then control.

Serum IgG level indicated gradual rise which was at its peak Laval in stage IV and as also in pooled group patients.

Total gamma globulin percentage was significantly decreased in NHL patients in polled as well as in stage uses data.

Serum proteins indicated significant increase in polled as well as stage uses data.

Albumin: globulin ration indicated gradual rise which was at its peak Laval in stage IV patients.

## References


1. Alexanian, R. Monoclonal Immunopathy in lymphoma. *Arch Intern Med.* 1975; 135: 62-72.
2. Advani SH, Dinshaw KA, Nair CN, Gopal R, Talwalkar GV, Lyyer YS, Bhatia HM and Desai PB. Immunodys function in non Hodgkin's lymphoma. *Cancer.* 1980; 45: 2843-2848.
3. Bjcarkhalm M. Immunodeficieny in Hodgkin's disease and its relation to prognosis. Scand.J Haematol suppl. 1978; 33.3.
4. Buchi G, Girotoo M, Termire G, Gario S, Grosso E, Antino R, Zappala C and Ferrero I. *Acta – Haematol.* 1984; 71: 322-28.
5. Crispen RG and Rosenthal SR. Vaccination and cancer mortality. *cancer – Immurnol – Immunother.* 1976; 1: 139–142.
6. Datta U, Aikat BK and Sehgal S. Immunoglobulin's in cases of leukemia's and lymphomas in north India (Chandigarh). *Indian J med Res*. 1971; 59: 1950–1958.
7. Gajl-Peczalska KJ, Hensen JA, Bloomfield CD and Good RA. Blymphocytes in untreated patients of malignant lymphoma and Hodgkin's disease. *J Clin Invest.* 1973; 52: 3064-3073.
8. Han Cock BW, Bruce L, Dunsmore JR. Follow-up Studies on the immune status of Patients with Hodgkin's disease after splencetomy and treatment in relapse and remission. *Br J Cancer.* 1977; 36: 347-354.
9. Jones SE, Griffith K, Domborowski P and Geinee JA. Immunodeficiency in patients with Non Hodgkins Lymphoma. *Blood.* 1977; 47: 335-344.
10. Levy R and Kaplan HS. Impaired Lymphocyte function in untreated Hodgkin's disease. *N Eng J Med.* 1974; 290: 181.
11. Lichtenstein A and Taylor CR. Serum Immunoglobulin levels in patients with non Hodgkin's Lymphoma. *Am J Clin Pathol.* 1980; 74: 12-17.
12. Lindemalm CS, Biberfeld P, Bjorkholm M, Holm G, Johansson B, Mellstedt H, Nilsson B and Ost A. Longitudinal studies of Blood lymphocytes functions in non Hodgkin's lymphomas. *Eur J Can Clin Oncol.* 1983; 19: 747-755.
13. Lowry OH, Roseborongh NJ, Farr PL and Randill RJ. *J Biochemistry*. 1951.

14. Lukes RJ and Collins RD. New approaches to classification of the lymphoma. *Br J can (Suppl-2)*. 1975; 31: 1-28.
15. Kohn J. Cellulose Acetate electrophoresis and Immunodiffusion technique edit by Ivar smith Vol. II. London: Willum Heinman medical book Ltd.; 1960, 56.
16. Kumar KR and Penny R. Cell medicated immune deficiency in Hodgkin's disease. *Immunology Today*. 1982; 3: 269-273.
17. Mancini G, Vaerman JP, Carbonara AO and Heremans JF. Immunochemical quantization of antigen by Single radial immune diffusion. *Immunochem*. 1968; 2: 235-254.
18. Mckinney MM and Parkinson A. A simple non chromatographic procedure to purify immunoglobulin full serum and asitic fluid. *J Immune method*. 1987; 96: 221-278.
19. Murray JL, Hurtubise PE, Young DC, Baleerzak SP, Lobuglio AF. Correlation of Prognostic factors and blood lymphocyte sub type in non Hodgkin's lymphoma. *Cancer*. 1980; 46: 1817-1824.
20. Piessens WF, Schur PH, Maloney WC and Churchill, WH. Lymphocyte surface immunoglobulin distribution and frequency in lymphoprolifertive disease. *New engl J med*. 1973; 288: 176-180.

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.

Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.com

Technical problems or general questions on publishing with IJPRP are supported by Red Flower Publication Pvt. Ltd's Author Support team (http://ww.rfppl.com)

Alternatively, please contact the Journal's Editorial Office for further assistance.

A Lal
Publication -in-Charge
Indian Journal of Pathology: Research and Practice
Red Flower Publication Pvt. Ltd.
41/48, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi – 110 091
India
Phone: 91-11-22754205, Fax: 91-11-22754205
E-mail: redflowerppl@gmail.com
Website: www.rfppl.com

*Case Report*

**Indian Journal of Pathology: Research and Practice**

# Marginal Zone Lymphoma of Urinary Bladder: A Case Report

**Sangeetha K Nayanar***

**Geetha K****

**Ramadas P*****

**Marie Therese M******

*MD(Path) DNB (Path), Asso. Professor, Department Of Pathology, Pariyaram Medical College, Kannur,Kerala-670503

**MD(Path), Professor, Department Of Pathology, Pariyaram Medical College, Kannur, Kerala-670503

***MCh(URO) DNB(URO), Asso. Professor, Department Of Urology, Pariyaram Medical College, Kannur, Kerala-670503

****MD, DNB(Path), Reader, Christian Medical College, Vellore,Tamil Nadu

Accepted on 21.11.2012

**Abstract**

Involvement of urinary bladder has been documented in 10-25% of Lymphomas and Leukemias, but lymphoid neoplasms arising primarily in the urinary bladder are rare. Low grade B non-Hodgkin lymphomas are the most common. This is a case report of a 68-year old male with primary low-grade B non-Hodgkin Lymphoma of the urinary bladder consistent with extra-nodal Marginal zone lymphoma.

**Keywords:** Urinary Bladder; Low grade B NHL.

## Introduction

10-20% of patients with Non-Hodgkin Lymphoma have infiltration of the urinary bladder at autopsy. Hodgkin Lymphoma involves the bladder in only 4% of patients. Primary Non-Hodgkin Lymphoma of the urinary bladder is rare. The disease is usually detected in middle-aged women undergoing cystoscopy for non-specific urinary symptoms. Most are seen as discrete tumor masses with fleshy white appearance rather than diffuse infiltrates. Majority of the tumours are of the low or intermediate grade.

*Case history*

68 year old male presented with irritative voiding symptoms. Ultrasonography showed gross thickening of the bladder wall with bilateral Hydronephrosis. CT scan of the abdomen revealed thickening of the bladder wall with extension of lesion to the right lateral pelvic wall. Cystoscopy showed only a small capacity bladder but no intraluminal lesions. Hence laparotomy was done and biopsy taken.

*Pathological findings*

Biopsy taken from the bladder wall showed bundles of detrusor smooth muscle infiltrated by a tumor composed of sheets of small lymphoid cells with scanty cytoplasm, round-

**Corresponding Author:**

Dr. Sangeetha K. Nayanar, Sri Sakthi, Talap, Kannur, Kerala - 670002, Phone: 04972700103, 9447170103.

E-mail : sgeetamauamar@yahoo.com

**Figure 1: Sheets of small lymphoid cells infiltrating detrusor smooth cells (H&E, x100)**

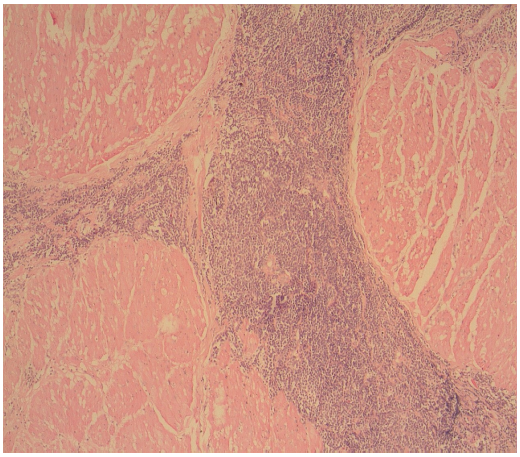
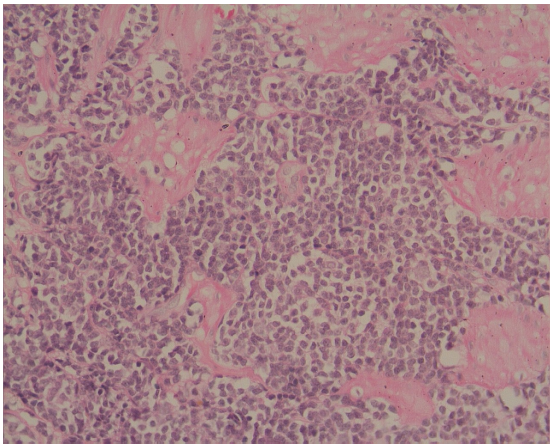
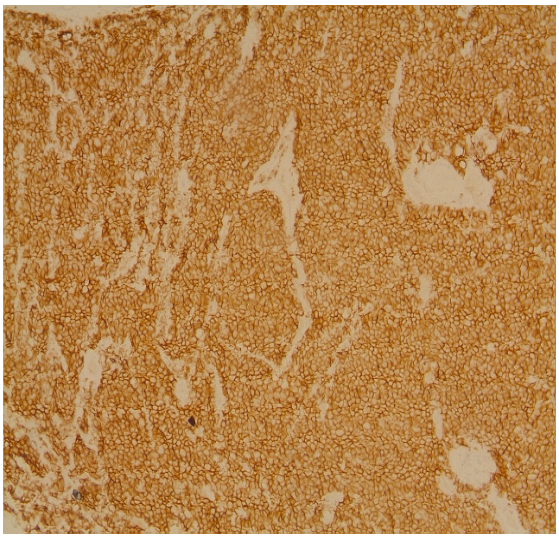


**Figure 2: Lymphoid cells show scant cytoplasm, clumped nuclear chromatin & sparse mitotic activity (H&E, x100)**



regular nuclei showing clumped chromatin. There were scattered larger cells with vesicular nuclei and one to three nucleoli resembling centroblasts. Mitotic activity was sparse. Immunohistochemistry done at Department of Pathology, Christian Medical College revealed that tumor cells were CD 20 positive and CD 3 negative with a MIB-1 proliferation index of 5%. The tumor cells were negative for CD5, CD10 and CD23. The final impression was low grade B non-Hodgkin lymphoma of urinary bladder . The immunomorphological features were consistent with Extranodal marginal zone lymphoma. The patient underwent

**Figure 3: Strong positive staining for CD 20(H&E, x200)**



chemotherapy with good response and is on follow-up.

## Discussion

The first recorded case of lymphoma of the bladder was reported by Eve and Chaffey in1885 [4]. Malignant Lymphomas of the bladder can be classified into one of three different clinical groups: 1) Primary lymphoma localized to the bladder, 2) Lymphoma presenting in bladder as the first sign of disseminated disease, 3) Recurrent bladder involvement by lymphoma in patients with a history of malignant lymphoma [4].

Grossly, bladder lymphomas are usually large masses centered in the dome or lateral walls. They are nearly always of the non-Hodgkin type and majority are low-grade B cell NHL. Recent data suggest that the most common type of low-grade B NHL is the extranodal marginal zone lymphomas of MALT type [1].

Lymphoepithelial lesions in MALT-type lymphoma involve transitional epithelium, and their presence in high grade lymphoma suggests a primary origin owing to transformation of low grade MALT-type [2]. They are more common in females and are associated with a history of chronic cystitis [3].

The differential diagnosis is with poorly differentiated carcinomas and other Small cell cancers. The prognosis of Primary Bladder Lymphoma has been favorable, with many patients alive and well several years after treatment [5]. Local radiotherapy appears to confer long-term control [3]. Ureteric obstruction and acute renal failure are serious complications [5].

**References**


1. Kempton CL, Kurtin PJ, Inwards DJ, Wollan P, Bostwick DG. Malignant lymphoma of the bladder: evidence from 36 cases that low-grade lymphoma of the MALT -type is the most common Primary bladder lymphoma. *Am J Surg Pathol*.1997; 21(11): 1324-33.
2. Bates AW, Norton AJ, Baithun SI. Malignant lymphoma of the urinary bladder: a clinicopathological study of 11 cases. *J Clin Pathol*. 2000; 53(6): 458-61.
3. Al-Maghrabi J, Kamel-Reid S, Jewett M, Gospodarowicz M, Wells W, Banerjee D. Primary low grade B-cell lymphoma of mucosa-associated lymphoid type arising in the Urinary bladder: report of 4 cases with molecular genetic analysis. *Arch Pathol Lab Med*. 2001; 125(3): 332-6.
4. Cohen DD, Lamarre C, Lamarre L, FS FS. Primary low grade B-cell lymphoma of the Urinary bladder: case report and literature review. *Can J Urol*. 2002 ; 9(6): 1694-7.
5. Hughes M, Morrison A, Jackson R. Primary bladder lymphoma: management and outcome of 12 patients with a review of the literature. *Leuk Lyphoma*. 2005; 46(6): 873-7.

## BOOKS FOR SALE

### CHILD INTELLIGENCE

By **Dr. Rajesh Shukla**

ISBN: 81-901846-1-X, Pb, vi+141 Pages

Price: Rs.150/-, US$50/-

Published by **World Informations Syndicate**

This century will be the century of the brain. Intelligence will define success of individuals; it remains the main ingredient of success. Developed and used properly, intelligence of an individual takes him to greater heights. Ask yourself, is your child intelligent! If yes, is he or she utilizing the capacity as well as he can? I believe majority of people, up to 80% may not be using their brain to best potential. Once a substantial part of life has passed, effective use of this human faculty cannot take one very far. So, parents need to know how does their child grow and how he becomes intelligent in due course of time. As the pressure for intelligence increases, the child is asked to perform in different aspects of life equally well. At times, it may be counter-productive. Facts about various facets of intelligence are given here. Other topics like emotional intelligence, delayed development, retardation, vaccines, advice to parents and attitude have also been discussed in a nutshell. The aim of this book is to help the child reach the best intellectual capacity. I think if the book turns even one individual into a user of his best intelligence potential, it is a success.

### PEDIATRICS COMPANION

By Dr. Rajesh Shukla

ISBN: 81-901846-0-1, Hb, VIII+392 Pages

Price: Rs.250/-, US$50

Published by **World Informations Syndicate**

This book has been addressed to young doctors who take care of children, such as postgraduate students, junior doctors working in various capacities in Pediatrics and private practitioners. Standard Pediatric practices as well as diseases have been described in a nutshell. List of causes, differential diagnosis and tips for examination have been given to help examination-going students revise it quickly. Parent guidance techniques, vaccination and food have been included for private practitioners and family physicians that see a large child population in our country. Parents can have some understanding of how the doctors will try to manage a particular condition in a child systematically. A list of commonly used pediatric drugs and dosage is also given. Some views on controversies in Pediatrics have also been included. Few important techniques have been described which include procedures like endotracheal intubations, collecting blood samples and ventilation. I hope this book helps young doctors serve children better.

**Order from**

Red Flower Publication Pvt. Ltd.

41/48, DSIDC, Pocket-II, Mayur Vihar, Phase-I

P.O. Box No. 9108, Delhi - 110 091 (India)

Tel: 91-11-65270068, 22754205, Fax: 91-11-22754205

E-mail: redflowerppl@gmail.com, redflowerppl@vsnl.net

*Case Report*

**Indian Journal of Pathology: Research and Practice**

# Isolated Tuberculous Osteomyelitis of Toe: A Case Report

**Veena P. Waiker***

**Udayashankar****

*Professor, Department of Plastic Surgery, M.S. Ramaiah Hospital, Bangalore.

**Professor, Department of Plastic Surgery, M.S. Ramaiah Hospital, Bangalore.

Accepted on 06.12.2012

**Abstract**

Isolatedextra pulmonary tuberculous osteomyelitis of the phalanx is rare as compared to that of proximal bones. We report acase of tubercular osteomyelitis of the middle phalanx of the toe in a 74 year old lady, presenting as post traumatic chronic ulcer which is even morerare. The difficulty in diagnosis and the related problems, the need to have a high index of suspicion of tuberculosis as one of the causes of chronic foot ulcer especially in developing countries has been emphasized.

**Key-words:** Tuberculosis; Osteomyelitis; Phalanx; Toe.

## Introduction

Skeletal tuberculousosteomyelitis hasbeen described, but involvement of the bones of hands and feet is rare while that of phalanx of the toe presenting as post traumatic non healing ulcer is very rare. There are very few reports regarding isolated involvement of phalanges [1,2]. It is a major health concern in developing countries even today. The increased incidence of immune-suppression due to various reasons hasagain led to the rise in tuberculosis.[3]

**Corresponding Author:**

Dr. Veena P. Waiker, 19 F-1, Vinayaka layouts 3rd Stage , Vijayanagar, Bangalore-560040. Mob. No. 9900531217.

E-mail : veenaprabhakar2003@yahoo.com

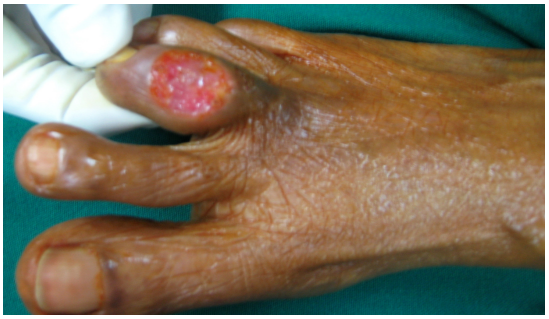


Musculoskeletal involvement may occur along with pulmonary lesion or as a delayed primary extra pulmonary lesion after many years. The chances of missing the diagnosis and progress of the disease are high in chronic ulcer. We hereby present a rare and isolated case of tuberculous osteomyelitis of toe which came to notice following a trivial injury.

### *Case presentation*

A 74 year old lady presented to our hospital with chronic non healing ulcer of one year duration, on the third toe of her right foot following trivial trauma. She complained of pain, discharge and swelling of the toe which was treated conservatively with dressings and analgesics. Intermittent courses of antibiotics

**Fig 1: Chronic non healing ulcer dorsum of third toe – right foot**



were administered based on culture and sensitivity reports by her family physician. There was no history of fever but she had loss of appetite and loss of about 3 kgs of weight in a period of six months. Physical examination revealed an ulcer of 1cm diameter overlying the dorsum of third toe of her right foot. (Fig 1) Interphalangeal joint movements were restricted and painful. There was surrounding oedema. Dorsalis pedis and posterior tibial artery pulsations were well felt bilaterally. There was no regional lymphadenopathy. The chest and abdomen were clinically normal. Plain radiograph of right foot which was done one month after trauma was found to be normal. Six months later it revealed cortical erosion of middle phalanx of third toe with osteoporosis of distal and proximal phalanges. (Fig 2) A diagnosisof post traumatic non healing ulcer due to chronic osteomyelitis was

**Fig 2: X-ray showing cortical erosion of middle phalanx**

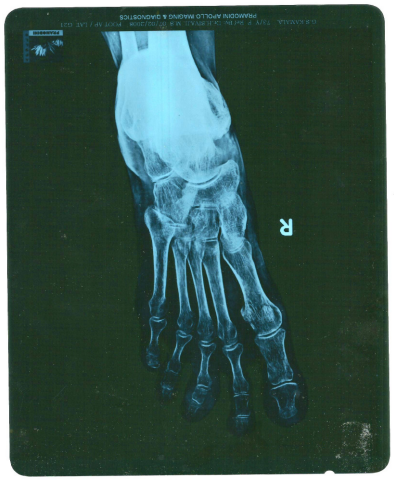
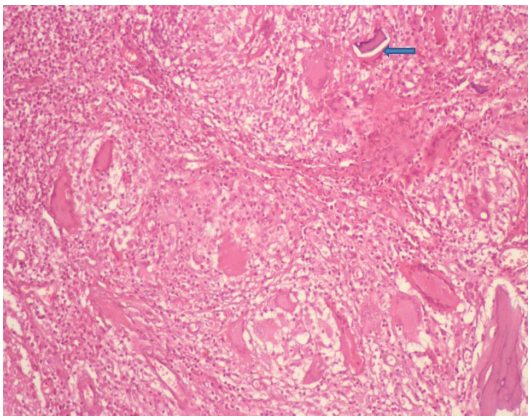


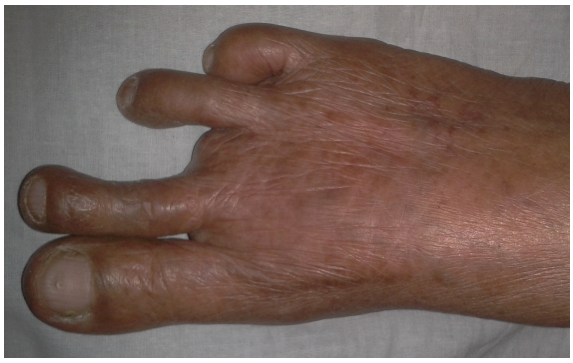
**Fig 3: Histopathology (High power) showing confluent tuberculous granulomas composed of epithelioid cells,Langhans giant cells and lymphocytes .A spicule of bone is seen (arrow)**



made having Marjolin's ulcer as differential diagnosis. On admission Haemoglobin was 11.1gms%, Random blood sugar 104.4 mg/dL and E.S.R. 8mm/hour. Chest roentgenogram was normal.

The involved toe was disarticulated at the metatarsophalangeal joint and closed with Fillet flaps. The specimen of bone and soft tissue was found to be infiltrated with granulomas composed of epitheloid cells, multinucleated giant cells and lymphocytes without caseous foci on histopathological examination which suggested tuberculous osteomyelitis.(Fig3) Bacteriological examination did not reveal any growth on culture and subsequent ZN stain was negative for AFB.The wound healed in a weeks' time. (Fig 4) Patient was treated with four drugs Rifampicin,Isoniazid,Ethambutol

**Fig 4: 5 years later – well settled scar**



and Pyrazinamide from the tenth post-operative day for two months followed by two drugs Isoniazid and Rifampicin in combination with Pyridoxine for another four months after prior liver function tests and ultrasound abdomen which were normal. Her appetite improved and she regained 4 kgs of weight in five months.

## Discussion

Extra pulmonary skeletal tuberculosis comprises of 1 to 5 percent of all tuberculous infections and usually occurs due to lympho haematogenous spread from lungs [4]. It may also affect otherwise healthy individuals. Long bones are less commonly involved compared to vertebral bodies due to the sluggish circulation. Tuberculous osteomyelitis of metatarsal bones and phalanges are rare but has been reported[5] whereas there are no reports on tuberculous osteomyelitis of phalanx presenting as post traumatic non healing ulcer. The common clinical symptoms are localised pain, swelling and discharge like in our patient. Occasionally it may be accompanied by constitutional symptoms such as fever and weight loss. Diagnosis of tuberculous osteomyelitis is usually delayed because the signs and symptoms are subtle [6]. The delay in diagnosis and administration of anti-tubercular drugs resulted in progress of the disease leading to loss of the toe in our patient. Laboratory tests, like erythrocyte sedimentation rate, complete blood count and fine needle aspirationmay not be conclusive. Radiological changes on plain X-ray are not seen initially. However, typical radiological appearances likeosteolytic areas with osteoporosis and periosteitis on x ray is difficult todifferentiate from chronic pyogenic osteomyelitis and tumours. An MRI scan is more sensitive in localising the lesion in the early stages [7] but has to be confirmed by an open biopsy.

Histopathological diagnosis was an incidental diagnosis in our patient, as the surgical specimen was sent for histopathology with Marjolin's ulcer and chronic pyogenic osteomyelitis as differential diagnosis. Subsequent Zeil Neilson.staining was done to confirm the histopathology report but did not reveal AFB. The diagnosis is said to be more accurate with histopathology of the specimen and PCR [8]. AFB may be absent on stain and culture as tuberculous osteiomyelitis is usually paucibacillary [9]. Hence, histopathological diagnosis in correlation with clinical and radiological findings prompted anti-tubercular therapy and healing of the surgical wounds with cure of the disease in our patient. Tuberculosis still remains a major health problem in developing countries despite the advances in anti-tuberculous drugs. In fact, the incidence of tuberculosis has risen again, which is probably associated with increase in incidence of diabetes[10],viral fever, HIV, increasing number of patients on immunosuppressive agents and emergence of drug resistant strains of mycobacterium.

Hence, we recommend the clinicians especially in developing countries to have a high index of suspicion and do an open biopsy for bacteriological and histological examination in chronic ulcers not responding to antibiotics and analgesics in otherwise healthy individuals. This helps in detecting asymptomatic cases,early diagnosis and timely administration of anti-tuberculous drugs which along with the required surgical procedure aids in wound healing and resolution of the disease.

## References

1. Mandeep S Dhillon, Sameer Aggarwal, Sharad Prabhakar, Vikas Bachhal. Tuberculosis of the foot. An Osteolytic variety. *Indian J Orthop.* 2012; 46(2): 206-211.
2. Rajeev Vohra, Harinder S Kang, Sameer Dogra, Radha R Sagar, Rajan Sharma. Tuberculous Osteomyelitis. *J Bone Joint Surg [Br].* 1997; 79-B: 562-6.
3. Davies PD. The world-wide increase in tuberculosis: how demographic changes, HIV infection and increasing numbers in poverty are

increasing tuberculosis. *Ann Med.* 2003; 35(4): 235-43.

4. MC Yuen, WK Tung. An uncommon cause of foot ulcer: tuberculous osteomyelitis. *Emerg Med J.* 2001; 18(2): 140-141.
5. Kushwaha RA, Kant S, Verma SK, Sanjay, Mehra S. Isolated metacarpal bone tuberculosis-a case report. *Lung India.* 2008; 25(1): 17-9.
6. Choi WJ, Han SH, Joo JH, Kim BS, Lee JW. Diagnostic dilemma of tuberculosis in the foot and ankle. *Foot Ankle Int*. 2008; 29(7): 711-5.
7. MC Yuen,WK Tung. An uncommon cause of foot ulcer: tuberculous osteomyelitis140. *Emerg Med J.* 2001; 18: 140–141.
8. Margaret D, Phillips MD. Presented by Stephen J Pomeranz, M.D.The MRI Mentor TM New Research and Trends in Advanced Imaging. 2011; 5(3).
8. Anil K Jain, Santosh Kumar Jena, MP Singh, IK Dhammi, VG Ramachadran, Geeta Dev. Evaluation of clinico-radiological, bacteriological, serological, molecular and histological diagnosis of osteoarticular tuberculosis. *Indian J Orthop*. 2008; 42(2): 173–177.
9. Shanmugasundaram TK. Bone and joint tuberculosis-Guidelines to management. *Indian J Orthop*. 2005;:39(3): 195-198.
10. Jeremy D Goldhaber-Fiebert,Christie Y Jeon,Ted Cohen,Megan B Murray. Diabetes Mellitus and Tuberculosis in countries with high Tuberculosis Burdens:Individual risks and Social Determinants. *Int J Epidemiol.* 2011; 40(2): 417-428.

*Case Report*

**Indian Journal of Pathology: Research and Practice**

# Primary Conjunctival Amyloidosis Mimicking Conjunctival Neoplasm: A Case Report

**Prem Singh, MD***

**Jyoti Bala, MD****

**Monica Garg MD*****

**H K Sidhu MS******

*Department of Pathology, M.M. Institute of Medical Sciences and Research, Mullana (Ambala), Haryana-133207, India

**Department of Pathology, M.M. Institute of Medical Sciences and Research, Mullana (Ambala), Haryana-133207, India

***Department of Pathology, M.M. Institute of Medical Sciences and Research, Mullana (Ambala), Haryana-133207, India

****Department of Ophthalmology, M.M. Institute of Medical Sciences and Research, Mullana (Ambala), Haryana-133207, India

Accepted on 15.07.2012

**Abstract**

Amyloidosis is a group of disorders characterized by the extra cellular deposition of a substance called amyloid in various tissues. When present as a conjunctival mass with recurrent haemorrhage the condition mimicks a neoplastic lesion. We report such a patient who had complaints of a conjunctival mass with recurrent subconjunctival haemorrhage. The diagnosis of amyloidosis was made by excisional biopsy and confirmed by Congo red staining. Further work up did not reveal any evidence of systemic amyloidosis.

**Key words :** Conjunctival amyloidosis; Congo red; Neoplastic leison.

## Introduction

Primary conjunctival amyloidosis is extremely rare entity with a characteristic feature of tendency to bleed. It is a chronic disease commonly involving tarsal and forniceal conjunctiva. When presenting as a conjunctival mass it mimicks a neoplastic lesion like lymphoma or papilloma [1]. The disease causes significant ocular discomfort and may also be a rare cause of ptosis. We report a case of conjunctival amyloidosis clinically mimicking a neoplastic lesion. The diagnosis was confirmed by biopsy of the lesion and histopathological examination (HPE).

**Corresponding Author:**

Dr Prem Singh,Department of Pathology, M.M. Institute of Medical Sciences and Research, Mullana (Ambala), Haryana, India. 1330203

E-mail: premsingh011@rediffmail.com

*Case Report*

A 60 years old female came to the Department of Ophthalmology with recurrent bleeding and chronic discomfort in the left eye for the last one year. She had noticed a slowly growing conjunctival mass in the left eye for

last 6 months. The patient had no preceding history of any ophthalmic complaints or systemic symptoms. There was no history of eye surgery or ocular trauma. Ophthalmic examination showed a circumscribed reddish mass in the left lower palpebral conjunctiva. Rest of the ocular and systemic examination was normal. The mass was excised and sent for HPE to rule out any neoplastic pathology. Grossly the excised mass measured 0.8 x0.5x0.2cm with a reddish pink colour. HPE revealed a polypoidal lesion lined by conjunctival epithelium with sub epithelial tissue showing deposits of amorphous, eosinophilic, pale hyaline material,with interspersed ectatic and congested blood vessels (figure 1). Areas of hemorrhage were also seen. Congo red staining revealed the characteristic salmon pink colour of the deposits (figure 2) which on polarized microscopy exhibited a characteristic apple-green birefringence suggestive of amyloid deposition. A diagnosis of amyloidosis was established. A further detailed work up revealed no evidence of systemic amyloidosis. Serum electrophoresis was normal with absence of monoclonal band.

## Discussion

Primary conjunctival amyloidosis is a rare disease which may mimic an allergic or neoplastic etiology and is apparently the most common non familial ophthalmological manifestation of amyloidosis. It results from extra cellular deposition of insoluble fibrous amyloid proteins in the organs and tissues [2]. It may occur as an uncommon complication of trachoma[3] or may be unusual cause of ptosis. The term amyloidosis was coined by Virchow in 1854. The first case of localized ocular amyloidosis was reported in 1871. The disease is usually unilateral as seen in our patient though bilateral involvement is not uncommon [2]. The characteristic feature of the disease is recurrent tendency to bleed[4]. The early diagnosis is difficult as yellowish deposits indicative of amyloidosis are not obvious at this stage[5]. The patients may also present clinically with lid swelling, ptosis, chronic ocular discomfort, irritation, foreign body sensation, papillary hyperplasia and recurrent conjunctival haemorrhage[1]. The classic "salmon-pink" conjunctival infiltrate has been associated with lymphoproliferative disorders; however amyloid should also be considered as it may be clinically indistinguishable from such disorders [6]. When presents as a conjunctival mass as in our case the differential diagnosis include lymphoma, papilloma and allergic conditions [1]. HPE supplemented by special stains is necessary to determine the final diagnosis. The possibility of amyloidosis in extra ocular sites must also be ruled out. Our case did not have any evidence of amyloidosis in a follow up period of two years.

**Figure 1: Biopsy section. Microphotograph showing subconjunctival deposits of pale, eosinophilic acellular material distinctive of amyloid. ( H& E stain,X 200)**

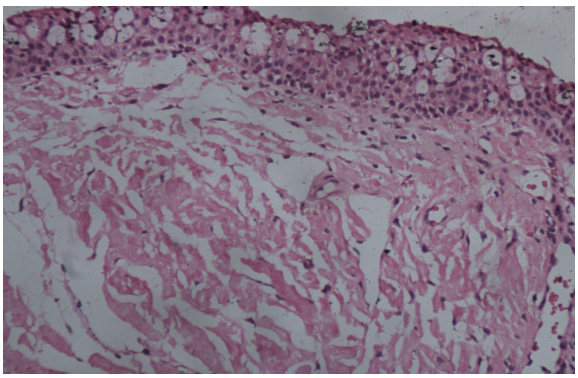
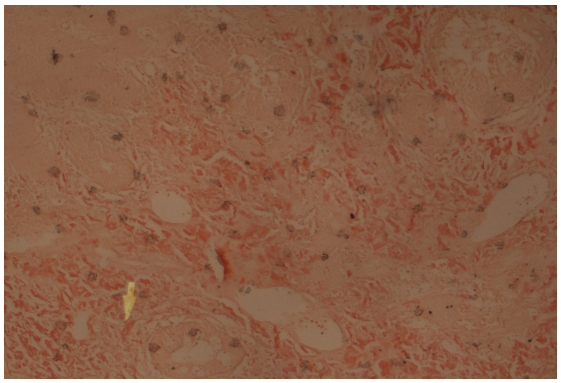


**Figure 2: Congo red staining of biopsy section showing typical red-orange color of amyloid deposits. ( Congo-red stain, X100)**



## Conclusion

Most of the patients with conjunctival amyloidosis present with yellowish pink haemorrhagic mass in the conjunctiva and such patients usually do not show any evidence of systemic amyloidosis. Hence conjunctival amyloidosis should be kept in the differential diagnosis of any patient presenting with a conjunctival mass and recurrent subconjunctival haemorrhage. Biopsy and HPE are infallible tools for diagnosing such a condition.

### References


1. Vemuganti GK. Inflammatory and non – neoplastic lesions of eyelids, eye ball and Orbit. *J Cytol.* 2007; 24: 26-30.
2. Rodrigues G, Sanghvi V, Lala M. A rare cause of unilateral upper and lower eyelid swelling: Isolated conjunctival amyloidosis. *Korean J Ophthalmol.* 2001; 5: 38-40.
3. Pecora JL, Sambursky JS, Vargha Z. Radiation therapy in amyloidosis of the eyelid and conjunctiva: A case report. *Ann Ophthalmol.* 1982; 14: 194-196.
4. Lee HM, Naor J, DeAngelis D, Rootman DS . Primary localized conjunctival amyloidosis presenting with recurrence of subconjunctival hemorrhage. *Am J Ophthalmol.* 2000; 129(2): 245-7.
5. Mesa-Gutierrez JC, Huguet TM, Garcia NB, Ginebreda JA. Primary localized conjunctival amyloidosis: a case report with a ten-year follow-up period. *Clinical Ophthalmol.* 2008; 2(3): 685-687.
6. Spitellie PH, Jordan DR, Gooi P, Brownstein S, Burns BF. Primary localized conjunctival amyloidosis simulating a lymphoproliferative disorder. *Ophthal Plast Reconstr Surg.* 2008; 24(5): 417-9.
7. Rosenblatt M, Spitz GF, Friedman AH, Kazam ES. Localized Conjunctival Amyloidosis: Case Reports and Review of Literature. *Ophthalmologica.* 1986; 192: 238-245. (DOI: 10.1159/000309653).

**Library Recommendation Form**

If you would like to recommend this journal to your library, simply complete the form below and return it to us. Please type or print the information clearly. We will forward a sample copy to your library, along with this recommendation card.

**Please send a sample copy to:**

Name of Librarian

Library

Address of Library

**Recommended by:**

Your Name/ Title

Department

Address

**Dear Librarian,**

I would like to recommend that your library subscribe to the **Indian Journal of Pathology: Research and Practice**. I believe the major future uses of the journal for your library would be:

1. As useful information for members of my specialty.
2. As an excellent research aid.
3. As an invaluable student resource.
4. **I have a personal subscription and understand and appreciate the value an institutional subscription would mean to our staff.**
5. Other

Should the journal you're reading right now be a part of your University or institution's library? To have a free sample sent to your librarian, simply fill out and mail this today!

Stock Manager

**Red Flower Publication Pvt. Ltd.**

41/48, DSIDC, Pocket-II, Mayur Vihar, Phase-I

P.O. Box No. 9108, Delhi - 110 091 (India)

Tel: 91-11-65270068, 22754205, Fax: 91-11-22754205

E-mail: redflowerppl@gmail.com, redflowerppl@vsnl.net

Website: www.rfppl.com

*Case Report*

**Indian Journal of Pathology: Research and Practice**

# Unicystic Ameloblastoma of Mandible with all the Variants Mimicking a Cyst: A Case Report

**Shambulingappa Pallagatti***

**Soheyl Sheikh****

**Rajesh Gupta*****

**Isha Singla******

**Ravinder Singh*******

**Deepak Gupta********

* M.D.S., Professor, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

**M.D.S., Professor, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

*** Post Graduate Student, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

**** Post Graduate Student, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

***** M.D.S., Assistant Professor, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

M.D.S., Reader, Department of Endodontics , M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India)

Accepted on 08.09.2012

**Abstract**

Unicystic ameloblastoma is a rare variant of ameloblastoma. We report a case of Unicystic ameloblastoma in a 29 year-old female with a pain and swelling in the left mandibular posterior region. Fine needle aspiration yielded no fluid. Periapical, panoramic and computer tomography scans showed well defined radiolucency present in relation to 36 and 37. Unicystic refers to those cystic lesions that show clinical, radiographic, or gross features of a cyst, but on histologic examination show a typical ameloblastomatous epithelium lining part of the cyst cavity, with or without luminal and/or mural tumor growth. Enucleation and Tumor resection was performed for the treatment. Our case report is an attempt to help the dental community in developing familiarity with the clinical presentation and at the same time advocating to develop a high index of suspicion in recognizing such cases.

**Keywords:** Unicystic Ameloblastoma; Odontogenic tumor; Histopathology.

**Corresponding Author:**

Dr. Rajesh Gupta, Post graduate student, Department of oral medicine and radiology, M.M. College of Dental Sciences and Research, Mullana, Ambala, Haryana (India) Pin 133203. Phone No: +91 9034808508 (Mobile), Fax: +91-1731- 304111, +91-1731- 304550.

E-mail: rajesh42gupta@gmail.com



## Introduction

Many benign lesions cause mandibular swellings and these can be divided into odontogenic and nonodontogenic origin. These

lesions include ameloblastoma, radicular cyst, dentigerous cyst, keratocystic odontogenic tumour, central giant cell granuloma, fibro-osseous lesions and osteomas [1].

The most common tumor of odontogenic origin is ameloblastoma which develops from epithelial cellular elements and dental tissues in their various phases of development [2].

It is a slow-growing, persistent and locally aggressive neoplasm of epithelial origin. Its peak incidence is in the 3rd to 4th decades of life and has an equal sex distribution. It is often associated with an unerupted third molar [3].

The majority of ameloblastomas arise in the mandible, and these are found at the angle and ramus region. There are three forms of ameloblastomas namely multicystic, peripheral, and unicystic tumors [2].

The unicystic ameloblastoma is a well-defined, often large monocystic cavity with a lining, focally but rarely entirely composed of odontogenic epithelium [4]. The unicystic ameloblastoma is considered as a variant of the solid or multicystic ameloblastoma, accounting for 6% to 15% of all intraosseous ameloblastomas [5]. More than 90% are located in the mandible [1]. This tumor has less aggressive biologic behavior and lower recurrence rate than the classic solid or multicystic ameloblastoma [6]. Impacted mandibular third molars are even more frequently associated with UA with figures ranging from 52 to 100% [7]. Unicystic tumors include those that have been variously referred to as mural ameloblastomas, luminal ameloblastomas, and ameloblastomas arising in dentigerous cysts [8]. Although the unicystic ameloblastoma is a "cystic" appearing lesion on gross examination and microscopic examination shows the presence of ameloblastoma within the cyst wall [6]. Here, we present a case of a large unicystic mandibular ameloblastoma in a 29 year old female.

**Figure 1: Extraoral photograph of the patient**

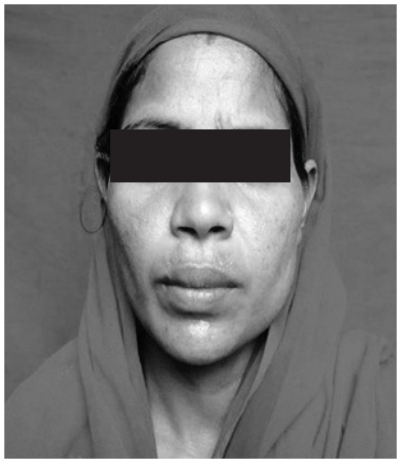
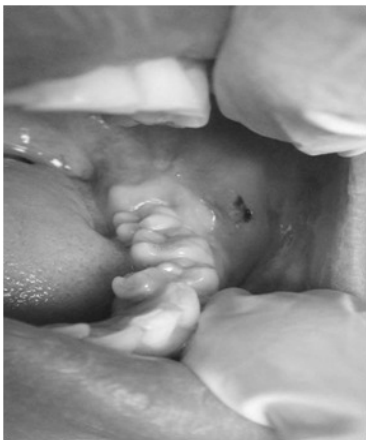


**Figure 2: Intraoral laceration with gingival swelling**



*Case Report*

A 29-year-old woman presented to the Department of Oral Medicine and Radiology, with pain and swelling in the lower left back region of the face since 3 months. There was

**Figure 3: Panoramic radiograph showing a well defined radiolucency with corticated borders and segmented appearance**

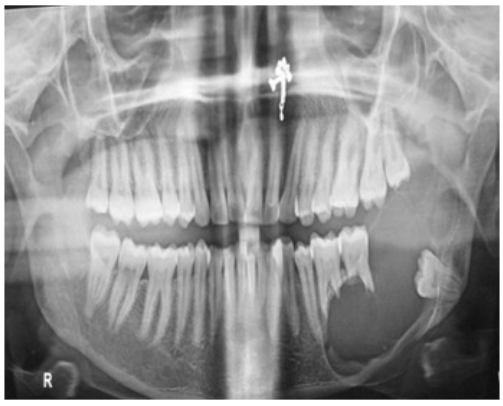
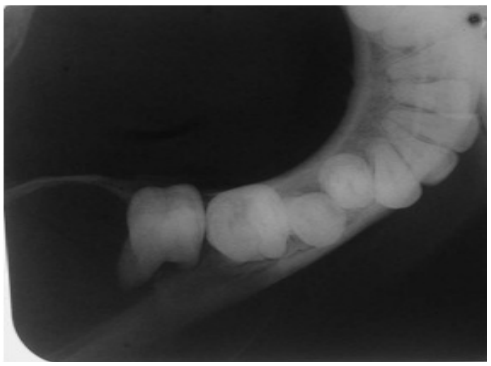


**Figure 4: Mandibular occlusal radiograph showing multilocular appearance and buccal cortical expansion**



no history of trauma and her past dental/ medical history was unremarkable. All vital signs were within normal limits.

The physical examination revealed facial asymmetry due to swelling on the lower left back region of face. On extra-oral examination, a single diffuse swelling was seen near the angle of left side of mandible. The swelling extended 3 cm from the angle of mouth to the posterior border of ramus anteroposteriorly and 4cm below the inferior border of left orbit to the inferior border of mandible superoinferiorly. The swelling was approximately 2x1 cm in size, well defined, oval in shape and had a smooth surface. The overlying skin was of same color as that of adjacent skin. The swelling was diffuse, firm, tender, non compressible and non reducible over the left side of the mandible. It was fixed to the underlying structures. Submandibular lymph nodes of left side were palpable, non-tender and not fixed to underlying structures.

On intraoral examination, a single linear laceration and gingival swelling was seen on the left buccal mucosa in relation to 37 and 38 region (Figure 2). On palpation, the gingiva around 37 and 38 region was firm and non tender. The teeth in the affected area was sensitive to percussion but no mobility could be demonstrated.

The patient was then subjected to the radiographic examination. The panoramic radiograph revealed a well defined radiolucency in relation to 36 and 37 extended from distal aspect of 37 upto the retromolar area antero-posteriorly and from the crest of the alveolar ridge to the lower border of mandible superoinferiorly. It was well defined,

**Figure 5: CT shows unilocular expansile lesion with the area of perforation in cortex**

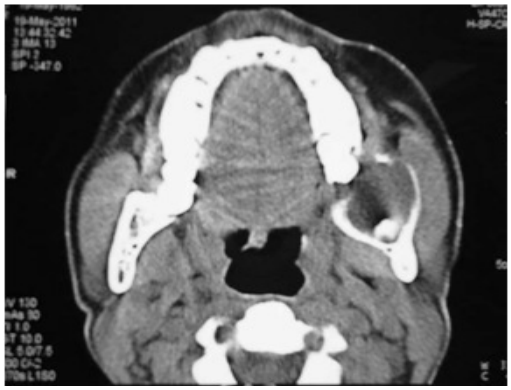


**Figure 6. Luminal UA shows cystic wall lined by ameloblastic epithelium**

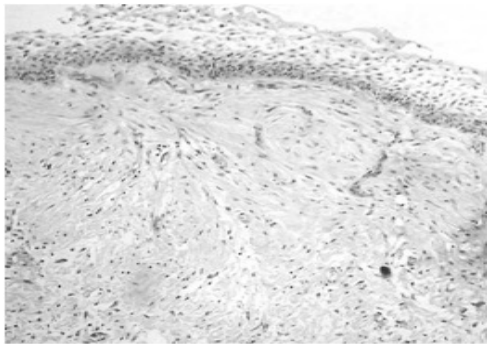


**Figure 7: Intraluminal UA shows proliferation of epithelium into lumen**

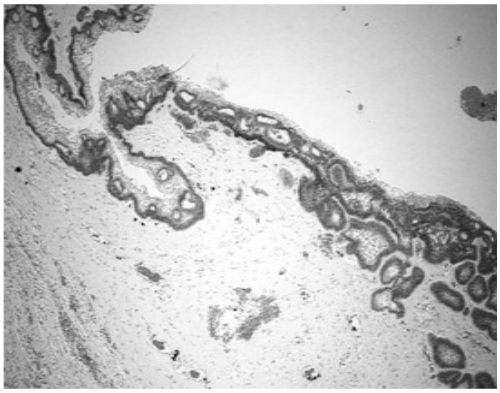
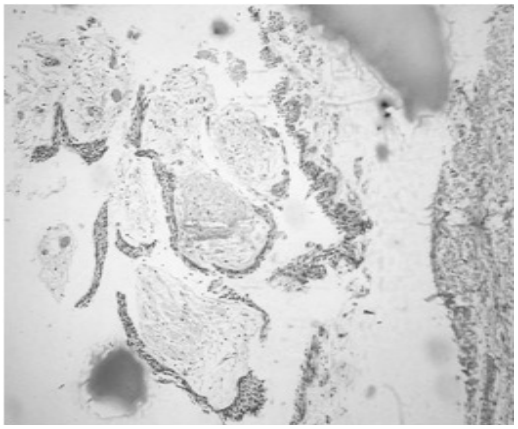


**Figure 8: Mural UA shows invasive islands of ameloblastic epithelium in CT wall**



corticated and had segmented appearance. Resorption of roots in relation to 36 and 37 was also seen. Impacted third molar was also seen on the left side of mandible (Figure 3). In the occlusal radiograph, the expansion of lingual cortical plate was evident. (Figure 4).

Computed tomography of the lesion showed a large unilocular expansile lesion of size 3.5x2.5x4.5cm was seen in relation to alveolar process of left side of mandible. Both buccal and lingual plates were thinned out with the area of perforation in cortex. Impacted third molar was also seen within the lesion (Figure 5).

Based on the clinical and radiological appearance, a provisional diagnosis of dentigerous cyst was made. Aspiration of the lesion was non-productive and a complete hemogram showed all the values within the normal range. An incisional biopsy was performed under local anaesthesia to establish a definitive diagnosis.

Histologically, it showed cystic wall lined by ameloblastic epithelium which in areas shows columnar basal cells with hyperchromatic nuclei, nuclear palisading with polarization and cytoplasmic vacuolation with intercellular spacing, thin layer of stellate reticulum like cells (Figure 6). The ameloblastic epithelium which is thickened at spaces showed papillary projections (Plexiform), extending into the lumen (Figure 7). The cystic stroma in one area shows an ameloblastic follicle with central acanthomatous change (Figure 8). All these features were consistent with unicystic ameloblastoma. Patient had subjected to enucleation and tumor resection was done.

## Discussion

The term Ameloblastoma was suggested by Churchill in 1934 [4]. There are almost upto fifteen different types of this tumor recorded till date. The most commonly occurring varieties of this tumour histologically are follicular, plexiform, granular, desmoplastic, basal cell, unicystic and the lesser occurring peripheral variant [4].

Unicystic ameloblastoma is a rare type of ameloblastoma, accounting for about 6% of ameloblastomas [2]. The concept of this tumor was first introduced by Robinson and Martinez in 1977 [9]. About 50% of the cases occur in the second decade of life [1]. Mandible is affected more than maxilla. They are most commonly encountered in the posterior mandible followed by the parasymphysis region, anterior maxilla, and the posterior maxilla [10]. Between 50 and 80% of cases are associated with tooth impaction, the mandibular third molar being most often involved [1]. Clinically and radiographically, the unicystic ameloblastoma often has the appearance of a dentigerous cyst [5]. The radiographic appearance is peculiar with the association of a circumscribed radiolucency with the crown of a tooth. The margins are well delineated, with well decorticated margins present in most of the cases [5]. In our case, tumor was associated with impacted mandibular third molar and consistant with all findings in the literature. A confirmatory diagnosis of unicystic ameloblastoma is made by histopathological evaluation of biopsy specimens. The following features are usually observed during microscopic examination.

Ackermann et al classified this entity into 3 histologic groups:

Group 1- Luminal UA lesions consist of a unilocular cyst lined by epithelium that in areas

shows ameloblastic transformation without infiltration into the connective tissue wall.

Group 2- Intraluminal/plexiform UA lesions consist of a unilocular cyst with the lining epithelium showing a nodular proliferation of plexiform ameloblastoma into the lumen without infiltration of tumor cells into the connective tissue wall.

Group 3- Mural UA lesions have invasive islands of ameloblastomatous epithelium in the connective tissue wall, that may or may not be connected to the cyst lining epithelium [5]. Our case was consistent with all the common features reported in the literature.

Odontogenic keratocyst, residual cysts, adenomatoid odontogenic tumor, giant cell lesions and sometimes solid ameloblastoma can be the possible differential diagnoses for a unilocular lesion with or without a 'dentigerous' relationship occurring within the jaws [10].

The treatment is decided by the clinical behavior and which in turn is dictated by the histological pattern of the ameloblastoma [10]. In cases of the luminal, intralumenal or plexiform pattern, enucleation generally suffices but if there is a mural component, bony resection is necessary to ensure adequate removal [1].

## Conclusion

The unicystic ameloblastoma is characterized by specific clinical, imaging, and histological features. For proper understanding of such cases, more in depth analysis and long term follow up is required. The clinician has to be alert regarding the unusual presentation of this neoplasm and should include unicystic ameloblastoma as differential diagnosis in any lesion ranging from simple abscess to any fibro-osseous lesions/neoplastic growth presenting in posterior mandible. The definite diagnosis requires histopathological examination. Also with the potential for recurrence, such cases should always be treated by complete resection.

## References


1. Ramesh RS, Manjunath S, Ustad TH, Pais S, Shivakumar K. Unicystic ameloblastoma of the mandible – an unusual case report and review of literature. *Head Neck Oncology.* 2010;14: 2:1.
2. Bhalerao S, Chaudhary R, Tamgadge A, Periera T, Tamgadge S. Unicystic Ameloblastoma –A case report. *IJCD.* 2011; 2(1): 65-8.
3. Ostric SA, Martin J, Stock C, Bittar SM. The Model Graft: Reconstruction of the Maxilla Using a Fibular Bone Graft Template. *The Journal Of Craniofacial Surgery.* 2006; 17(1): 145-204.
4. Dandekar RC, Shankar AA. Unicystic ameloblastoma: Mimicking a cyst. *Journal of Nepal Dental Association.* 2010 ; 11(1): 66-9.
5. Pizer ME, Page DG, Svirsky JA. Thirteen-Year Follow-Up of Large Recurrent Unicystic Ameloblastoma of the Mandible in a 15-Year-Old Boy. *J Oral Maxillofac Surg.* 2002; 60(2): 211-15.
6. Williams TP. Unicystic Ameloblastoma of the Mandible: A Long-Term Follow-up. *J Oral Maxillofac Surg.* 1997; 55(4): 349-50.
7. Philipsen HP, Reichart PA. Unicystic ameloblastoma. A review of 193 cases from the literature. *Oral Oncology.* 1998; 34(5): 317-25.
8. Chana JS, Chang YM, Wei FC, Shen YF, Chan CP, Lin HN, Tsai CY, Jeng SF. Segmental Mandibulectomy and Immediate Free Fibula Osteoseptocutaneous Flap Reconstruction with Endosteal Implants: An Ideal Treatment Method for Mandibular Ameloblastoma. *Free Fibula And Endosseous Implants.* 2005; 113(1): 80-7.
9. Konouchi H, Asaumi JI , Yanagi Y, Hisatomi M, Kawai N, Matsuzaki H, Kishi K. Usefulness of contrast enhanced-MRI in the diagnosis of unicystic ameloblastoma. *Oral Oncology.* 2006; 42(5): 481–6.
10. Yunus M, Baig N, Haque A, Aslam A, Atique S, Bostan S, Syed AM. Unicystic Ameloblastoma: A Distinct Clinicopathologic Entity. *Pakistan Oral & Dental Journal.* 2009; 29(1): 9-12.

# Subject Index

# Author Index