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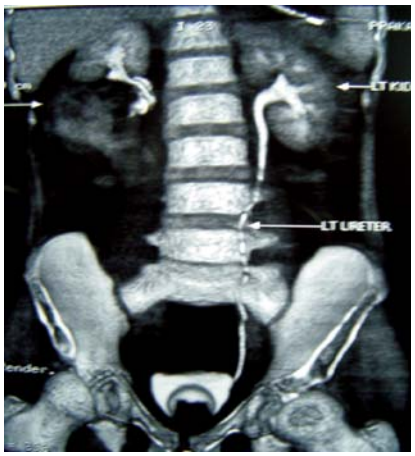

SRI RAMACHANDRA UNIVERSITY
(Established under Section 3 of the UGC Act, 1956)
PORUR, CHENNAI - 600 116.

This Journal is dedicated to the Founder Chancellor

Shri N.P.V. Ramasamy Udayar

SRI RAMACHANDRA JOURNAL OF MEDICINE

NOVEMBER 2007

CONTENT

GUIDELINES FOR AUTHORS

Scope of the Journal

The Sri Ramachandra Journal of Medicine - a scientific journal, entertains communications on all aspects of original biomedical research contributing to the advancement of knowledge in medical sciences. The scope of the journal allows publication of papers on medical education at undergraduate and postgraduate levels in either medical or paramedical courses; innovations in techniques; epidemiologic investigations and case reports. Readers are encouraged to write comments on papers published in the journal in the form of correspondence. Brief communication containing significant findings will be given priority. Review articles are also invited on topics of current interest. The journal is issued thrice in every calender year. All papers are subjected to peer review by the Editorial Board and also experts in the field before acceptance for publication. All papers are accepted subjected to editorial changes.

Articles submitted to the journal should abide by the following manuscript submission guidelines.

Submission of Manuscript :

Each manuscript submission should include the following documents.

Part I - Title Page
Part II - Manuscript file
Part III - Acknowledgment, declaration by authors, patient consent and supplemental file.

All contents related to manuscript submission should be in English on a White paper of A4 size (210 x 297) with margins of 25mm (1 inch) wide on all the four sides. Print should be on oneside only with double spacing throughout. Pages should be numbered consecutively, beginning with title page. Lettering should in Times New Roman with a font size of 12. Three copies should be submitted to the editor. A copy of the title page and manuscript file must be emailed (as an attachment) with a covering letter address to the editor.

PART I - Title Page must include : a) Title of the article b) Name of each contributor with the highest degree and institutional affiliation. c) Name, cellphone, e-mail of the corresponding author.

PART II - Manuscript file : Should include the text of the article followed by tables and figures. The table/figure number (eg: Table 1, Figure 1) should be appropriately mentioned in the text. The references should be numbered as they appear in the article and must be written in Vancouver style. The references should be kept after the tables/figures.

PART III - Acknowledgment: May include the names with details of affiliation, if any. They will appear in the article, but before the references.

Declaration by the authors: All the authors should submit a declaration regarding originality of the work, submission to other journals, whether the articles were already published and financial conflicts of interest which might influence the manuscript.

Supplemental file: These articles / texts which might help the review process, they should be relevant to the article submitted.

Nature of Articles - 1. Original articles: Articles of original research are welcome in this category. Articles should not exceed 4000 words. It must include an abstract of 250 words which should be structured as a) Aim of the study, b) Methodology, c) Results and d) Discussion. Minimum of three MesH words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

2. Review articles: These articles addressing an issue / theme of current interest. They should not exceed 4000 words. Should include an unstructured abstract of 400 words with three MesH words. Article may include upto 100 references.

3. Case reports: Case reports reflecting a major clinical problem are welcome for this section. Word count should be restricted to 300 with references upto 5. May include 2 photographs and 1 table. Photographs having visible identification of patients must have written consent from the patient/close relatives. Case reports having more than 1 case will be given preference. Photographs should be at least 5 by 7 inches. Photographs may be submitted in a digital file, preferable in a JPEG (or) Tiff format. Photographs should be labeled appropriately.

4. Letter to the Editor: Correspondence to the editor regarding an article published in the journal are invited in this category. The content should be restricted to 300 words with references upto five.

Address for correspondence: Office of the Editor, Sri Ramachandra Journal of Medicine,
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It gives a great pleasure for the editorial board of Sri Ramachandra Journal of Medicine to bring out the next issue of the journal in this calender year. It was possible because of the support & encouragement from all quarters of this university. In this issue we have brought out three original articles and two special articles written by faculty of our University, one of which was adjudged the best paper in a National Conference. It highlights the enormous potential & talents available in this University. We hope that these articles will become beacons for others to fellow. We hope to get our journal indexed shortly, which will give further impetus to authors to submit high quality manuscripts. The editorial is getting excellent comments from other Institutions about the journal.

We welcome any suggestions (or) constructive criticism from any quarter which will enrich the contents of this prestigious journal. Please be free to transfer your suggestions to the email id of Editorial Board, srjm@srmc.edu

I thank the Chancellor, Management, Dean of Faculties & others for their untiring support & encouragement for this academic venture.

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Inauguration of WHO collaborating centre
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&
AERB accreditation for Biodosimetry Laboratory to Department of Human Genetics,
Sri Ramachandra University

THE EPITHELIAL RETICULAR CELL OF THE THYMUS

Saratha Kathiresan

These cells are also called the EPITHELIOCYTES. Some are named as THYMIC NURSE cells, because they play a role in the maturation of the lymphocytes, making them responsible for cell mediated immunity.

Embryologically, their origin is from the endodermal cells of the third pharyngeal pouch. Their epithelial origin is confirmed by the presence of basement membrane and the desmosomes with tonofibrils. The cells later become flat and spindle shaped.

These cells are seen in the following area: (1) outside the capsule (2) just deep to the capsule in the subcapsular zone (3) within the trabeculae forming septae (4) as a sheath, covering the blood vessels within the gland, and probably play a role in the formation of the partial blood-thymic barrier (5) in the cortico medullary zone, (6) lastly, they form a lattice like structure both in the cortex and medulla (Figure 1).

Fig. 1: Thymus gland showing lobular architecture. Hasall's corpuscles as seen in medulla (TPA stain X 20)

The lymphocytes lie in this network of epithelial reticular cells. Since there is crowding of the lymphocytes in the cortex, the reticular cells are not clearly visible in the cortex. But in the medulla, there are only a few lymphocytes and hence epithelial cells are clearly seen and they form the Hassall's corpuscles.

As it is a part of the blood thymic barrier, these cells prevent the antigens present in the blood from reaching the T lymphocytes. The epitheliocytes also promote proliferation of T cells and T cell differentiation.

According to recent studies, several differences are noted in their structure(1) . Hence they are classified into five types.

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Type I - Line the inner aspect of the capsule, the septa and the blood vessels. These form the blood – thymic barrier.

Type - II and Type - III are seen outside the cortex and inner parts of the cortex. These are the network on which the lymphocytes are placed and hence are not seen clearly.

Type IV – are cells in the deeper part of the cortex – in the Cortico-medullary junction and the medulla (Figure 2).

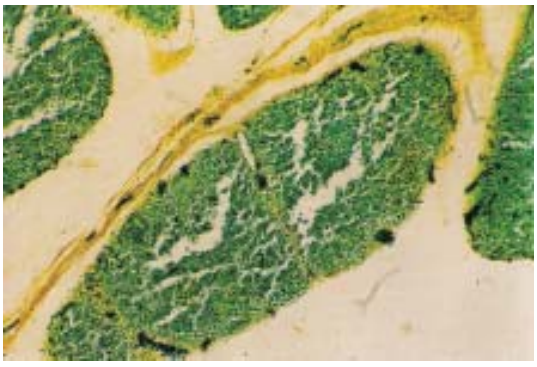

Fig. 2: Epithelioreticular cells highlighted by amidoblock dye in the subcapsular region, in the septae and corticomedullayr junction (TPA stain X 20)

Type V – are cells in and around the Hassell's corpuscles. They probably destroy T cells reacting with self antigens by phagocytosis. That is why some authors(2) call the Hassall's corpuscles as the 'graveyard' for the incompetent lymphocytes. These cells predominate in the medulla during early gestational period in human foetal thymus. Hassell's corpuscles first appears during the 17th week of gestation and increases in size subsequently.

The thymic micro-environment is mainly created by these epitheliocytes. Ultrastructural evidence suggest that these cells provide the three dimensional framework for the thymic cells. By their contact with the lymphocytes and probable secretion of certain hormones they induce INTRATHYMIC LYMPHOCYTIC DIFFERENTIATION and influence the events associated with the maturation of T cells.

The identity of these cells has been established by their consistent ultrastructural features such as 1) presence of tonofilaments and desmosomes, (2) basal lamina associated with cell membrane. These cells have long cytoplasmic process which connect with adjacent cells. These connections are shown in electron and light microscopic studies using special staining method called TPA (Tannic acid - phosphomolybdic acid - Amido black) technique developed by Clermont & Leblond to show epithelial cells in tissues other than thymus. These cells form a three dimensional frame work of thymic parenchyma. These reticular cells are distinguished from the reticular cells

of mesodermal origin in the spleen and lymph nodes. Their epithelial origin is proved by presence of keratin in Hassall's corpuscle and by the presence of tonofilaments and desmosomes. Hence these cells are also called "EPITHELIOCYTES"

Several workers have described ultrastructural differences between the cortical and medullary epithelial reticular cells. It is not clear whether the cells of the same origin are seen differently according to their functions in varied situations (1, 3).

Under the Electron Microscope, two main types are described – PALE EPITHELIAL RETICULAR CELL (PER) and DARK EPITHELIAL RETICULAR CELL (DER) according to their electron density, created by the increased density of cytoplasmic ground substance(3,4). The pale epithelial cell shows the heterochromatin along the inner nuclear membrane as a thin rim rarely clumped (Figure 3).

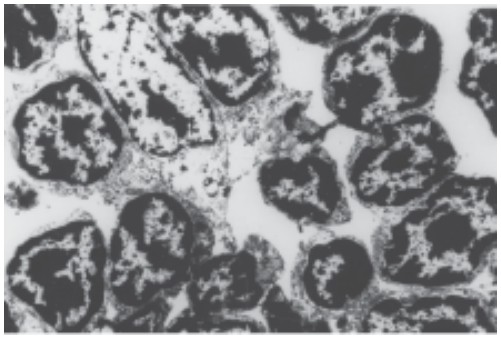

Fig. 3: Ultrastructural appearance of a pale epithelioreticular cell seen on electron microscopy.

Nuclei are distinct and there is space distribution of ribosomes. Some pale cells form the Hassell's corpuscles. The dark cells are associated with collagen fibres (figure 4). The long cytoplasmic processes extend from the cell body to encompass the bundle of collagen fibres. The collagen fibers are definitely extracellular in position.

Both pale and dark epithelial reticular cells have in common rough endoplasmic reticulum, moderately developed golgi bodies, membrane bound vesicles, electron dense granules and lysosome like bodies.

A few of these cells show vacuoles and small cystic inclusion in their cytoplasm. These vacuoles may contain

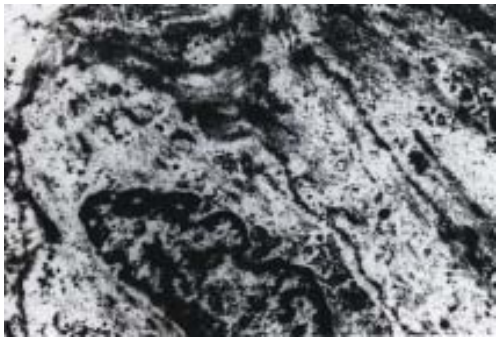

Fig. 4 : A dark epithelioreticular cell with desmosomal junction seen on electron microscopy.

degenerating material which may be lymphocytes reacting with self antigens and hence getting destroyed by some factors released from these epithelial reticular cells. Hence, the epithelial reticular cells are called THYMIC NURSE CELLS(5).

These are epithelial cells, which show evidence of hormonal secretion(3). At least eight hormones have been isolated since 1966, but the details of the synthesis, production and its transportation are not clear.

Still, thymus is considered as a PRIMARY LYMPHOID ORGAN, along with the bone marrow.

The proliferation of T lymphocytes and their conversion into cells capable of reacting with antigens are events dependant on the hormones produced by these epithelio-reticular cells. The hormone affects LYMPHPOIESIS in the peripheral lymphoid organs. If thymus is removed during neonatal period, the peripheral lymphoid organs do not develop in the normal way.

Recent studies have identified some of these hormones originating from epitheliocytes:

a. THYMULIN - enhances function of T cells
b. THYMOPOIETIN stimulates production of cytotoxic T cells
c. THYMOSIN alpha I – stimulates lymphocyte and also antibody production.
d. THYMOSIN Beta – 4
e. Thymic humoral factor controls the multiplication of helper and suppressor T cells.

Apart from actions on the lymphocytes, hormones or any other substance formed in the thymus probably also influence the adenohypophysis gland and ovaries. In turn, the activities of thymus is influenced by the hormones from these organs.

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TRANSLATIONAL MEDICAL EDUCATION AND RESEARCH : SRI RAMACHANDRA UNIVERSITY INITIATIVES.

Sadras Panchatcharam Thyagarajan

ABSTRACT:

Translational medicine *is a branch of medical research that attempts to more directly connect basic research to patient care. Translational medicine is growing in importance in the healthcare industry. In the case of drug discovery and development, translational medicine typically refers to the "translation" of basic research into real therapies for real patients. The emphasis is on the linkage between the laboratory and the patient's bedside, without a real disconnect. This is often called the "bench to bedside" definition. Translational medicine can also have a much broader definition, referring to the development and application of new technologies including therapeutics in a patient driven environment - where the emphasis is on early patient testing , evaluation and management .*

Translational Medicine encompasses (a)Basic science studies which define the biological effects of therapeutics in humans (b)Investigations in humans which define the biology of disease and provide the scientific foundation for development of new or improved therapies for human disease (c)Non-human or non-clinical studies conducted with the intent to advance therapies to the clinic or to develop principles for application of therapeutics to human disease (d)Any clinical trial of a therapy that was initiated based on (a) to (c) with any endpoint including toxicity and/or efficacy.(e) Appropriate product development for clinical use in various stages of investigational clinical trial before initiating Phase III trials as required by the regulators and (f) The adoption of best practices that lead to greater understanding of the link between medical learning (how one acquires and applies relevant attitudes, knowledge and skills in medicine) and patient outcomes via real-world clinical decision-making and other physician actions.

The requirements of Translational education & research are (i) Academic setup with components of Clinical and translational Science (ii) Institutional culture and commitment for Clinical and Translational science (iii) Education, training and Career development opportunities (iv) Clinical research Informatics (v) Intra and Inter Institutional Collaboration and (vi) appropriate manpower with the capacity to envisage the whole process from "bench to bedside" to be generated by due educational program.

Sri Ramachandra University has conceived a M.Sc in Translational Medicine and a Post graduate Fellowship in Translational Health sciences for consideration of support by the Department of Biotechnology, Government of India. To promote Translational research, a Central Research Facility has been created for intra institutional collaboration and multi-investigator cum interdepartmental research projects. Institute- Industry collaboration has also been strengthened for drug discovery program and/or clinical trials.

Key words : *Research, Education*

INTRODUCTION:

Until recently, basic science advances have made oversimplified assumptions that have not matched the true etiological complexity of most common diseases; while clinical science has suffered from poor research practices, overt biases and conflict of interest. The advent of molecular medicine and the recasting of clinical science along the principles of evidence-based medicine provide a better environment where translational research may now materialize its goals.

The status of translational research has drawn increasing attention recently in top biomedical journals [1-5] and in the policy making of the NIH, as reflected also in the NIH Roadmap [6]. Translational medicine encompasses all the disciplines that intervene in moving scientific progress from the bench to the bedside and in conveying stimulating information from the bedside back to the bench [5]. While basic sciences are conceived as having made amazing leaps forward, this progress has not resulted in many major cures [7]. At the other end, clinicians are considered too unfamiliar with the capacities of modern science to bring fruitful questions to the attention of basic scientists [5]. Nevertheless, recent evolutions in basic and clinical science have created a new window of opportunity for the growth of translational medicine.

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What is Translational Medicine?

Translational medicine is a rapidly emerging discipline focused on bridging technologies and discoveries in the laboratory with clinical research and practice. The same principles apply in academic, biotechnology and pharmaceutical environments. Numerous definitions of translational medicine exist. The National Institute of Health (NIH) at USA has stated that " To improve human health, scientific discoveries must be translated into practical applications. Such discoveries typically begin 'at the bench' with basic research- in which scientists study disease at a molecular or cellular level- then progress to the clinical

level, or the patient's 'bedside', thus terming translational medicine as 'bench to bedside research"[6]. The Medical Research Council(MRC), UK has summed up the concept succinctly as, "The process of the bidirectional transfer of knowledge between basic work (in the laboratory or elsewhere) with that of the person,in health or disease"[36].

Historical Background:

In embarking on its Roadmap for Medical Research, the National Institutes of Health (NIH) has pledged to accelerate the application of "scientific advances to real-world [medical] practice" through translational research.[6,8] As a cornerstone of its Roadmap Initiative, the NIH introduced Clinical and Translational Science Awards (CTSAs) in the year 2002 designed to overcome the "bench-to-bedside" barriers of translational research by promoting interdisciplinary and cross-institutional collaboration, with the goal of "enhancing the adoption of best practices in the community."[9] Historically, "bench to bedside" translational research has been equated with the conversion of scientific discoveries into promising diagnostic and therapeutic modalities.

Over the past decade, a multitude of editorial commentaries, journal articles, and blue ribbon reports have called for a concerted effort to advance translational medical educational research[10-20]. Yet progress has been slow. Major obstacles to progress include lack of funding[21], scarcity of experienced medical education researchers[22] and the absence of a "national research infrastructure to build and sustain a medical education research enterprise."[22] Other obstacles are related to the inherent structural and methodological difficulties of designing and conducting educational research, particularly controlled interventional studies, on medical students, residents and practicing physicians. Recruitment of subjects, long-term follow-up, the lag between the educational intervention and end-point/measurement timing, cross-contamination between control and experimental groups,, and the inability to control for an ever-changing learning environment make it challenging to produce high quality studies that demonstrate a clear link between the educational intervention and outcomes that matter, including health outcomes of patients[17,23]. Finally, there is currently no widely accepted "theory of medical education and its influence on outcomes" to use as a foundation for designing and implementing research studies[19]. Limited by these obstacles, translational medical education research has generally lacked the methodological depth, breadth and rigor necessary to inform with conviction the development and adoption of best educational technologies and practices in physician lifelong learning and professional development. Country specific initiatives are now being taken to operationalise translational education and medical research in view of basic sciences maturing in complexity and Clinical science maturing in its evidence base.

Basic sciences: Maturing in complexity

Basic science advances in the last few years have indicated that most common diseases entail extremely complex patterns of pathogenesis, involving the regulation of dozens and hundreds of genes and their protein products. In the light of advances in genomics, proteomics, and bioinformatics, basic science of the 1980s and 1990s where single or few pathways were investigated would currently seem naïve at best. We don't know yet whether the molecular medicine of the early 21st century may seem equally oversimplified even within a decade or two. The complex new patterns of disease etiology and regulation still pose considerable problems of validation and testing of generalizability [24,25]. The role of environmental parameters and their interactions at the molecular level with intrinsic factors is yet largely unknown. These interactions may prove to be even more formidable. The intractability of several chronic diseases, the unpredictable emergence of new diseases such as AIDS, SARS, and mad cow disease and the equally unpredictable re-emergence of old diseases such as tuberculosis makes such a target an utopia. Nevertheless, provided that we approximate the depth of the complexity of the molecular issues involved, translational medicine may have indeed a more solid starting point now in its efforts.

Clinical sciences: Maturing in evidence base

Clinical research has been even more revolutionized in the last decade, in particular with the advent of evidence-based medicine. It is now acknowledged that a large corpus of clinical information that has haunted the top medical textbooks and experts' opinions was wrong, outdated, and/or dangerous for human health [26]. Evidence-based medicine has placed emphasis on robust scientific principles, the dissection of strengths and limitations of various clinical research designs, and the identification of bias in medical research [27]. We are now aware that serious errors may underpin much of clinical research, and even randomized trials may succumb to biases [28]. Moreover, clinical scientists have now tried to systematize their knowledge base. Efforts such as the Cochrane Collaboration [29,30], an international coalition that aims to generate systematic reviews on all aspects of health care, has been hailed as equivalent in scope to the Human Genome Project [31]. Such systematic efforts can tell us reliably for each disease and condition whether we have enough evidence for its effective management, and whether this evidence is biased or not. Furthermore, rigorous approaches have been recently developed to quantify the burden of disease for various conditions [32]. It is thus becoming evident that for several trivial issues, there is often a waste variety of expensive treatments, while for many serious conditions there is no effective intervention at all and little research is targeted at them [33,34].

Priorities and cross-links between diseases

With genuine progress in the basic and clinical sciences, translational efforts have a better chance of being successful. Even under these circumstances though, the question remains on who will do this research and where it will be done. A very large portion of all current research is done in the USA. Scientific papers with US authorship attract approximately half of all citations in the Web of Science (over 30 million citations in the last decade alone, followed by England with less than 6 million) [35]. A handful of developed countries make up another 40–45% of the total citations. NIH is by far the greatest governmental funding body for biomedical research globally and hence has laid out a road map as mentioned earlier for translational education and research.

NIH Roadmap for Re-engineering the Clinical Research enterprise-Translational research :

Starting in 2002, the NIH in the USA began a process of charting a "roadmap" for medical research in the 21st century [6,8], identifying gaps and opportunities in biomedical research that crossed the boundaries of then extant research institutions. A key initiative that came out of this review is a move to strengthen *Translational Research*, defined as the movement of discoveries in basic research (the *Bench*) to application at the clinical level (*the Bedside)*

This NIH roadmap framed guidelines for the above mandate under the following five areas:

i) **Components of a Clinical and Translational Sciences, Academic Home ,** describing the methodology to identify the desirable components which would work.Together and how to prioritize these components, and govern them;

ii) **Institutional Culture and Commitment for Clinical and Translational Science:** To get the institutions to change and to sustain the change with a mission mode approach including the space allocation, finances, service benefits of the personnel and the educational pathways;

iii) **Education, training and career development:To evolve** a more efficient and effective education, training and career development pathway for the clinical and translational sciences and to design the pathway not only for principal investigators, but also for all members of multidisciplinary teams;

iv) **Clinical research Informatics:**To identify areas where informatics would be most helpful, and to consider what type of institutional leadership would be needed to take full advantage of informatics in the clinical and translational sciences.

v) **Intra and Inter-Institutional Collaboration:**To consider how the project sites might work together to develop the tools and training programs needed for clinical and translational sciences and then to share and distribute their findings to a wider community. Also to design modalities to enhance discipline of Clinical and translational sciences by collaboration at institutional, regional and national levels.

MRC Guidelines for Translational Medical Research:

More recently, the Medical Research Council (MRC), UK in the year 2007 came out with Guidelines for accelerating the translation of Medical Research [36]. Approximately 50 members of MRC's community including industry and representatives from the Health Departments came together in February 2007 to discuss these issues. Delegates discussed the translational research process, MRC's role and what more MRC could do. The main issues to emerge were the need for:

i) Cultural change within the research community and recognition that translating research findings and communicating findings to research users was part of a researcher's role. Structures and funding mechanisms needed to be put in place to encourage and reward researchers to move into and become active in these areas.

ii) Guidance on the process of translation and an accompanying vocabulary to describe more precisely translational activities. Process maps could be used to educate and assist researchers in identifying the next steps in progressing research findings and also provide metrics against which MRC could evaluate its process.

iii) A separate funding stream to build capacity and provide project support for early translational research (e.g. experimental medicine; biomarkers; proof-of-concept studies). Where possible, these new initiatives should be undertaken through partnerships with industry.

iv) MRC's should continue to build on its high quality basic research portfolio but a much greater focus must be placed on the management of the findings from MRC funded research. Resources needed to be provided for the proactive brokering of partnerships between researchers and research users and for catalysing bottlenecks in the process in order to facilitate translation.

Translational research and the information ecosystem

Much of the ability of biomedical researchers and health care practitioners to work together – exchanging ideas, information, and knowledge across organizational, governance, socio-cultural, political, and national boundaries – is mediated by the Internet and its ever-increasing digital resources. These resources include scientific literature, experimental data, summaries of knowledge of gene products, diseases, and compounds, and informal scientific discourse and commentary in a variety of forums. Together this information comprises the scientific

"information ecosystem" [6]. Despite the revolution of the Web, the structure of this information, as evidenced by a large number of heterogeneous data formats, continues to reflect a high degree of idiosyncratic domain specialization, lack of schematization, and schema mismatch.

The lack of uniformly structured data affects many areas of biomedical research, including drug discovery, systems biology, and individualized medicine, all of which rely heavily on integrating and interpreting data sets produced by different experimental methods at different levels of granularity. Complicating matters is that advances in instrumentation and data acquisition technologies, such as high-throughput genotyping, DNA microarrays, protein arrays, mass spectrometry, and high-volume anonymized clinical research and patient data are resulting in an exponential growth of healthcare as well as life science data. This data has been provided in numerous disconnected databases – sometimes referred to as data silos. It has become increasingly difficult to even discover these databases, let alone characterize them.Together, these aspects of the current information ecosystem work against the interdisciplinary knowledge transfer needed to improve the bench-to-bedside process.

Curing and preventing disease requires a synthesis of understanding across disciplines:

In applying research to cure and prevent diseases, an integrated understanding across subspecialties becomes essential. Consider the study of neurodegenerative diseases such as Parkinson's Disease (PD), Alzheimer's Disease (AD), Huntington's Disease (HD), Amyotrophic Lateral Sclerosis (ALS), and others. Research on these diseases spans the disciplines of psychiatry, neurology, microscopic anatomy, neuronal physiology, biochemistry, genetics, molecular biology, and bioinformatics. Hence both Translational Education and Medical Research requires interdisciplinary information system [37].

Major Components and role of Translational Medicine:

Translational Medicine encompasses:

a) Basic science studies which define the biological effects of therapeutics in humans

b) Investigations in humans which define the biology of disease and provide the scientific foundation for development of new or improved therapies for human disease

c) Non-human or non-clinical studies conducted with the intent to advance therapies to the clinic or to develop principles for application of therapeutics to human disease

d) Any clinical trial of a therapy that was initiated based on #1–3 with any endpoint including toxicity and/or efficacy.

e) Translational research may play a role as appropriate product development for clinical use in various stages of investigational clinical trial. For example, identity, purity and potency of a drug product must be studied during the early stages of the clinical trial. However, these tests must be in place before implementing phase 3 trials as required by the regulators.

f) The critical role that translational medical education and research can play in ensuring the adoption of best practices that lead to greater understanding of the link between medical learning (how one acquires and applies relevant attitudes, knowledge and skills in medicine) and patient outcomes via real-world clinical decision-making and other physician actions is necessary if we are to fully realize the NIH's "bench to beside" mission

Industries and Translational Medicine:

Traditionally, basic research has been separated from the clinical practice of medicine by a series of hurdles or fences. New drugs were developed independently of the clinic, and often "thrown over the fence" for safety testing and clinical trials. The move toward translational medicine is focused on removing these fences, and stimulating "bench to bedside" research. A large number of developments show the growing influence of translational medicine across academia and industry. A couple of these over the past year include a 50 million pound sterling investment by Wyeth and Scottish Enterprise in the Translational Medicine research. Many pharmaceutical companies are building translational medicine groups to facilitate the interaction between basic research and clinical medicine, particularly in clinical trials.

International Funding support for Translational Medicine Research:

The National Institutes of Health has awarded UW-Madison's new Institute for Clinical and Translational Research (ICTR), one of the largest grants in the history of the School of Medicine and Public Health and has identified UW-Madison as a key player in an ambitious NIH plan designed to transform the country's clinical and translational research enterprise. With $ 41 million over five years, ICTR will aggressively address clinical and translational research in Wisconsin by first building a network of key partners from across campus and around the state including even international partners.

In the UK, Collaboration across a consortium of research universities and launch of six major MRC translational medicine centres has evidenced that government also is tuning into the potential for translational medicine to overhaul medical research.

Government of India Initiative for Translational Education and research:

Department of Biotechnology, Government of India has announced awards for institutions desiring to create

centres or new expended or remodeled departments for translational research. These centres will have to focus on translational biology and clinical research in an interlinked way. The purpose is to enable and accelerate the translation of basic scientific and engineering knowledge from lab to patients. To facilitate more translational research, the centre will facilitate PhD's to engage in strategic basic sciences based on patients, and animal models of disease.

The support where appropriate can cover physical infrastructure, renovation, PhD fellowships, post doctoral fellowships, strengthening platform technologies and short term training for skill acquisition and R&D costs on five years basis extendable thereafter based on performance and institutional commitment. The participating scientists could belong to a nodal department and to other collaborating departments. DBT will consider support for 5-10 years to any new faculty recruited to strengthen research and working at least 60% of their time on the centre's programmes. A clear defined R&D strategy is important for a successful award; disconnected, multiple proposals even of good quality will fall short of our prescribed benchmark.

The Indian Council of Medical Research, which has now become part of the Department of Health Research, Govt. of India has announced its policy decision to launch Translational Medicine program. It has jointly announced with DBT to set up an Institute of Translational Research recently.

Sri Ramachandra University Initiatives in Translational Education and Research:

(a) Translational education:

In 2006, Sri Ramachandra University responded to the call of the Department of Biotechnology, Government of India by developing the curricula, syllabi an the academic structure for conducting(i) M.Sc (Translational Science) and (ii) Post Graduate Fellowship in Translational Health Sciences. It was also planned to bring in place (a) Division of Translational research, (b) Division of Clinical Trials and (c) Division of Outcomes, Clinical Epidemiology and Health outcomes as umbrella structures for the Basic and Clinical Sciences departments to undertake these programs.

(b)Translational Medical research:

A Central research Facility along with a Central Animal House have been established and being strengthened since May,2007 to facilitate intra-institutional collaboration and multi-investigator cum interdepartmental research projects to realise the requirements of Translational Medical Research. Some of the success stories in implementing Research projects as per the concept of Translational Medicine are (i) The Department of Science & Technology supported Population Based Risk factor analysis & Prevention (PURSE-HIS) study for Cardio Vascular Diseases under Dr.S.Thanikachalam of Cadiac Care Centre in collaboration with the departments of Periodontal diseases, Genetics, Nutrition, Biochemistry and Biotechnolgy (ii) The Department of Biotechnology and Department Atomic Energy supported Cardiomyocyte tissue-engineering and Artificial Heart project under Dr.K.R.Balakrishnan of Cardiothoracic Surgery with Department of Patholgy, SRU and Central Leather Research Institute; (iii)The Department of Biotechnology supported Chondrocyte tissue-engineering project under Dr.S.Arumugham of Department of Sports Medicine & Orthopedics with the Department of Pathology and Central Leather Research Institute and (iv) The Herbal Research Laboratory under Dr.Hanna R.Vasanthi with Herbal/Indigenous drug development/ validation projects supported by Department of Scientific & Industrial Research, AYUSH etc., in collaboration with Departments of Cardiology, College of Pharmacy and Central Leather Research Institute. Many more projects/programmes are in the pipeline to be supported by Indian Council of Medical Research/Department of Biotechnology in collaboration with National Centre for Biological Sciences, Bangalore, National Institute of Immunology, Delhi besides interdepartmental participation.

CONCLUSION:

As basic and clinical sciences mature, translational research has a chance of making an important difference for human health. However, priorities need to be selected with broad horizons in mind. A global perspective including that of the developing world should be assumed not only in priority setting, but also on the conduct of research. Universal guidelines that are consistent with the realities of the 21st century biotechnology industry and academic science should be adopted. The rules should be clear and they should reward creativity, maximize transparency, and exploit local strengths, not to stifle progress with irrelevant administrative burdens. The ability to create a truly international scientific society with high standards, transparent processes and academic independence may create a healthier world for all humans across the globe.

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POPULATION BASED STUDY OF EPISIOTOMY

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ABSTRACT

Objectives: *To estimate episiotomy rate in a rural population and to find out if higher episiotomy rate is associated with place of delivery and category of health care provider.*

Design: *Population based cross sectional study*

Setting: *Rural population near Chennai.*

Sample: *Included 442 mothers who had vaginal delivery between August 2004 and July 2005.*

Methods: *Cluster sampling was used to select the study sample. Information about episiotomy during last child birth and other details were obtained by personal interview and from available medical records.*

Results: *Overall episiotomy rate was 67% (95% CI 62.6 – 71.4). For women whose delivery was conducted by doctors the episiotomy rate was 77.4% and conducted by nurses it was 53.1%. Episiotomy rate was very high (91.8%) when delivery was conducted in private medical college hospitals and the rates were lower when conducted in secondary and primary level institutions. Adjusted odds ratio for episiotomy was 38 when doctors conducted delivery compared to trained birth attendants and 8.9 when delivery was conducted at private medical college hospitals compared to primary health centres.*

Conclusion *Episiotomy rate in the study population is high. Probably similar high rates are found in other parts of India. The probability of episiotomy is very high when doctors conducted the delivery and when place of delivery is private medical college hospital. Evidence based restrictive practice of episiotomy to less than 30% should be adopted by all, particularly in tertiary care teaching hospitals which should serve as role models.*

Key words: *Episiotomy, Cross sectional studies, Rural population, Epidemiology*

INTRODUCTION

Episiotomy was introduced as an obstetric procedure more than 200 years ago. However it became a common practice only from the beginning of 20th century. It was then thought that all primi gravida should receive an episiotomy to protect foetal head and the pelvic floor. Popularity of episiotomy is mainly because it seems to substitute a straight, neat surgical incision for the ragged laceration that otherwise might result(1). Research carried out over the last 20 years has highlighted the problems associated with the procedure, which include increased blood loss, perineal pain and dyspareunia. A number of observational studies and randomized controlled trials show that routine episiotomy is associated with an increased incidence of anal sphincter and rectal tears(2,3,4). The long held belief that postoperative pain is less and healing improved with episiotomy compared with perineal tear appears not to be true(5).

It is now very important to improve new birthing techniques that maintain the integrity of the perineum which do not involve surgical procedures(6). A randomized controlled trial done recently, concluded that avoiding episiotomy at tears presumed to be imminent increases the rate of intact perineum, reduces postpartum perineal pain and does not have any adverse effects on maternal or fetal morbidity(7). Episiotomy at a perineal tear presumed to be imminent does not have any advantage with regard to pelvic floor function and should be avoided(8). Very little information is available about episiotomy rates in India. This study was done to estimate episiotomy rate in a rural population and to find out if higher episiotomy rate is associated with place of delivery and category of health care provider.

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METHODS

This cross sectional study was done in a designated rural population near Chennai. This population is served by 10 health sub centres, 1 primary health centre, and few private hospitals. They also have access to taluk hospitals and district hospitals. A few private and Government medical college hospitals are available within about 30 kilometers from the study area.

Initially the plan was to use simple random sampling method for selection of study subjects. However in view of logistic constraints common in population based studies, cluster sampling method was used to select randomly from the whole population, 442 mothers who had vaginal delivery during the last one year (August 2004 to July 2005).

Information about place of delivery, who conducted the delivery, if the delivery was normal or induced / instrumental, whether pre term, term or post term, parity, birth weight, age of the mother at birth of last child and other baseline information were obtained from the selected subjects after getting their informed consent.

Socioeconomic status was assessed by Standard of Living Index (SLI), which includes 11 items on housing details, basic amenities, and ownership of land, livestock and durable goods. The scoring ranges from 0 to 67 classified as low, medium and high(9). Each study participant was asked if she had episiotomy (If the opening of the birth canal was cut at the time of vaginal delivery). At the end of the interview, medical record / discharge summary if available was verified for recorded evidence of episiotomy.

SPSS version 10 was used for data entry and analysis. Episiotomy rates were calculated as percentages with 95% confidence interval overall and for subgroups. X^2 test was used as statistical test of significance for comparison between percentages. Odds ratios and adjusted odds ratios with 95% confidence intervals were found using logistic regression analysis. Approval for this study was obtained from the Medical Ethics Committee of Sri Ramachandra Medical College & Research Institute, Chennai.

RESULTS

The mean age of the 442 women selected for the study was 23.9 years (SD 3.5). Among them 14.7% were 20 years and below, 84.6% were between 21 to 35 years and 0.7% were 36 years and above. For 39.6% of women it was first delivery, for 40.0% second delivery, for 14.5% third delivery and for 5.9% it was more than three deliveries.

Primary health care setting was the place of delivery for 20.5%, secondary and tertiary care settings were the place of delivery for 42.6% and 31.7% of deliveries respectively. In terms of public and private sectors, 62.2% of deliveries were conducted at Government (public sector) and 32.6% at private hospitals (private sector). The remaining 5.2% of deliveries occurred at home.

Out of 442 mothers who had vaginal delivery during the (last) one year, 296 underwent episiotomy with an episiotomy rate of 67% (95% CI 62.6%, 71.4%). The episiotomy rate was highest when delivery was conducted by doctors to the extent of 77.4% followed by 53.1% when conducted by nurses and 5.0% when conducted by trained birth attendant. (Trained birth attendants are women selected from rural communities and given training for conducting labour). The differences in the episiotomy rates are found to be statistically significant.

The episiotomy rate was very high when delivery was conducted in private medical college hospitals to the extent of 91.8% followed by government medical college hospitals, private hospitals, district hospitals, taluk hospitals, primary health centres and health sub centres. When classified as primary, secondary and tertiary health care settings the episiotomy rate was highest in tertiary care setting. In terms of public and private sectors the episiotomy rate was much higher for private sector. The differences in the episiotomy rates are found to be statistically significant (Table 1).

Table - 1 Episiotomy rates and place of delivery.

Place of delivery	Episiotomy rate	95% CI	p value
Type of health institutions			
Private medical college hospitals	91.8	84.1 - 95.5	
Government medical college hospitals	74.7	65.8 - 83.6	
Private hospitals	74.7	66.0 - 83.4	
District Hospitals	69.5	57.8 - 81.2	< 0.01
Taluk hospitals	67.6	51.9 - 83.3	
Primary health centres	55.1	44.1 - 66.1	
Health sub centres	23.1	0.2 - 46.0	
Levels of Health Care			
Tertiary level institutions	80.7	74.2 - 87.2	< 0.01
Secondary level Institutions	71.8	65.4 - 78.2	
Primary level institutions	50.5	40.2 - 60.8	
Private / Public institutions			
Private	80.6	74.1 - 87.1	< 0.01
Public	64.7	59.1 - 70.3	

The episiotomy rate was 55.6% in pre term deliveries and 67.1% in term deliveries. Episiotomy was done in 60.6% of normal delivery and it was much higher (83.6%) in induced / instrumental delivery. Episiotomy rate was 61.7% when the birth weight was below 2500 grams and it was much higher for birth weight between 2500 to 3500 grams and for more than 3500 grams (Table 2).

Table – 2 Episiotomy rates, duration of pregnancy, type of delivery and birth weight

	Episiotomy rate	95% CI	p value
Duration of Pregnancy			
Preterm	55.6	23.1 - 88.1	0.5
Term	67.1	62.7 - 71.5	
Post term	100	—-	
Type of Delivery			
Normal	60.6	55.2 - 66.0	< 0.01
Induced / instrumental	83.6	77.0 - 90.2	
***Birth weight (grams)**			
< 2500	61.7	51.1 - 72.3	0.3
2500 – 3500	70.3	65.2 - 75.4	
> 3500	76.7	61.6 - 91.8	

*(Information available only for 414)

In primipara the episiotomy rate was highest to the extent of 83.4%. For second, third and more than third order of deliveries the episiotomy rates were 66.7%, 37.5% and 30.8% respectively and the differences are statistically significant

(p < 0.01). Episiotomy rate was highest among mothers who were 20 years of age and below and it was highest for mothers with high standard of living index (Table 3).

Table 3 Episiotomy rates, parity, age of mother at last birth and standard of living index

	Episiotomy rates%	95% CI	p value
Parity			
Primi	83.4	77.9 - 88.9	< 0.01
Second	66.7	59.8 - 73.6	
Third	37.5	25.6 - 49.4	
More than three	30.8	13.1 - 48.5	
Age of mother at (last) child birth			
20 years & below	76.9	66.7 - 87.1	0.18
21 – 35 years	65.2	60.4 - 70.0	
36 years & above	66.7	13.4-100.0	
Standard of living index			
High	76.3	69.9 - 82.7	< 0.01
Middle	69.3	62.0 - 76.6	
Low	50.4	41.4 - 59.4	

Even after controlling for type of delivery, duration of pregnancy, birth weight, parity, age of the mother at birth of last child and standard of living index, the probability for episiotomy was higher to the extent of 12.6 and 38.0 times when nurses conducted the delivery and doctors conducted the delivery respectively compared to when trained birth attendants conducted the delivery. Similarly the probability for episiotomy was higher to the extent of 1.7, 2.0, 1.8, 2.4 and 8.9 times when delivery was conducted at taulk hospitals, district hospitals, private hospitals, government medical college hospitals and private medical college hospitals respectively compared to when the delivery was conducted at primary health centres (Table.4).

The probability for episiotomy was also higher when delivery was conducted in tertiary level institutions or in secondary level institutions compared to primary level institutions. Probability for episiotomy was higher in private institutions compared to public institutions, however the odds ratio is not statistically significant (Table.4).

As mentioned earlier information about episiotomy was obtained directly from the participants since in rural populations most individuals do not preserve the medical records given to them at the time of discharge from the

Table- 4 Probability of episiotomy based on category of health care providers conducting delivery and place of delivery.

	Un adjusted Odds ratio	95% CI	p value	*Adjusted Odds ratio	95% CI	p value
Category of health care providers						
Trained birth attendants	1.0			1.0		
Nurses	21.5	2.8 – 164.8	0.03	12.6	1.4 – 112.1	0.02
Doctors	65.0	8.5 – 493.7	< 0.01	38.0	4.3 – 336.7	< 0.01
Place of delivery						
Type of health care institution						
Primary Health centres	1.0			1.0		
Taluk Hospitals	1.7	0.7 – 4.0	0.21	1.7	0.7 – 4.6	0.29
District Hospitals	1.9	0.9 – 3.8	0.09	2.0	0.9 – 4.6	0.11
Private Hospitals	2.4	1.3 – 4.6	0.07	1.8	0.8 – 3.9	0.16
Govt. Medical Colleges	2.4	1.3 – 4.6	0.08	2.4	1.1 – 5.1	0.03
Private Medical colleges	9.1	3.0 – 27.9	< 0.01	8.9	2.6 – 30.7	< 0.01
Levels of Health Care						
Primary Level Institutions	1.0			1.0		
Secondary Level Institutions	2.5	1.5 – 4.2	< 0.01	2.2	1.2 – 4.2	0.01
Tertiary Level Institutions	4.1	2.3 – 7.4	< 0.01	4.1	2.0 – 8.2	< 0.01
Private / Public Institutions						
Public	1.0			1.0		
Private	2.3	1.4 - 3.7	< 0.01	1.8	1.0 – 3.1	0.05

*Adjusted for type of delivery (normal or induced/ instrumental), term (pre term, term or post term), birth weight (low, normal or high), parity (primi, second para, third para and more than three para), age of the mother at birth of last child (20 years & below, 21 to 35 years and 36 years & above) and standard of living index (high, medium and low)

hospital. As expected out of 442 mothers who had vaginal delivery during the (last) one year, only 173 (39.1%) had medical records of delivery available with them. Among those who had medical records available, there was recorded evidence of episiotomy for 92 mothers with an episiotomy rate as high as 53.2%. The recorded evidence of episiotomy tallied totally with the participants' own version of having undergone episiotomy.

Even when the 92 mothers with recorded evidence of episiotomy alone were analyzed, the same trend is seen with highest episiotomy rate of 61.8% when doctors conducted the delivery and 96.2% when the delivery was conducted in private medical college hospitals. Similarly the same trend is seen with 1.2 times higher probability for episiotomy when doctors conducted the delivery compared to birth attendants and 197 times higher risk for episiotomy when the delivery was conducted in Private medical college hospitals compared to primary health centres.

Post natal complications were more common among women who had episiotomy (14.5%) compared to those who did not have episiotomy (4.8%) and the difference is statistically significant ($p < 0.05$). Most common complications were continued perineal pain and wound infection.

Out of the 442 women, 363 had episiotomy at least in one of their deliveries with an overall episiotomy rate of 82.1% (95% CI 78.5 – 85.7). Among women who had 2 deliveries, 60.8% had episiotomy both times. Among women who had 3 deliveries, 26.6% had episiotomy all three times.

DISCUSSION

The study population though located in a designated rural area, being near Chennai city, the population has access to primary, secondary and tertiary levels of health care and also availed both private and public sector facilities. This feature made it possible to estimate episiotomy rates in different institutions and categories of health care providers.

An ideal rate of episiotomy, if there is one, has yet to be defined that balances optimal maternal and fetal outcomes(10). Consensus is still being arrived at on what should be the acceptable and reasonable episiotomy rate and what are the specific maternal and fetal indications for episiotomy. However there is evidence that episiotomy rate of more than 30% is not acceptable and episiotomy should be done on selective basis than done as a routine(11). Many authors suggest use of episiotomy in not more than 30% of vaginal deliveries(3, 12). An overall episiotomy rate of 67% found in this study population is high and may be similar high rates of episiotomy are found in other parts of India. Higher the level of health care institution higher is the episiotomy rate found. In tertiary health care set up, the episiotomy rate is found to be very high (80.7%) and particularly in private sector medical college hospitals it is extremely high (91.8%). Similarly it is very high when doctors conduct the delivery (77.4%). Whatever way sub grouping is done, either by duration of pregnancy, type of delivery, birth weight, parity, age of mother at birth of last child or standard of living index, the episiotomy rate is high except when the parity is more than three (Tables 2 & 3).

Cochrane systematic review on episiotomy for vaginal birth concludes that restrictive episiotomy policies appear to have a number of benefits than routine episiotomy policies(13). With restrictive episiotomy use, the episiotomy rate, anal sphincter laceration rate were all reduced by 50%(14). Evidence does not support maternal benefits traditionally ascribed to routine episiotomy(15). The use of episiotomy increased the risk of extensive perineal tears without a reduction in the risk of shoulder dystocia(16). Despite a relative paucity of clinical evidence justifying its routine use, high percentage of all vaginal deliveries include an episiotomy in different parts of the world. A study done in Jordon has found an episiotomy rate of 39%(17). In Lagos, Nigeria episiotomy rate is 54.9%(18) and in Brazil it is 94.2%(19). Episiotomy has been routinely used in the United States for nearly a century. As recently as 1987, episiotomy was used in 62% of all vaginal deliveries. Study done in Pittsburgh, USA found a decline in episiotomy rate from 59.7% to 45.0% from 1995 to 2000(10). Study done in the Department of Gynecology and Obstetrics, Charles University and Faculty Hospital Pislen found episiotomy rate of 75%(20). Public hospitals in Hong Kong have an episiotomy rate of 85.5%(21).

Medline analysis from 1970 to 2005 concludes that there is no evidence in literature favoring a liberal policy over a restrictive policy for the use of episiotomy both in terms of fetal and maternal indications and the only specific indication that could be retained after analysis was the short perineum when the distance between the fourchette and the center of the anus is less than 3 cm(22). We can reasonably conclude that episiotomy rate of more than 30% in any institution could be due to other reasons than due to real fetal or maternal indications. This study indicates persons conducting delivery and indirectly place of delivery as important extraneous factors for high rates of episiotomy. The probability for episiotomy was higher to the extent of 38.0 times and 12.6 times when delivery was conducted by doctors and by nurses respectively compared to that of trained birth attendants even after controlling for possible confounders. Similarly the risk of episiotomy was higher to the extent of 8.9 and 2.4 times when delivery was conducted at private medical college hospitals and government medical college hospitals respectively compared to when delivery was conducted at primary health centres. Risk of episiotomy was 4.1 and 2.2 times higher when delivery was conducted in tertiary level institutions and secondary level institutions compared to primary level institutions respectively (table

5). This may be due to the fact that doctors conduct deliveries more often in tertiary and secondary level institutions.

The higher rates of episiotomy in higher levels of health centers and more in private sector than in public sector is the trend seen in many other countries also. In Canada the rate of episiotomy was higher among the obstetrician group compared to the family physician group(23). In USA, during intra-partum care women managed by family physicians were less likely to have an episiotomy as compared with managed by obstetricians(24), and women admitted to obstetrician supervised teaching services were more likely to have higher episiotomy rate than family practice teaching services(25). A study done in Australia shows that privately insured women, are twice as likely to experience episiotomy as publicly insured women after controlling for clinical and other factors(26). A study done in US, shows that the strongest predictor of episiotomy use was practitioner type, with women attending private physicians having an adjusted 7 fold increased risk for episiotomy after controlling for year of delivery, maternal age, race, birth weight, mode of vaginal delivery, parity, and history of cesarean delivery(10)

Tertiary and secondary level health care institutions adopt better intra natal care compared to primary level institutions. However in the case of episiotomy it is found to be in the reverse. What could be the reasons? It may be because of the type of training received by doctors and nurses as students in medical college hospitals and may be because of the interventionist attitude currently among some specialists and practitioners. The possibility of commercial advantage of episiotomy in some institutions also cannot be ruled out.

CONCLUSION

There is an urgent need for evidence based practice guidelines for specific maternal and fetal indications for episiotomy. As suggested by Faruel Fosse H, a program aiming at continuous improvement in quality of care after episiotomy including various actions like training courses, audits, presence of a staff leader, episiotomy rate feed back per midwife or obstetrician could help reduce the use of episiotomies(27). Evidence based restrictive practice of episiotomy to less than 30% should be adopted by all particularly in tertiary care teaching hospitals which should serve as role models.

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DEPRESSION AND COPING: A STUDY ON HIV POSITIVE MEN AND WOMEN

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ABSTRACT

Aim of the study: *This study is aimed at evaluating the level of depression and coping pattern in HIV positive patients.*

Methodology: *51 newly diagnosed HIV patients (M = 34 / F = 17), were selected for the study from the HIV Clinic, SRU. Hamilton Depression Rating Scale and Ways of Coping were used to evaluate the levels of depression and to identify their different coping styles. Descriptive and Inferential statistics were used to analyze the data.*

Results: *Statistical analysis based on ANOVA indicates no significant difference in the level of depression in relation to gender; mean scores reveal severe level of depression in all patients included in this study. Among the 8 types of coping, there is significant difference in Confrontative coping, Seeking social support, Accepting responsibility ($p = <0.001$) and Escape-Avoidance, Self control ($p = <0.005$) in relation to gender; where men tend to escape or avoid circumstances, whereas women seek more social support*

Discussion: *Retro-positive patients have severe depression. Women face lot of conflicts, as they are more responsible in maintaining relationships in the family; whereas men deny or they feel guilty of their illness or high-risk behaviour and are more concerned about financial issues. It is evident that their coping styles are maladaptive in nature. Men escape from problem situations; they try to control the situation or people around them. They also try to detach and distance themselves from stressors. Women seek support from others in the family or society and they too avoid or detach conflicting situations. It is evident that both men and women do not try to cope by accepting responsibility, planning and solving the problems or through positive reappraisal for improving or maintaining their personal growth.*

Key words: *Psychological adaptation, HIV, Depression*

INTRODUCTION:

HIV/AIDS is a major concern and has only recently attracted the attention of psychosocial research, especially among subjects at higher risk. A number of clinical psychiatric syndromes have been identified in relation with HIV infections. As with any other life threatening illness, the HIV patient must adapt to a set of disease specific factors such as medical, psychological and social as well as the general threat of death. All these factors may often lead to various psychiatric conditions like anxiety and depression; and they tend to adapt maladaptive coping styles. These patients may not recognize or report depressive symptoms. Instead they may present with behavioural changes, which may indicate the presence of underlying depression.

In a meta-analysis people with HIV were twice as likely to to be diagnosed with major depressive disorder than those with HIV seronegativity(1). A study of 100 AIDS patients depicted that the depressive disorders were more prevalent among female patients and they also had more prominent psychosocial problems(2). Studies have also reported a link between passive coping strategies (e.g., denial) and HIV-1 disease progression. Coping by means of denial was found to correlate with lower CD4 count(3).

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The role of depression in HIV-1 disease progression has been examined. Seropositive men showed that baseline depression was associated with faster progression to AIDS (4) and that elevated depression at every visit increased the risk of mortality (5). Baseline measure of depression can vary and produce inconsistent results due to other moderating factors (e.g., coping, social support, premorbid vulnerability) (3). Studies reported one of the first prospective findings that stressful events and social support were related to HIV-1 disease progression to AIDS (6). Conscientiousness was related positively to medication adherence and active coping and negatively to depression, avoidant coping and perceived stress (7).

A study showed that greater health worries, less comfort with how one contracted HIV, more HIV-related symptoms, less social support, and lower spiritual well-being was associated with significant depressive symptoms (8). Psychiatric morbidity, coping responses, and disability in male and female outpatients recently diagnosed with HIV/AIDS, showed no significant gender differences in the prevalence of mood disorders. Men, however, were more likely than women to meet diagnostic criteria for alcohol abuse or dependence, and to engage in certain risky sexual behaviours. Women were more likely to suffer from post-traumatic stress disorder, and to use coping strategies of planning and religion to deal with the illness (9).

Although there is growing literature on the psychological responses and the psychopathology associated with HIV/AIDS, few investigations have focused on the role of gender. Thus this study was aimed to evaluate the level of depression among retropositive male and female patients and their coping styles.

MATERIALS AND METHODS:

The present study hypothesizes that there will be significant difference between male and female patients in their level of depression and coping styles. The sample consists of 51 newly diagnosed Retropositive patients, including both males (N = 34) and females (N = 17). They were selected through purposive sampling, from the HIV clinic, SRU after being diagnosed by the concerned physician. The age range of the sample is 20 to 50 years. Past history and family history of mental illness is excluded. After a month of diagnosis and post-test counseling, informed consent was obtained and their socio demographic details were collected.

Later the following assessment tools were administered in 2 sessions (during monthly follow up).

- Hamilton Depression Rating Scale (HDRS) – is a 24 item scale developed by Max Hamilton (10). The clinician rates and scores (as per the manual). The level of depression is interpreted based on the scores obtained; where Normal is 7 and below, Mild is 8 to 13, Moderate is 14 to 18, Severe is 19 to 22, and Very severe is 23 and above.

- Ways of Coping – is a 66 item questionnaire developed by Lazarus and Folkman (11). The scale consists of 8 types of coping viz., Confrontative Coping (CC), Distancing (D), Self Control (SC), Seeking Social Support (SSS), Accepting Responsibility (AR), Escape – Avoidance (EA), Planful Problem Solving (PPS) and Positive Reappraisal (PR). Raw scores for each subscale is converted to a relative score and is interpreted based on ranking method (as per the test manual). Hence, lesser the score indicates that the individual adapts that particular ways of coping more than other coping styles.

Descriptive Statistics and ANOVA were used to analyze the collected data.

Results:

Table 1 shows the socio demographic details of the sample. The selected sample consists of 34 males and 17 females. The majority of the group falls in the age range between 31 to 40 years and most of them belong to low socioeconomic status with education up to high school. Majority of the sample population are married, many accepted the presence of high-risk behaviours and reported of opportunistic infections.

Figure 1 gives the graphical representation of the mean scores of depression and various coping styles among male and female patients. The mean score shows that females have higher level of depression when compared to male patients. The low means scores of the sub scales of coping indicate that the sample population highly tend to Escape or Avoid, Confrontate, Seek Social Support or Distance themselves as part of their coping style. It is evident that men tend to cope more by Escape Avoidance, Confrontative Coping and Distancing; whereas women tend to cope by Seeking Social Support, Escape Avoidance and Distancing.

Table 1: Frequency and Percentage Distribution of the Demographic details of HIV positive patients

VARIABLES		Frequency	Percentage
Sex	Male	34	66.7
	Female	17	33.3
Age	20 – 30 years	19	37.3
	31 – 40 years	24	47.0
	41 – 50 years	8	15.7
Education	< 10th std	39	76.0
	< 12th std	6	12.0
	Graduation	6	12.0
Socioeconomic Status	Low	34	66.7
	Middle	17	33.3
Marital Status	*Married*	*41*	*80.0*
	Single	*6*	*12.0*
	Separated	*1*	*2.0*
	Widowhood	*3*	*6.0*
Spouse HIV Status	Positive	18	40.0
	Negative	12	26.7
	Not Known	5	33.3
Family Support	Present	34	66.7
	Absent	17	33.3
High-risk Behaviour	Present	24	47.0
	Absent	14	27.5
	Denied	13	25.5
Opportunistic Infections	Present	36	70.6
	Absent	15	29.4

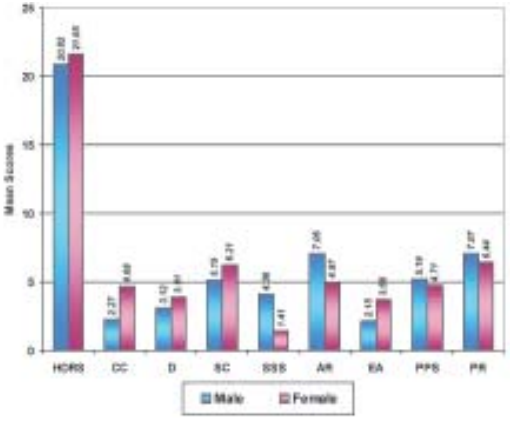

Figure 1: Mean scores of Male and Female patients on Depression and Various Ways of Coping

(HDRS – Hamilton Depression Rating Scale, CC – Confrontative Coping, D – Distancing, SC – Self Control, SSS – Seeking Social Support, AR – Accepting Responsibility, EA – Escape Avoidance, PPS – Planful Problem Solving, PR – Positive Reappraisal)

Table 2 shows that there is significant difference between males and females in Confrontative Coping, Seeking Social Support and Accepting Responsibility ($p = < 0.001$); Escape Avoidance and Self Control ($p = < 0.005$). There is no significant difference between men and women in their level of Depression, Distancing, Planful Problem Solving and Positive Reappraisal.

Table 2: Summary of ANOVA on Depression and Various Coping Styles of HIV positive patients

Variables	Source of Variation	df	Sum of Squares	Mean Square	F	Sig.
Depression (HDRS)	Between Groups	1	7.686	7.686	1.590	.213
	Within Groups	49	236.824	4.833		
	Total	50	244.510			
Confrontative Coping (CC)	Between Groups	1	65.922	65.922	34.150	.000**
	Within Groups	49	94.588	1.930		
	Total	50	160.510			
Distancing(D)	Between Groups	1	7.147	7.147	1.813	.184
	Within Groups	49	193.147	3.942		
	Total	50	200.294			
Self Control (SC)	Between Groups	1	12.706	12.706	9.252	.004*
	Within Groups	49	67.294	1.373		
	Total	50	80.000			
Seeking Social Support (SSS)	Between Groups	1	79.412	79.412	48.338	.000**
	Within Groups	49	80.500	1.643		
	Total	50	159.912			
Accepting Responsibility (AR)	Between Groups	1	49.422	49.422	15.077	.000**
	Within Groups	49	160.618	3.278		
	Total	50	210.039			
Escape Avoidance(EA)	Between Groups	1	26.510	26.510	10.456	.002*
	Within Groups	49	124.235	2.535		
	Total	50	150.745			
Planful Problem Solving (PPS)	Between Groups	1	2.669	2.669	1.108	.298
	Within Groups	49	118.037	2.409		
	Total	50	120.706			
Positive Reappraisal(PR)	Between Groups	1	4.532	4.532	3.732	.059
	Within Groups	49	59.507	1.214		
	Total	50	64.039			

** Significant at the 0.001 level (p = < 0.001)* Significant at the 0.005 level (p = < 0.005)

DISCUSSIONS:

Depression: The total mean score of the sample population indicates severe level of depression (10). Mean scores reveals that females have more depression than males, though the difference is not statistically significant. This difference may be due to societal and cultural expectations from women as a wife and mother are highly demanding. Mothers play a major role in child rearing especially breast-feeding and are also worried about their family after their death. Feelings of guilt are also present as they are unable to take care of themselves and the family, which results in helplessness, hopelessness and worthlessness (2, 8, 12).

Depression in men is also evident due to fear of death, helplessness and guilt feelings which are prominent as most men have high-risk behaviours and that they are the cause of transmission within the family. During the depressive phase more concern is focused on how to inform and face the family members rather than on how to manage themselves with the illness (4). Financial burden is also a contributing factor for depression in men (13).

Coping: Coping styles can be adaptive or maladaptive in nature and it differs in each individual, depending on the stress experienced by the individual. Each coping style is discussed as per the total mean score obtained by the group (Figure 1 and Table 2).

The following coping styles have significant difference in relation to gender.

Confrontative Coping: They may take risk and try to alter the problem situation or change other's mind and accept responsibility rather than by facing the problem. Men tend to get hostile towards problems; which may be due to lack of support. They express their anger towards their family members even though they are not the cause (12, 14).

Seeking Social Support: Women seek support from family members and at times even from the medical team in order to deal problems related to illness (e.g. controlling or advising husband, issues related to children, etc). Men seek less support and have greater use of denial due to various stressful life events (3). Both seek social support from people

of their personality trait or characteristics (e.g. women seek support from those who blame her husband and men from those who have similar problem or high-risk behaviour) (6, 12, 14).

Accepting Responsibility: Men tend to deny, but some women are forced to accept their mistakes on the basis of religion (3). In crisis situation women are able to take up the role of a man by supporting the family financially; whereas only few men take up such familial responsibilities.

Escape – Avoidance: Men tend to cope by escaping, avoiding and confrontative coping when compared to other styles, which may be due to the phase of denial on the part of the patient after being diagnosed (3, 12, 15). There is less need to seek support from others, as they tend to avoid problematic issues (7, 16). They are unable to face the problem, as they may be blamed for their risk behaviours. Women avoid or escape by blaming others. Men try to escape by smoking or consuming alcohol; some may even involve more in high-risk behaviours (9).

Self Control: Men try to control their actions and behaviour in order to avoid problem situation. They hide their feelings and do not want to reveal their HIV status to others (17). They fear rejection, has poor social interaction, leading to depression.

There is no significant difference between males and females in the following types.

Distancing: Both men and women try to detach and distract themselves from the actual problem (3). They tend to reduce the importance of the situation in order to avoid the distress faced by the individual. They tend to attribute their problems to fate or sin.

Planful Problem Solving: All patients were found to use this coping style rarely (18). Among them women use this strategy more than men, in order to analyze the ways to live with their illness and face the consequences (9).

Positive Reappraisal: Women tend to emphasize on religious aspects, tries to cope with their spiritual beliefs (9, 19). They rarely try to change themselves or aim for personal growth. As this illness is not curable, they believe that nothing can be improved by changing themselves.

Both men and women with HIV infection use Planful Problem Solving (19) and Positive Reappraisal the least, as it requires high sophistication of adaptive coping strategy.

CONCLUSION:

There is severe level of depression present in retropositive patients. No statistical difference is evident between males and females. The sub types in coping indicate that patients use more of maladaptive coping strategies like escaping, avoiding, controlling self and others. The sample population does not use adaptive coping styles like positive reappraisal, planning and problem solving.

This study highlightens certain clinical features of HIV infection, which generally goes unnoticed. Further investigation on other psychosocial variables and the efficacy of psychological management is planned to reduce the level of depression and ameliorate their ways of coping.

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A STITCH IN TIME SAVES NINE- PATIENT NOT RESPONDING TO ANTIBIOTIC TREATMENT THINK OF FUNGUS.

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ABSTRACT

The increased incidence of fungal infections in past two decades has been overwhelming. Earlier it was the pathogenic dimorphic fungi or the agents causing superficial fungal infections which were known as pathogens for humans. However, starting from 1980s, the opportunistic fungi started causing more infections especially in the immunocompromised host. More recently newer and less common fungal agents are being increasingly associated with infections in immunosuppressed host.

Materials and Methods: *There were 255 samples sent specifically for fungal culture to the Microbiology Department in the last one year from varied specialties in our institute. All the samples were subjected to microscopy (10% KOH or India ink preparation depending upon the sample) to look for fungal elements.*

Results: *The total number of fungi isolated were 100 (39%) out of 255 specimens sent for fungal culture in the last one year. The predominant fungus isolated was Candida species (42%) from respiratory and soft tissue specimens. It was closely followed by Aspergillus sp (23%) which was from ENT specimens.*

Conclusion: *Strong suspicion of fungal infection, prompt diagnosis and treatment can salvage the patients from morbidity and mortality.*

Key words : *Fungi, Microbiology, Culture*

INTRODUCTION

The last 2-3 decades of the 20th century witnessed increase in the incidence of opportunistic fungal infections mainly due to modern medical advances, changes in man's environment and his immune defense.[1]

The new millennium in medical mycology certainly belongs to opportunistic fungal infections in immunocompromised patients. As such only a few of the vast empire of fungi present in nature are able to produce human disease. These fungi are distinguished by their ability to adapt to the elevated body temperature and to overcome the defense mechanisms of the human host.

These fungi manifest almost exclusively in the immunocompromised host. Invasive fungal infections are now more prevalent than ever, due to an increasingly large population of patients at high risk to secondary immunosuppression, underlying diseases and chronic conditions such as cancer bone marrow transplants or solid organ transplant. HIV infections and chronic corticosteroid administration make patients vulnerable to opportunistic fungal infections.

As these fungi are ubiquitous saprophytes in man's environment, all immunocompromised hosts world wide remain predisposed to fungal infections.

The majority of opportunistic mycoses are caused by exogenous fungi.[1]

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Significantly, among them the members of the order mucorales have latent pathogenic potentiality to incite rapidly progressing and fulminant infection generally in immunosuppressed hosts.

Normally fungal infections are of low virulence and are confined to local infections. But in the immunocompromised patients there is an increasing tendency for the fungi to spread from local sites and produce invasive fungal infection with devastating consequences to the patient.

The distribution of fungal infections in a community varies according to a number of factors, including climatic conditions, environmental and socio-economic status of individual communities, their habits and genetic factors.[2]

An understanding of the distribution of these infections, their prevalence and spread would contribute greatly towards control and treatment of these conditions.

The ability of fungi to produce disease in the human host is apparently an accidental phenomenon. In general, invasion of the human host is not an essential pre-requisite for the maintenance and dissemination of the species.

This article highlights about our experience on the fungal isolates we have come across in the last 1 year in SRMC and in whom, suspicion of fungal infection prompt intervention and aggressive medical/ surgical treatment has lead to a positive out come in the patients.

Importance of a properly collected sample

Whenever possible, the laboratory personnel/ mycologists himself or herself should examine the patient, elicit appropriate history and obtain clinical sample. Otherwise the clinician in-charge should provide detailed history, findings of clinical examination, the disease suspected and any other relevant background information which are likely to be of use to the laboratory personnel in proper processing and further workup.

Collection and transportation of clinical samples.

The type and quality of the clinical specimens are the most important factors in determining the outcome of the exercise. Hence, utmost care and discretion are essential in collection and proper transportation of the clinical samples.

- The specimen should be collected aseptically, placed in sterile containers, delivered to the laboratory as early as possible, preferably within 2 hours and processed within a few hours of collection. Prompt processing minimizes the loss in viability and enables an accurate estimation of the quantity of fungus, prevents or reduces the overgrowth of bacterial contamination.
- Swabs are not generally acceptable except in cases of ear canal, nasopharynx, vagina and cervix.
- Specimens should be transported in sterile , humidified, leak proof containers. Transport media are generally not suitable for transportation of samples from fungal infections.
- Specimens should be processed and inoculated on to isolation media as soon as possible.

Materials and Methods

There were 255 samples sent specifically for fungal culture to the Microbiology Department in the last one year from varied specialties in our institute.

All the samples were subjected to microscopy (10%KOH or India ink preparation depending upon the sample) to look for fungal elements. Clinicians were informed of any positive finding over the phone immediately so that antifungal could be started without delay.. The specimens were then inoculated in media like Sabouraud's dextrose agar, with antibiotics and special media like oat meal agar whenever required. All the tubes were put in duplicate one set at 37°C and one set at 25°C. The tubes were observed for any growth from day 2 onwards till 6 weeks.

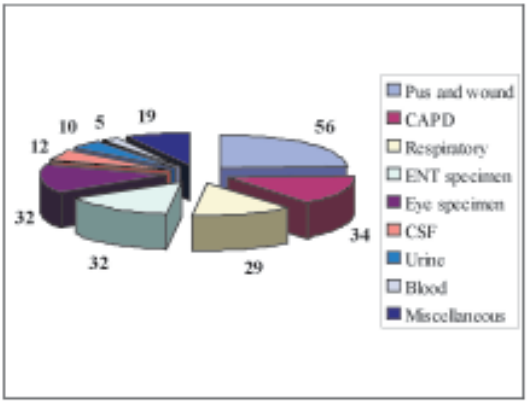
Once the growth was observed slide culture was put up to identify the species.

Results

The total number of fungi isolated were 100 (39%) out of 255 specimens sent for fungal culture in the last one year.

The samples consisted of pus, urine, blood, tissues, swabs, CSF, peritoneal fluid, CAPD catheters, respiratory samples etc.(Table 1)

Table 1. Distribution of the types of specimens received



Details of few important fungal isolates and the out come is shown in table 2

Table 2 Unusual fungal isolates, and the out come

Specimen	KOH	Isolate	Management	Outcome
Maxillary Sinus tissue	positive	*Rhizopus oryzae*	Surgical debridment and ampho B	Recovered
Tissue from discharging sinuses	positive	*Absidia corymbifera*	Surgical debridement and ampotericinB	Sinuses healed.
CAPD catheter tip	positive	*Cuninghamella*	Catheter replacement	Condition improved
CAPD fluid	positive	*Aspergillus terreus*	Catheter replacement	Condition improved
Pus swab	positive	*Fusarium solani*	Surgical debridement and ampotericinB	Condition improved
Conjunctival swab	positive	*Engyodontium album*	voriconazole	Recovered
Corneal scraping	positive	*Hormonema dermatoides*	Natamycin	Recovered
Corneal scraping	positive	*Curvalaria lunata*	Natamycin	Recovered
Ethmoidal sinus tissue	positive	*Scedosporium apiospermum*	Surgical debridement	Improved

The predominant fungus isolated was *Candida* species (42%) from respiratory and soft tissue specimens.

It was closely followed by *Aspergillus* sp (23%) which were from ENT specimens.

We describe here the rare isolates which we isolated in our Institute.

Rhizopus arryizhus

Rhizopus arrhizus is the most common etiologic agent of human disease and accounts for approximately 90% of rhinocerebral zygomycosis associated with diabetic ketoacidosis. Individuals with hematologic malignancies, undergoing iron chelation therapy, and those sustaining traumatic injuries are also at risk. A recent report also links long term voriconazole use in immunocompromised patients with hematologic disease as a potential predisposing factor [4].

A 46 year old male with uncontrolled diabetes was admitted with complaints of pain and swelling over the left eye following a tooth extraction. Patient was diagnosed to have left periorbital cellulitis with associated sinusitis for which functional endoscopic sinus surgery with ethmoidectomy and debridement was done ; the tissue grew *Rhizopus arrhizus*.

Macroscopic morphology

Colonies on potato dextrose agar at 25°C are woolly and initially white, quickly becoming gray and then developing small black dots in the mycelium which are mature sporangia. Growth is very rapid, filling the tube or petri dish within 2 to 3 days.**(Fig 1a)**

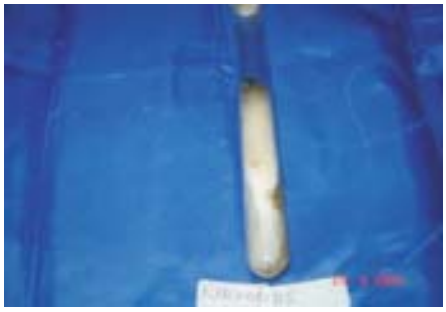


Fig 1a. Culture in SDA white cottony growth

Microscopic morphology

Hyphae are hyaline, broad (5-15 μm), ribbon-like, irregularly branched, and aseptate to sparsely septate. Sporangiophores and rhizoids are borne from creeping aerial hyphae known as stolons**. (Fig 1b)**

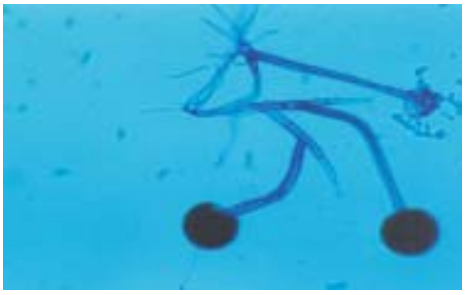


Fig 1b. LPCB mount showing Rhizoids and sporangium (Magnification 20X)

Similar to the other genera belonging to the phylum Zygomycota, treatment of *Rhizopus* infections remains difficult. Due to its property to invade vascular tissues, infarction of the infected tissue is common and mortality rates are very high. Early diagnosis is crucial and surgical debridement or surgical resection, as well as antifungal therapy, are usually required. Amphotericin B is the most commonly used antifungal agent [4, 5,]. Liposomal amphotericin B [6] and other lipid-based amphotericin B formulations such as amphotericin B colloidal dispersion [7] have also been used in some cases of zygomycosis.

Absidia corymbifera

A 60 year old male diabetic had non healing ulcers which grew *Absidia corymbifera* on culture and patient responded to treatment. (surgical debridement followed by amphotericin B)

Pathogenicity and Clinical Significance

Absidia corymbifera is a relatively rare cause of human zygomycosis. Zygomycosis is an opportunistic mycoses that manifests with pulmonary, rhinocerebral, cutaneous, gastrointestinal, renal or meningeal involvement. Disseminated zygomycosis may originate from these infections. Zygomycosis is very rarely observed in immunocompetent host [8].

Since *Absidia* spp. are cosmopolitan and ubiquitous in nature, they are also common laboratory contaminants. Thus, their isolation in culture requires cautious evaluation. Nevertheless, the growth of *Absidia*, particularly from clinical samples of patients with immunosuppression or diabetes mellitus, should be regarded as potentially significant. Also, the visualization of typical hyphae of zygomycetes group of fungi on direct microscopic examination, of particularly a sterile body site, should be considered significant even if the culture yields no growth.

Macroscopic Features

Absidia corymbifera grows rapidly. The rapid growing, flat, woolly to cottony, and olive gray colonies mature within 4 days on potato glucose agar. The texture of the colony is typically woolly to cottony. From the surface, the colony is grey in color. The reverse side is uncolored and there is no pigment production. *Absidia corymbifera* grows more rapidly at 37°C than at 25°C. **(Fig2a)**

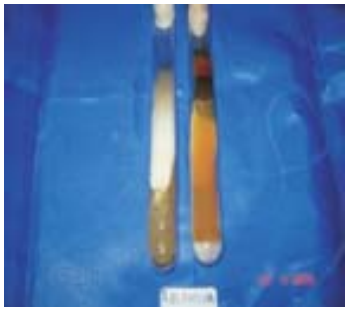


Fig2a. Culture in SDA white cottony growth

Microscopic Features

Similar to that of the other members of the class zygomycetes, *Absidia corymbifera* has wide (6-15 μm in diameter) nonseptate hyphae. A few septa may occasionally be present. Rhizoids are rarely observed. When present, the sporangiophores arise on stolons from points between the rhizoids, but not opposite the rhizoids. The sporangiophores are branched and arise in groups of 2-5 at the internodes. They often produce arches. Sporangiophores carry pyriform, relatively small (20-120 μm in diameter) sporangia. A septum is usually present just below the sporangium in the sporangiophore. The sporangiophore widens to produce the funnel-shaped apophysis beneath the sporangium. The apophysis of *Absidia corymbifera* is very well-developed and typical. The columella, the tip of the sporangiophore that extends into the sporangium, is semicircular in shape and has a small projection on top. **(Fig 2b)**

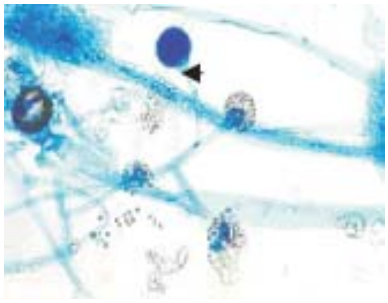


Fig 2b. Slide culture with LPCB mount showing basal apophyses (Magnification 20X)

Similar to the other members of the class Zygomycetes, amphotericin B appears as the sole antifungal drug which is consistently active against *Absidia corymbifera* . In general, it is resistant to azoles, including the newer derivatives such as voriconazole (9) flucytosine is also ineffective against *Absidia corymbifera* .

Cunninghamella

64 year old diabetic male with CRF on CAPD developed fungal peritonitis. The CAPD catheter tip was sent for fungal culture which grew *Cunninghamella*.

Description and Natural Habitats

Cunninghamella is a filamentous fungus found in soil and plant material, particularly at Mediterranean and subtropical zones. It has also been recovered from animal material, cheese, and Brazil nuts. In addition to being a common contaminant, *Cunninghamella* is an opportunistic fungus that may cause infections in immunocompromised hosts [10, 11].

The genus *Cunninghamella* currently contains seven species; of which *Cunninghamella bertholletiae*, is the only known human and animal pathogen.

Pathogenicity and Clinical Significance

Cunninghamella spp are among the causative agents of zygomycosis. Although the term mucormycosis has often been used for this syndrome, zygomycosis is now the preferred term for this angio-invasive disease. Trauma, diabetes mellitus, immunosuppression due to various reasons (hematological malignancies, organ transplantation, AIDS), and desferoxamine therapy are the major risk factors for development of zygomycosis. Among the other genera belonging to the class zygomycetes, *Cunninghamella* is particularly sensitive to desferoxamine therapy. Lastly and importantly, *Cunninghamella* infections have been reported in a number of cases receiving antifungal prophylaxis with itraconazole [12].

Cunninghamella bertholletiae may cause rhinocerebral, pulmonary, cutaneoarticular, and disseminated forms of zygomycosis. The infection usually starts after inhalation of the spores or inoculation of the fungus following the primary breakdown of the skin integrity due to a trauma [13].

Macroscopic Features

Cunninghamella colonies are rapidly growing (mature in 4 days), cottony, and white to tannish-gray in color. The reverse is pale. *Cunninghamella elegans* produces purely gray colonies. **(Fig3a)**

Fig 3a. Culture in SDA white fluffy growth

Microscopic Features

Nonseptate or sparsely septate broad hyphae, sporangiophores, terminal vesicles, sporangioles and sporangiospores are visualized. Sporangiophores are erect and form short lateral branches each of which terminates in a swollen vesicle. **(Fig3b)**

Very few data are available and there is as yet no standard method for in vitro susceptibility testing of *Cunninghamella* spp. In an in vitro study where two *Cunninghamella echinulata* strains were tested, the rank of MICs was found to be voriconazole, ketoconazole, amphotericin B, itraconazole [9].

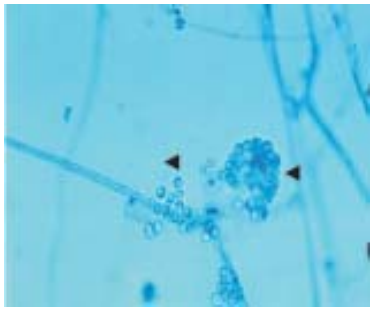


Fig 3b. Slide culture showing sporangioles (Magnification 20X)

Aspergillus nidulans

An 8 year old male with end stage renal disease and obstructive uropathy developed fungal peritonitis. Aspirated fluid from peritoneum was sent for fungal culture from which *Aspergillus nidulans* was isolated.

Macroscopic morphology

Colonies on potato dextrose agar at 25°C are dark green with orange to yellow in areas of cleistothecial production. Reverse is purplish to olive. **(Fig4a).**

Fig 4a. Culture showing honey coloured pigment in SDA

Microscopic morphology

Hyphae are septate and hyaline. Conidial heads are columnar. Conidiophores are brown, short (60-150 μm in length), and smooth-walled. Vesicles are hemispherical, small (8-12 μm in diameter), with metulae and phialides occurring on the upper portion. Conidia are globose (3-4 μm) and rough. *A. nidulans* is a homothallic species capable of producing the teleomorph (sexual stage) without mating studies. The ascomycetous telemorph (*Emericella nidulans*) produces brown to black globose cleistothecia (100-250 μm) that are engulfed with globose Hülle cells. Ascospores are reddish brown, lenticular (4 × 5 μm), with two longitudinal crests **(Fig4b**)

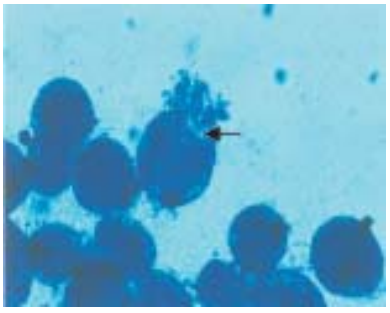


Fig 4b. Globose cleistothecia and the crest in slide culture (Magnification20X)

Aspergillus terreus

A 57 year old man was diagnosed as a case of fungal granuloma of left maxilla.

Debridement was done and tissue sent for fungal culture, which grew *Aspergillus terreus*.

Macroscopic morphology

Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with conidial production **(Fig 5a)**

Fig 5a. Culture on SDA showing beige to buffcoloured colonies

Microscopic morphology

Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar. Conidiophores are smooth-walled and hyaline, 70 to 300μm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 μm), globose, and smooth. **(Fig 5b)**

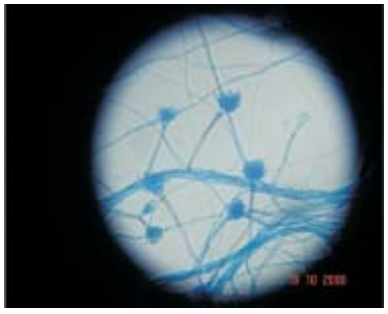


Fig 5b. Slide culture showing biseriate conidial heads (Magnification 20 X)

This species is noteworthy for its refractoriness to amphotericin B therapy [14,15]. Isolates that are initially white and producing only accessory conidia may be mistaken for *Histoplasma capsulatum* [14]

Acremonium species

35 year old female came with complaints of headache and nasal block for 2 months.Microdebrider assisted Functional Endoscopic Sinus Surgery was done. Tissue sent for fungal culture grew *Acremonium* species.

Description and Natural Habitats

Acremonium spp. are filamentous, cosmopolitan fungi commonly isolated from plant debris and soil.

Pathogenicity and Clinical Significance

Acremonium is one of the causative agents of eumycotic white grain mycetoma. Rare cases of onychomycosis, keratitis, endophthalmitis, endocarditis, meningitis, peritonitis, and osteomyelitis due to *Acremonium* have also been reported [16, 17]. This fungus is known to cause opportunistic infections in immunocompromised patients, such as bone marrow transplant recipients [18]. Infections

of artificial implants due to *Acremonium* spp. are occasionally observed [19]. Since *Acremonium* species are cosmopolitan in nature, they are also encountered as contaminants. Thus, their isolation in culture requires cautious evaluation.

Macroscopic Features

The growth rate of *Acremonium* colonies is moderately rapid, maturing within 5 days. The diameter of the colony is 1-3 cm following incubation at 25°C for 7 days on potato glucose agar. The texture of the colony is compact, flat or folded, and occasionally raised in the center. It is glabrous, velvety, and membrane-like at the beginning. Powdery texture may also be observed. By aging, the surface of the colony may become cottony due to the overgrowth of loose hyphae. The color of the colony is white, pale grey or pale pink on the surface. **(Fig 6a)**

Fig 6a. SDA showing white compact growth

Microscopic Features

Acremonium spp. possess hyaline, septate hyphae which are typically very fine and narrow. Vegetative hyphae often form hyphal ropes. Unbranched, solitary, erect phialides are formed directly on the hyphal tips, the hyphal ropes, or both. At the apices of the phialides are the hyaline conidia 2-3 × 4-8μm in size. They usually appear in clusters, in balls or rarely as fragile chains. The conidia are bound by a gelatinous material. They may be single or multicellular, fusiform with a slight curve or resemble a shallow crescent. These structural properties of conidia vary depending on the species. **(Fig 6b)**

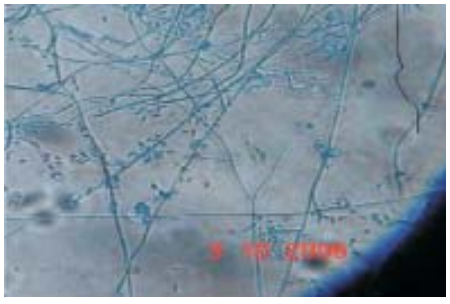


Fig 6b. LPCB mount showing narrow septatehyphae with conidia (Magnification20X)

The novel azoles, such as voriconazole, and posaconazole appear to exhibit favorable in vitro activity against *Acremonium* strains

In vivo response, on the other hand, depends on both antifungal therapy and surgical intervention. Among the available antifungal agents, amphotericin B remains as the mainstay of therapy. *Acremonium* spp. may also respond to azoles, which are occasionally used in combination with amphotericin B. The limited data obtained so far for voriconazole appear promising [16].

Fusarium solani

A 45 year old recipient of renal transplant done five years ago developed non healing ulcers over the heel. Specimen from the site grew *Fusarium solani*. Patient improved after wound, debridement and antifungal therapy.

Description and Natural Habitats

Fusarium is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops [21]. While most species are more common at tropical and subtropical areas, some inhabit soil in cold climates.

As well as being a common contaminant and a well-known plant pathogen, *Fusarium* spp. may cause various infections in humans. *Fusarium* is one of the emerging causes of opportunistic mycoses [10, 21, 22].

Species

The genus *Fusarium* currently contains over 20 species. The most common of these are *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium chlamydosporum* [10].

Pathogenicity and Clinical Significance

As well as being common plant pathogens, *Fusarium* spp. are causative agents of superficial and systemic infections in humans. Infections due to *Fusarium* spp. are collectively referred to as fusariosis. The most virulent *Fusarium* spp. is *Fusarium solani* [23]. Trauma is the major predisposing factor for development of cutaneous infections due to *Fusarium* strains. Disseminated opportunistic infections, on the other hand, develop in immunosuppressed hosts, particularly in neutropenic and transplant patients [24, 25]. *Fusarium* infections following solid organ transplantation tend to remain local and have a better outcome compared to those that develop in patients with hematological malignancies and bone marrow transplantation patients [26].

Keratitis [27], endophthalmitis [28], otitis media [29], onychomycosis [30], cutaneous infections [31] particularly of burn wounds, mycetoma, sinusitis [32], pulmonary infections [33], endocarditis, peritonitis, central venous catheter infections, septic arthritis, disseminated infections [10, 21, 22], and fungemia due to *Fusarium* spp. have been reported [10].

Macroscopic Features

Fusarium spp. grow rapidly on Sabouraud's dextrose agar at 25°C and produce woolly to cottony, flat, spreading colonies.. From the front, the color of the colony may be

white, cream, tan, salmon, cinnamon, yellow, red, violet, pink, or purple. From the reverse, it may be colorless, tan, red, dark purple, or brown. **(Fig 7a)**

Fig 7a. SDA showing reddish pigment

Microscopic Features

Hyaline septate hyphae, conidiophores, phialides, macroconidia, and microconidia are observed microscopically. In addition to these basic elements, chlamydospores are also produced by some species.(10.) Phialides are cylindrical, with a small collarette, solitary or produced as a component of a complex branching system. Macroconidia (3-8 x 11-70 μm) are produced from phialides on unbranched or branched conidiophores. They are 2- or more celled, thick-walled, smooth, and cylindrical or sickle- (canoe) shaped. **(Fig 7b)**

Fig 7b. LPCB mount showing conidia and chlamydiospores (Magnification 20X)

Macroconidia have a distinct basal foot cell and pointed distal ends. They tend to accumulate in balls or rafts. Microconidia (2-4 x4-8 μm), on the other hand, are formed on long or short simple conidiophores. They are 1-celled (occasionally 2- or 3-celled), smooth, hyaline, ovoid to cylindrical, and arranged in balls (occasionally occurring in chains). Chlamydospores, when present, are sparse, in pairs, clumps or chains. They are thick-walled, hyaline, intercalary or terminal, [11].

Macroscopic and microscopic features, such as, color of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* species [10].

The only antifungal drugs that yield relatively low MICs for *Fusarium* are amphotericin B [34], voriconazole [35], and *natamycin* [36].

Fusarium infections are difficult to treat and the invasive forms are often fatal. Amphotericin B alone or in combination with flucytosine or rifampin is the most commonly used antifungal drug for treatment of systemic fusariosis [26]. Lipid formulations of amphotericin B, such as *liposomal amphotericin B* and *amphotericin B lipid complex* are also used.

Scedosporium apiospermum

A 45 year old male came diagnosed as fungal granuloma of left maxilla and maxillary carcinoma.

Scedosporium is a filamentous fungus which occasionally causes infections in humans.

The genus *Scedosporium* contains two species; *Scedosporium apiospermum* and *Scedosporium prolificans*. *Pseudallescheria boydii* is the telemorph of *Scedosporium apiospermum*.

Pseudallescheria is a filamentous fungus that is found worldwide. It has so far been isolated from soil [37], sewage, contaminated water, and the manure of farm animals. It is an emerging opportunistic pathogen and can cause various infections in humans. *Pseudallescheria boydii* is the telemorph of *Scedosporium apiospermum* and *Graphium eumorphum*.

Pathogenicity and Clinical Significance

Scedosporium apiospermium and *Sc. Prolificans* are the two organisms responsible for scedosporiasis. The recognition of these rare human fungal pathogens is important because both are resistant to a variety of antifungal agents. The microorganism *Scedosporium apiospermium* is the asexual state of *Pseudallescheria boydii*. The most common clinical manifestations of *Scedosporium apiospermium* infection are chronic cutaneous mycetoma, (also known as 'Madura foot') and pulmonary infection. The latter often occurs in the setting of previous tuberculosis. Other infections associated with this organism include sinusitis, arthritis and osteomyelitis of long bones and brain abscess[38].

Macroscopic Features

Colonies of *Pseudallescheria boydii* grow rapidly at 25°C. The texture is wooly to cottony. From the front, the color is initially white and later becomes dark gray or smoky brown. **(Fig 8a)**

Fig 8a. Culture in SDA shows smoky brown colonies.

Microscopic Features

In the asexual stage (*Scedosporium apiospermum* or *Graphium eumorphum*), the asexual reproductive structures; septate hyaline hyphae (2-4 μm in diameter), conidiophores and (annello)conidia are produced. The conidiophores of *Scedosporium apiospermum* are simple while those of *Graphium eumorphum* are long, erect, narrow, and cemented together forming synnemata (the erect structure consisting of united conidiophores). Conidia (4-7 × 5-12 μm) of both *Scedosporium apiospermum* and *Graphium eumorphum* are unicellular and oval in shape. The conidia of *Scedosporium apiospermum* are often formed singly on the conidiophores. **(Fig 8b)**

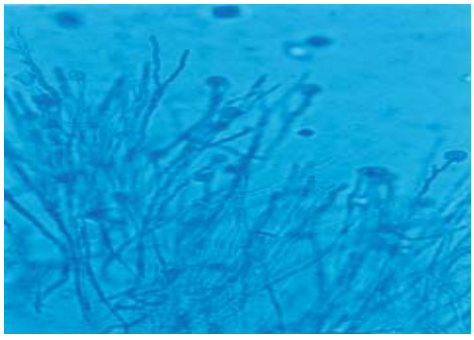


Fig 8b. Slide culture and LPCB mount of *Scedosporium apiospermum* showin Singleconidia on the conidiophore (Magnification20X)

Scedosporium apiospermum infection is resistant to the traditional antifungal agents such as amphotericin B, fluconazole and flucytosine but miconazole and the new triazole, voriconazole has been now successfully used for the treatment caused by this fungal agent [39]

Curvularia

45 year old male came with trauma developed fungal keratitis, corneal scraping was done and the specimen was sent to the Microbiology Department.

Description and Natural Habitats

Curvularia is a dematiaceous filamentous fungus. Most species of *Curvularia* are facultative pathogens of soil, plants, and cereals in tropical or subtropical areas, while the remaining few are found in temperate zones. As well as being a contaminant, *Curvularia* may cause infections in both humans and animals [40,41].

The genus *Curvularia* contains several species, *Curvularia lunata* is the most prevalent cause of disease in humans and animals.

Pathogenicity and Clinical Significance

Curvularia spp. are among the causative agents of phaeohyphomycosis. Wound infections, mycetoma, onychomycosis, keratitis, allergic sinusitis, cerebral abscess, cerebritis, pneumonia, allergic bronchopulmonary disease, endocarditis, dialysis-associated peritonitis, and disseminated infections may develop due to *Curvularia* spp. *Curvularia lunata* is the most commonly encountered species. Importantly, the infections may develop in patients with intact immune system. However, similar to several other fungal genera, *Curvularia* has recently emerged also as an opportunistic pathogen that infects immunocompromised hosts [13, 22].

Macroscopic Features

Curvularia produces rapidly growing, woolly colonies on potato dextrose agar at 25°C. From the front, the color of the colony is white to pinkish gray initially and turns to olive brown or black as the colony matures. From the reverse, it is dark brown to black [14]**(Fig 9a).**

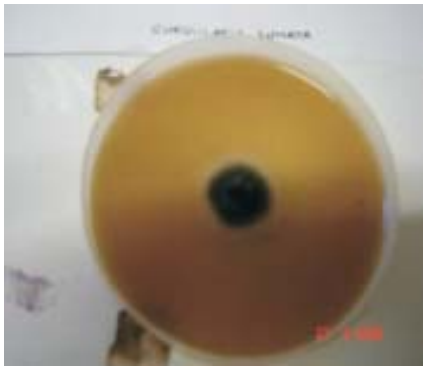


Fig 9a. Black colonies of C*urvalaria* on SDA

Microscopic Features

Septate, brown hyphae, brown conidiophores, and conidia are visualized. Conidiophores are simple or branched and are bent at the points where the conidia originate. This bending pattern is called sympodial geniculate growth. in the conidium. The central septum may also appear darker than the others. The swelling of the central cell usually gives the conidium a curved appearance [10, 11, 14].

The number of the septa in the conidia, the shape of the conidia (straight or curved), the color of the conidia (dark vs pale brown), existence of dark median septum, and the prominence of geniculate growth pattern are the major microscopic features that help in differentiation of *Curvularia* spp. among each other **(Fig 9b).** For instance, the conidia of *Curvularia lunata* have 3 septa and 4 cells, while those of *Curvularia geniculata* mostly have 4 septa and 5 cells.

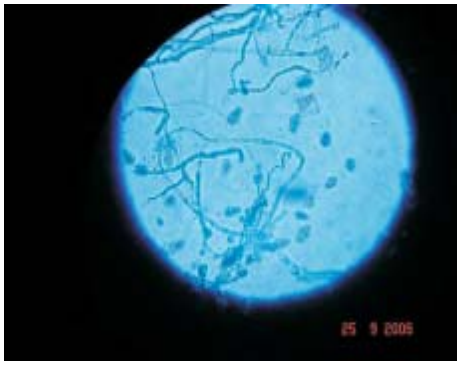


Fig 9b Slide culture and LPCB mount showing curved conidia.

Treatment modalities for *Curvularia* infections have not been standardized yet. Amphotericin B, itraconazole, and terbinafine have so far been used to treat *Curvularia* infections. However, the prognosis is usually poor, particularly for immunocompromised patients. For treatment of allergic sinusitis, surgical treatment and administration of steroids are usually required as well as antifungal therapy. Surgery may be required in other infections.

Engyodontium album

A 55 year old diabetic female came with watering of eyes and slow loss of vision. The patient had been diagnosed earlier of having orbital apex syndrome due to mucormycoses. The conjunctival swab grew *Engyodontium album*.

Engyodontium album is an unusual pathogen but is a rather common inhabitant of waste and moist material, relatively frequently being isolated from substrates such as paper, jute, linen, and painted walls. Its dispersal is by dry, hygrophobic conidia,and hence it may be isolated from house air. The infection reported to be caused by *E. album* include keratitis, brain abscess and eczema vesiculosum. [42]. *Engyodontium* colonies appear white and cobweb like.

Colony characteristics

Colonies moderately expanding, white., appearing lanose to floccose, up to 2mm high, sometimes zonate; reverse ochraceous-buff or uncoloured. (FIg. 10b)

Microscopy

Conidiophores are ascending, 2-4 um wide, somewhat stiff, hyaline and thin walled, with branching pattern subverticillate to verticillate. conidiogenous cells consisting of an elongate to subcylindrical, tapering basal part, and a well developed rachis geniculate, with up to 1 um long denticles. Conidia hyaline, smooth walled, subspherical. **(Fig. 10a and 10c)**

Fig 10a. 10% KOH showing thin septate hyphae (Magnification 40X)

Fig10 b. Culture of *Engyodontium album* on SDA showing white floccose growth

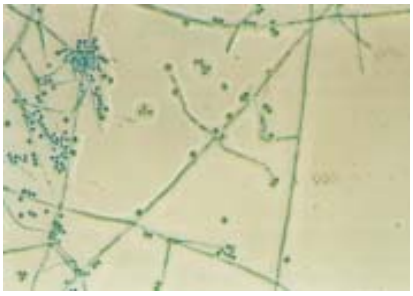


Fig 10c. Slide culture and LPCB mount showing terminal zig zag rachis with conidia (Magnification 20X)

Hormonema dematioides

A 45 year old male diabetic with fungal keratitis grew Hormonema dematioides from the corneal scraping.

Colony characteristics. Colonies expanding, flat, smooth, moist, tough, initially with pinkish tinges, soon becoming olivaceous black.**(Fig 11a)**

Microscopy

Expanding hyphae nonseptate, with irregularly dichotomus branching. Mature hyphae wide, densly septate, cells often becoming wider than long, locally converted into thick walled chlamydospores. Conidia formed percurrently on undifferentiated hyphae from inconspicuous scars, hyaline, non septate, smooth- and thin walled, ellipsoidal, of variable size, often budding, finally becoming septate and olivaceous brown.**(Fig.11b)**

Pathogenicity

A cutaneous pheohyphomycosis was reported and a fatal peritonitis by Shin et al reported fungemia after exposure to birds.

Itraconazole and voriconazole have low MICs for Hormonema dematioides.

Fig 11a. Colonies on chocolate agarexpanding, flat, smooth

Fig 11b. Mature hyphae showing septations and conidia. Magnification (20X)

Bipolaris australiansis

A case of fungal sinusitis grew *Bipolaris australiansis*

Description and Natural Habitats

Bipolaris is a dematiaceous, filamentous fungus. It is cosmopolitan in nature and is isolated from plant debris and soil.

Species

The genus *Bipolaris* contains several species. Among these, three well-known pathogenic species are *Bipolaris spicifera*, *Bipolaris australiensis*, and *Bipolaris hawaiiensis*.

Pathogenicity and Clinical Significance

Bipolaris is one of the causative agents of phaeohyphomycosis. The clinical spectrum is diverse, including allergic and chronic invasive sinusitis, keratitis, endophthalmitis, endocarditis, endarteritis, osteomyelitis, meningoencephalitis, peritonitis, otitis media (in agricultural field workers),and fungemia as well as cutaneous and pulmonary infections and allergic bronchopulmonary disease. *Bipolaris* can infect both immunocompetent and immunocompromised host [29,43]. *Bipolaris* may also be isolated as a laboratory contaminant.

Macroscopic Features

Bipolaris colonies grow rapidly, reaching a diameter of 3 to 9 cm following incubation at 25°C for 7 days on potato dextrose agar. The colony becomes mature within 5 days. The texture is velvety to woolly. The surface of the colony is initially white to grayish brown and becomes olive green to black with a raised grayish periphery as it matures. The reverse is also darkly pigmented and olive to black in color [41]**(Fig 12a)**

Microscopic Features

The hyphae are septate and brown. Conidiophores (4.5-6 μm wide) are brown, simple or branched, geniculate and sympodial, bending at the points where each conidium arises from. This property leads to the zigzag appearance of the conidiophore. The basal scar indicates the point of attachment to the conidiophore. **(Fig12b)**

Amphotericin B and ketoconazole are used in treatment of *Bipolaris* infections. Surgical debridement may be indicated in some cases, such as sinusitis [43]

Fig 12a. Culture of Bipolaris showing black colonies

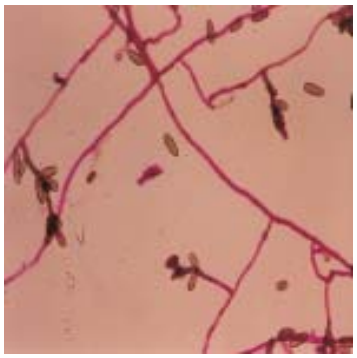


Fig 12b. Slide culture & polyvinyl chloride staining showing septate conidia.(Magnification 20X)

CONCLUSION

The diagnosis of fungal disease is a multi –disciplinary approach requiring cooperation and collaboration of many people with diverse expertise. As most of fungi causing fungal disease are saprophytic in nature, close communication with physician is important to interpret the result of the laboratory. The demonstration of fungi in the tissue by histopathology is important to prove the invasive character of the saprobes. As conventional techniques fail to diagnose most of the invasive fungal infection, the attention of scientists, especially molecular biologists is also required to improve the sensitivity and specificity of diagnosis.

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SCREENING, STABILIZATION AND EXPANSION OF SECRETORY HYBRIDOMAS IN CULTURE AS A STEADY SOURCE OF MONOCLONAL ANTIBODIES (Mabs).

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ABSTRACT:

Aim of the study: *To optimize techniques for selecting, screening and obtaining stable hybridoma cultures and their expansion into medium-scale cultures; as a steady source of Monoclonal Antibodies (Mabs) and also for obtaining affinity purified Mabs from the hybridoma culture supernatants.*

Methodology: *On days 4 and 7, post fusion, 100 µl of supernatant from the limiting dilution wells was replaced. Sub cloning was performed in culture units where more that one clone was observed. 12 hybridoma clones that showed the best culture properties were chosen for expansion into 2 ml cultures and one clone was further expanded into medium scale 5 ml cultures in flasks. Culture supernatants were concentrated by sucrose dialysis or by ultra concentrators and were screened by double diffusion and ELISA. The supernatant from 5ml culture was processed by ammonium sulphate fractionation followed by affinity chromatography. Protein estimation and back calculation for culture volumes was performed to estimate the secretory extent of the 20E5 clone in culture. All the clones obtained were frozen in liquid nitrogen.*

Results: *Limiting dilutions and sub-cloning while ensuring the true monoclonality of the hybridomas, supernatant screening for the immunoglobulin isotype and antigenic specificity provides us with tools for further defining and characterization of the obtained antibodies. The presence of feeder cells, gradual acclimatization of the developing hybridomas to serum free culture conditions stabilized the cultures.*

Discussion: *Monoclonal antibodies (Mabs) are antibodies of the same isotype produced by a single clone of transformed plasma cell with specificity to a single antigenic determining site of a complex antigen. The technology for the production of such Mabs involves immortalization of the antibody secreting plasma cell by fusing the same to genetically compatible, syngenic and non-secretory myeloma cell type; aptly named the hybridoma technique. Pre-fusion parameters are by and large optimized and case-sensitive; but, several post-fusion parameters are important for stabilizing hybridomas to expanded cultures. These parameters can be utilized irrespective of the original antigenic choice, modes of immunizations, hybridization modalities or the secretory extent of the obtained hybridomas.*

Key words: *Hybridomas, cell culture, monoclonal antibodies, stabilization, affinity purification.*

INTRODUCTION:

The revolutionary technological development in 1975 by Kohler and Milstein led to the advent of Hybridoma technology and the production of Monoclonal Antibodies (Mabs). (1) Mabs are those obtained from a single clone of hybridoma cell line and are known for their specificities to a single epitope of a complex antigen. A homogenous collection of such Mabs, all being directed to a particular antigenic determining site of a complex antigen have become invaluable tools for research, diagnostics and therapeutics for human health care, (2) and also for several other clinical applications. (3) The original techniques for the development of hybridomas and to obtain Mabs continuously have undergone dramatic change in recent years with efforts towards efficient means of arriving at stable hybridomas in culture. (4) The generation of stable hybridomas is affected by variables such as: Cell cycle status of B cells in immunized mice at the time of cell isolation, Myelomas used as fusion partner, composition of fusogen, presence of contaminating fibroblasts and macrophages in primary culture, concentration of fused and unfused cells in primary culture, presence or absence of growth factors and serum, presence of feeders and presence or absence of inhibitory substances in culture medium. Two important post fusion techniques necessary for Generating Stable Hybridomas in Culture are (a) the sub cloning of hybridomas and (b) screening of limiting dilution wells for antibody activity; which will enable to expand desired hybridomas as a continuous source of Mabs.

Immediately after fusions of genetically compatible myeloma and B-lymphocytes, limiting dilution is performed to arrive at true single clones of hybridomas thereby ensuring a supply of antibodies that are truly monoclonal. When a culture well of initial limiting dilution contains more than one type of antibody secreting hybrid cells it is cloned to ensure that the antibody secreted is monospecific and homogenous. Sub cloning also ensures that the non producers, originating from the undesired fusions or spontaneous variants, do not over grow the desired hybridomas. Cloning may be achieved in soft agar, by limiting dilution or by using Fluorescence activated cell sorting (FACS). Since cloning in soft agar is tedious and FACS proves expensive, sub cloning is usually done by limiting dilution. (5, 6)

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The purpose of screening is to identify wells containing hybridomas that secrete antibody of desired specificity. The early identification of monoclonal antibodies with the desired specificities is the most critical step in monoclonal antibody production. The initial screening for antibody activity should be done as soon as growth of hybrid cells is seen under the microscope or upon a change in culture medium pH. Although cells have been diluted to limit the number of independent hybrid cells per well, it is important to realize that, a positive clone (secreting the desired antibody) may be detected soon after fusion, but then might be lost due to overgrowth of a negative or other positive clone and also no activity may be detected during the first assay due to the cells of a positive clone being a minority. It is therefore essential to test negative supernatants from actively growing cultures on two or three occasions.

The type of assay used to detect the antibody depends on the nature of the antigen and the type of antibody desired. During the initial screening, for the selection of positive hybrids for cloning, speed, convenience and reproducibility are essential. Technically simple, sensitive and convenient assays are used to screen large number of supernatants to identify the wells containing desired antibodies. Generally screening assays use labeled reagents to detect antibodies. These assays are performed in solid phase and assay the antibodies using reagents labeled with either radioisotopes (radioimmunoassay-RIA) or enzymes (enzyme linked immunosorbent assay- ELISA). Once a clone has shown to secrete antigen specific Mab, efforts should be taken to preserve this clone, allowing them to grow to confluency and supernatants should be screened periodically for antibody activity (7, 8)

Freshly isolated hybrid cell cultures grow slowly and the volume of cell culture is expanded slowly either *in vivo* (in animals) or *in vitro* (in culture flasks). Once the monoclonality of the cultures has been established, the clones are grown sequentially in increasing volumes of culture medium to develop a suitable stock of antibody producing cells. The colonies or cloning wells are transferred to flasks containing medium and diluted with medium as and when there is a change in pH. The Mabs are secreted and it accumulates in the spent medium of the cultured flasks. *in vivo* expansion is done by injecting these tumorigenic lines intraperitonially into histocompatible mice and large amounts of hybridoma derived antibody are then secreted into the ascetic fluid from which they can be purified (8)

Accustoming the hybridoma cells to serum free (SF) medium can be done in different ways and requires adapting cells to SF medium and to establishing the adapted cells in SF medium (needs at least 4-6 weeks of continuous passaging until Mab producing sub lines have established themselves in stable growth). Accustoming can be done in different ways such as by replacing the serum containing medium entirely with SF medium without any adaptation phase; by reducing the serum content slowly over a number of passages- more gentle method but requires a comparatively longer time or also by plating out the same number of cells in medium with decreasing concentration of serum and to which serum replacement has already been done. However the growth and behavior of cells in SF media cannot be predicted and the doubling time of hybridomas may remain the same or may be prolonged under such conditions. Antibody production can be just as good under SF conditions or higher productivity remaining stable for longer periods can be observed. (5, 9)

Apart from the presence of contaminating viruses and mouse Ig along with the secreted Mab in ascites fluid, *in vivo* expansion of hybridomas proves lethal to the animal and is at present not practiced owing to ethical reasons. *in vitro* expansion of hybridomas result in secretion of Mab into culture supernatants that are collected and assayed periodically. Immunoglobulins can be isolated and purified by ammonium sulphate precipitation, ultracentrifugation, ultra filtration, and column chromatography (gel filtration, ion exchange or affinity) (5, 10)

Here, we describe the screening, stabilization and expansion of secretory hybridomas in culture as a steady source of monoclonal antibodies (Mabs). Double Diffusions and ELISA were used for screening the supernatants both for the immunoglobulin isotype identification as well as for the specificities of the antibodies. One hybridoma clone, 20E5 was chosen for sequential transfer, acclimatization and expansion to higher culture volumes in serum free conditions. The supernatant was collected and affinity purification of Mab 20E5 was performed along with deriving at the rate of immunoglobulin secretory activity of the said clone in culture.

MATERIALS AND METHODS:

The details of protocols used for obtaining hybridomas such as the antigenic choice, methods of immunization and parameters for limiting dilutions are discussed elsewhere. (11) Briefly, murine splenocytes were immunized *in vitro* with protein extracts of Chinese Hamster Ovary (CHO) mitotic cells. Sp2/0 cells (histocompatible, non-secretory syngenic myeloma cells) were used as fusion partners and limiting dilutions performed in the presence of syngenic feeder cells in selective medium containing Hypoxanthine, Aminopterin and Thymidine (HAT medium).

Maintaining Hybridomas in Culture

On days 4 and 7, post fusion, 100 μl of supernatant from the limiting dilution wells was aspirated by suction and the wells were refed with 200 μl of fresh HAT medium, supplemented with conditioned medium with/ without serum and incubated at 37 ºC; 5% CO_2. The wells containing more than one hybridoma clone were identified and sub cloned. The hybridomas from such wells were

Figure 1: Syngenic Feeder Cells after 1 week of plating as a stabilizing zone for the single hybridoma cells immediately after limiting dilution and also as a source of conditioning medium for further early expansions of the positive secretory clones. (Inverted Phase Contrast; Magnification: 40 X)

aspirated after gentle mixing, and limiting dilution was done on 96 well plates containing feeder cells (Figure 1) in HAT medium. For the expansions, 13 actively growing hybridomas were aspirated from 96 well plates, and added into 2ml HAT in 24 well plates. One ml of supernatant from the wells containing actively expanding hybridomas were collected into clean eppendorf tubes periodically and screened. The wells were re fed with one ml hybridoma SF medium (with 10% FBS) and the plates incubated at 37 ºC; 5 % CO_2. The supernatants collected were concentrated by sucrose dialysis (for larger supernatant volumes) or using ultra concentrators with cut-off of 50K and screened for antibody by Ouchterlony double diffusion (ODD) and ELISA.

Figure2:Atypical young hybridoma clone stabilizing in culture in the presence of feeder cell.The spherical cells are the hybridomas whereas the cells with extended morphology are the feeder cells. (Inverted Phase Contrast; Magnification 40 X)

One clone 20E5 (Figure 2) was chosen for further expansions into T-25 flasks and the supernatant was concentrated followed by fractionation of antibodies and finally to obtain affinity purified Mabs. 8 ml of supernatant from actively expanding Hybridoma was collected into dialysis tubing and was placed on sucrose powder in a pertidish. The tubing was covered completely with sucrose powder and left undisturbed at room temperature. The moist sucrose powder was periodically replaced, and carefully monitored until the supernatant loaded showed a marked decrease in volume. The concentrated supernatant was then collected in a clean eppendorf tube, labeled and stored at 4 ºC. Supernatant samples from clones in 2ml cultures, of about 0.5 ml volumes, ultra concentrators with a molecular weight cut-off of 50 KDa were used for concentrations. The supernatants were loaded into the ultra concentrators and centrifuged at 10,000 rpm for 5 minutes at 4 ºC. Following centrifugation, the filtrate was discarded and the sample was reloaded and centrifuged. The retentate containing the concentrated proteins were collected in a clean eppendorf tubes, labeled and stored at 4 ºC.

The concentrated samples were screened for specific activity to anti- mouse IgG whole serum and anti- mouse whole serum by ODD. 1 % Agarose was prepared in PBS as slides. Wells were punched in the gel and scooped. 10 μl of supernatant was added to the central wells and 10 μl of anti- mouse IgG whole serum and anti- mouse whole serum were loaded in the peripheral wells. The slides were left in a humid chamber at room temperature overnight. The following day, the Agarose slides were observed for the presence or absence of precipitin bands. The slides were documented by several washes in PBS to remove unbound proteins, stained with Coomassie Brilliant Blue, and heat-dried. Ouchterlony Double Diffusion was performed in 5ml, 1% Agarose Gels in Saline on standard microscope glass slides for several supernatants from about 10th day of limiting dilution. Typically, about 5 supernatants were screened at a time in one gel slide as a continuous process right up to the final concentrated supernatants of the 13 clones. As the process was routine and standard technique, gel preservation followed by documentation was not made. However, the ELISA screening of the same supernatants are well documented as we used a software based approach and the details of the Plate lay-out, Programme used, Absorbance Readings directly on the wells are well documented and data stored.

The same samples were then screened for specific activity to the CHO mitotic cytosolic protein antigen by ELISA. The antigenic coating to the wells of the ELISA plates is done by dissolving the antigen in a coating buffer. A 50% antigen was prepared as 1:1 v/v ratio of CHO mitotic cytosolic protein in the coating buffer and 100 μl was added to each of the wells of the ELISA plate. The plates were incubated at 4ºC overnight. Following incubation, the plates were washed once with 200μl washing solution and the unreacted sites blocked with blocking solution. The plate was incubated at 37ºC for an hour following which, the plate was decanted and washed thrice with 200 μl of wash solution. 50μl of each of the concentrated supernatant was added to the respective wells and incubated at 37 ºC for an hour. The plate was decanted and washed thrice with 200 μl of wash solution. 100 μl of 1x anti-mouse IgG

HRP conjugate was added to each well and incubated at 37 ºC for an hour. Following incubation, the wells were decanted, washed thrice with 200μl of wash solution and 100 μl of substrate solution (TMB/H_2O_2 in substrate buffer) was added, incubated at room temperature for 10 minutes. The reaction was arrested following development of blue color which turned yellow upon addition of stop solution and OD read at 450 nm.

Fractionation of Mabs by Ammonium Sulphate Precipitation

The supernatant from 20E5 expanded culture was centrifuged at 10000rpm for 10 minutes at 4 ºC. To the supernatant 45% v/v of saturated ammonium sulphate was added and incubated at 4 º C overnight. The following day the tubes were centrifuged at 10,000 rpm for 30 minutes. After centrifugation, the supernatant was discarded and the pellet was dissolved in 1ml PBS, loaded in dialysis membrane and left in 250 ml PBS overnight at 4 º C. PBS was changed periodically and extent of dialysis was monitored by the extent of precipitate formed with barium chloride in the spent PBS.

Affinity Purification of Mabs by Chromatography

The Protein-A CL- Agarose Column was equilibrated with 15ml of equilibration buffer. Following equilibration of the column, 1.5 ml of the fractionated antibody sample was mixed with equal volume of equilibration buffer and loaded into the column. The undesired proteins present in the sample were washed using the equilibration buffer. The desired fraction containing monoclonal antibodies of the IgG isotype were eluted using 15ml of the elution buffer. The eluted sample was collected in a 15ml centrifuge tube, and neutralizing buffer 25 μl/ml of sample was added, and the tube was labeled and stored at -20 ºC. The column was washed with storage buffer and stored at 4 ºC. Protein estimation of the affinity purified Mabs was done by Bradford method.

Freezing of Hybridomas

The cells from actively, expanding hybridomas were collected in 0.5 ml of Freezing medium; transferred to sterile cryovials, with the well numbers labeled on the cryovials and stored in cryopack at -70º C for 3 hours to ensure the reduction of temperature of -1 ºC every one minute. After three hours the vials were transferred to canisters and stored in liquid nitrogen in cryocans.

RESULTS:

Re-feeding of limiting dilution wells was done on day 4 and 7 post fusion as guided by the rate of cell growth. This was done by removing of about half the culture medium by suction, followed by its replacement with fresh HAT medium. (With 10 % conditioned medium). Nonetheless, few of the wells showing rapid change in color of medium and increased cell density were refed more often. (Figure 3)

Figure 3: Micro culture plates of 96 wells, ten days after limiting dilution showing distinct change in medium color indicating wells with metabolic activity associated with cell division and growth. Pink colored wells indicate the original color of the medium where there is no apparent cellular activity and various shades of yellow indicate sufficient activity necessitating the need for screening such supernatants for Mab.

A small number of wells showing multiple clones of cells were observed. (Figure 4) These were sub cloned by limiting dilution to ensure monoclonality of the hybridomas. To obtain, sufficient quantities of supernatants for screening, the hybridomas were expanded into serum free DMEM, supplemented with HAT in 24 well plates. The plates were incubated at 37 ºC; 5 % CO_2 and regularly observed for the development of clones.

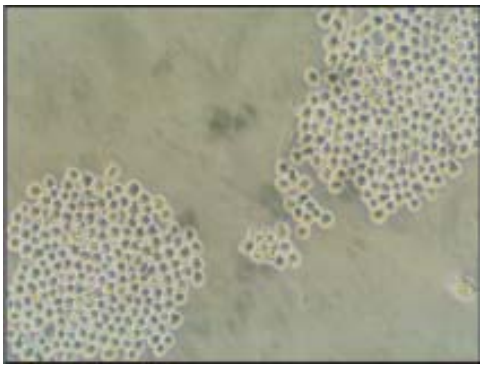
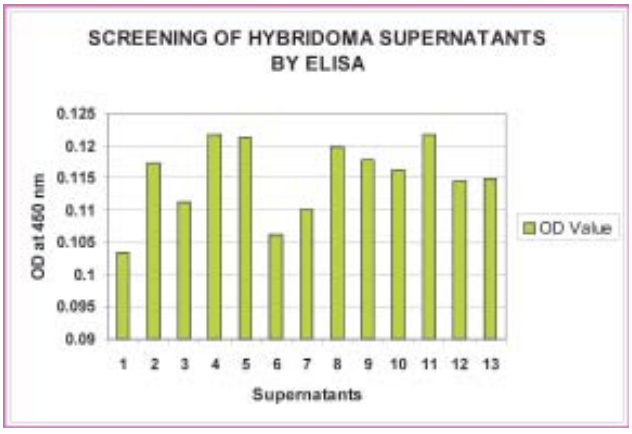


Figure 4: A culture unit showing two simultaneously developing healthy hybridoma clones necessitating the need for sub-cloning to ensure the true monoclonality of the hybridoma cell line and subsequent monospecificity of Mabs secreted. (Inverted Phase Contrast; Magnification 40 X)

Supernatants from T-25 flasks with expanded hybridoma cultures were concentrated 5 folds using sucrose. 8ml of supernatant loaded initially yielded 1.5 ml of concentrated sample. Concentration of supernatants resulted in its decreased volume and an apparent change in its color (appeared darker) was observed. The supernatants of lower volumes (from 1ml cultures) were concentrated 10 folds by using ultra concentrators yielded a final volume of 100 μl from an initial volume of one ml. The concentrated samples were collected in eppendorf tubes, labeled and stored at 4 ºC.

ODD results showed the presence of precipitin line between wells containing the supernatant and those containing anti mouse IgG whole serum and anti mouse whole serum. The precipitin bands obtained between wells containing supernatant and anti mouse IgG whole serum were sharper, than the one in between the wells containing the supernatant and anti mouse whole serum indicating the presence of Mabs of the IgG isotype. The ELISA results obtained showed the presence of monoclonal antibodies of the IgG isotype in all 13 the supernatants tested. However, the concentration of IgG present in the supernatant varied within close range. In Figure 5, the numbers denote supernatants obtained from the specific wells of 96 well plates that contain hybridomas derived from splenocytes exposed to particular antigenic volume. For eg 10 D5- denotes the supernatant from the wells that contain hybridomas obtained from D5 well of 96 well plates that have been exposed to 10μl of antigen.



S.No		1	2	3	4	5	6	7	8	9	10	11	12	13
1.	Supernatant from well	10 D5	10 F9	20 E5	10 C8	15 E5	10 G6	10 F3	20 C7	20 C8	20 D9	10 G10	15 D4	10 F5
2.	OD value	0.103	0.117	0.111	0.122	0.121	0.106	0.11	0.119	0.117	0.116	0.121	0.114	0.115

Figure 5: ELISA OD values obtained by screening the hybridoma supernatants for specific antigen.

One clone 20E5, apart from being an antigen specific hybridoma, showed the best cultural characteristics, rapid doubling time, and thus selected for expansion into T-25 flasks and supernatant collected after sufficient metabolic activity was observed. (Figure 6) The Mabs were isolated from culture supernatants by ammonium sulphate precipitation, purified by dialysis and IgG isotype isolated by affinity chromatography. Salt fractionation yielded 8ml of suspension from 15ml of supernatant. Finally, 1 ml of Affinity Purified Mab (in Phosphate Buffered Saline) with a protein concentration of 92.5 mg / ml was obtained by Protein-A affinity chromatography. Therefore, the hybridoma clone 20E5 was secreting 06.16 mg / ml of Mab into the supernatant in culture. (Figure 7)

Figure 6: A healthy expanding hybridoma clone with secretory properties about ten days further to limiting dilution in the presence of feeder cells. (Inverted Phase Contrast; Magnification 20 X)

Figure 7: A semi confluent culture of the Hybridoma clone in the presence of feeder cells, 15-20 days further to limiting dilution. (Inverted Phase Contrast; Magnification 20 X)

The healthy hybridomas were frozen in 1.0 ml of cell freeze medium in cryovials and were stored in liquid nitrogen.

DISCUSSION:

Polyclonal antisera owing to greater avidity to a polyvalent antigen has wide applications in areas where such multiple interactions are required as in the case of heamagglutination, or whole-bacterial agglutination and complement mediated lysis. The advantages of such polyclonal antibody generation are distinct in typical *"in vivo"* situations. Monoclonal antibodies (Mabs) on the other hand have become indispensable for finer *"in vitro"* applications as well as in increasing usage in human diagnostics and therapeutics. The technical modalities of the generation of hybridomas have seen much refinement since their original description. Major challenges here are to ensure a stable, healthy hybridoma culture as a source of monoclonal antibodies, the time and cost involved in such production and also the isotype of the Mabs produced. Some of these were addressed by the recent developments such as *"in vitro"* immunizations of murine splenocytes which eliminated repeated animal handling, circumvented the need for booster doses of antigenic administration and test sera check for a generated immune response in the experimental animals. Also, a distinct advantage is the considerable reduction in time required for stimulation of eretentate and can thus be concentrated. Concentration resulted in increased antibody concentrations in lower supernatant volumes thereby increasing the sensitivity.

Initial screening of hybridoma supernatants to check for the presence of antibodies was done using Ouchterlony double diffusion. Subsequent screening was done using techniques with relatively higher sensitivity, (that did not require concentration of supernatants) such as ELISA. ELISA with Antimouse IgG confirmed the IgG isotype of the secreted Mabs and ELISA with the antigen gave us the specific nature of the antibodies. While ODD required concentrated supernatants for identification of secreted Mabs, ELISA being a more sensitive technique did not require the same.

The IgG isotype antibodies are preferable to the initial IgM response as for a variety of applications not only owing to the high affinity but also because secondary Antimouse IgG conjugates (either with dyes or enzymes) are readily available. (13) Such IgG responses were achieved by a slightly extended duration of immunization in this study without compromise on the health of the splenocytes. Mabs were fractionated using ammonium sulphate precipitation method and were further affinity purified using a Protein A column.

The hybridomas are fragile initially after fusion and are different from the parental cell types. Several factors such as random chromosome loss can either turn secretory cells non-secretory or the hybridomas might even collapse and fail to expand. A very important step in Hybridoma technology is to obtain the hybrid cells essentially as a continuous culture. The present study highlights on the parameters that can be applied for obtaining continuous stable cultures. While previously reported studies give us a detailed account on the production of Human Mabs, (14, 15) the combination of those techniques for immunization and immortalization of the clones in conjunction with those described above for the stabilization and expansion of secretory hybridomas can result in enhancing the applicatory potential of the Mabs for research, diagnostics and therapeutic.

CONCLUSION:

The screening techniques utilized and described in this study gave us the differentiation of positive secretory clones and nonsecretory clones. Also, the isotype of the antibodies and their specificities were ascertained by the screening techniques. Stabilization of the desired hybridoma clones as cell-lines in culture was optimized by expansions from 96 well plates as 0.2ml cultures to 5 ml cultures in T-25 flasks. While sucrose dialysis was employed to concentrate larger volumes of the culture supernatants, smaller volumes were concentrated by ultra-concentrators with 50 KDa cut-offs that gave us culture supernatants with detectable quantities of Mabs even by double diffusions while ELISA was employed for more sensitive screening. Healthy expanding hybridomas were identified and cryopreserved.

The culture supernatant from the clone 20E5 was harvested, fractionated and affinity purified which yielded antigen specific Mabs of the IgG isotype. The techniques employed and described above are not antigen-specific and can readily be employed for obtaining a constant source of Mabs specific for any antigen which are invaluable biological reagents for a variety of purposes towards human health care.

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FORMULATION AND EVALUATION OF CHURNA FOR DIGESTIVE PROPERTY

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ABSTRACT

Back ground:

Ayurvedic medicines play an important role in gastro intestinal problems due to safety and efficacy in it. Hence churna meant for digestive property has been formulated by standard procedures and evaluated by physical and analytical methods.

Method:

The formulation consists of fine powder (sieve 60 size) of dried rhizomes of Zingiber officinale, fruits of Foeniculum vulgarae, barks of Cinnamomum zeylanicum and fruits of Trachyspermum ammi in appropriate proportions (2:2:1:1) and mixed well. Physical parameters viz, total ash, acid insoluble ash, water extractive values, alcohol soluble extractive values and crude fibre content besides heavy metal analysis were carried out. The microbial load of formulation for Escherichia coli was also determined. The efficiency of churna for digestive property is determined by finding the amylolytic activity and lipolytic activity and compared with GASTRAP a marketed formulation for gastritis.

Results:

Ash values and extractive values were found to be within prescribed limits. The arsenic level was found to be 0.205ppm. Churna did not show the presence of any Escherichia coli and other microorganisms. The churna showed pronounced amylolytic and moderate lipolytic activity when compared to GASTRAP proving its efficiency for digestive problem.

Key words: *Ayurvedic Medicines, Digestion, Complementary Therapies*

INTRODUCTION

Churna is defined as a fine powder of drug or drugs in Ayurvedic system of medicine. Drugs mentioned in patha, are cleaned properly, dried thoroughly, pulverised and then sieved. The churna is free flowing and retains its potency for one year, if preserved in an airtight containers. Triphala churna, Trikatu churna, Drakeshadi churna and Sudharsana churna are some of examples. Churna formulation are similar to powder formulations in Allopathic system of medicine. In recent days churna is formulated into tablets in order to fix the dose easily. These forms of medicament are prescribed generally because of their particle size. Smaller the particle size greater is the absorption rate from g.i.t and hence the greater is bioavailability. It is prescribed by the Ayurvedic physician for treating conditions such as diabetes, indigestion, constipation etc. Indigestion is a common ailment affecting the general population and in allopathy system antacids are commonly prescribed. Since the usage of such aluminium containing antacids cause deleterious effects like Alzheimer's disease upon long term usage, we explored an alternative and safe remedy for indigestion. Hence we prepared a churna with natural ingredients commonly used by mankind for culinary purposes. Thus the present study examined the favourable influence of four spices formulated into churna said to have digestive property. The common ingredients of these churna were Ginger (*Zingiber officinale*), Ajowan (*Trachyspermum ammi*), Cinnamon (*Cinnamomum zeylanicum*) and Fennel (*Foeniculum vulgare*). The formulated churna derived from above said drugs is reported to have a wide range of biological activity. Ginger contains aromatic principle like Zingiberine and bisaboline while pungent principles are gingerols and shogaols. Other components are nerol, geraniol, d-camphor, β-Phellandrene, linalool, α-farnesene, [1] Shagoal, [2] and also diarylheptanoids such as gingerone A&B. This is used in the treatment of flatulence, colic, indigestion, vomiting, constipation. It also maintains the tonicity of intestine muscle [3,4]. Ajowan was found to contain essential oil that contains 50% thymol. This is used in traditional medicine for the treatment of indigestion and also as antispasmodic [5]. Cinnamon contains cinnamaldehyde, which is a phenylpropene derivative [6]. It was found to possess antibacterial property and is mostly used as carminative. Fennel contains anethole and fenchone. This is mainly used as a carminative [7,8,9,10].

An earlier report on the digestive and carminative property of the mentioned ingredients prompted us to formulate and evaluate the digestive enzyme activity namely amylolytic, lipolytic and proteolytic activity in comparison with GASTRAP (marketed formulation) used as a digestive agent.

MATERIALS AND METHODS

PREPARATION OF CHURNA:

The raw materials such as rhizomes of *Zingiber officinale* (2 parts), fruits of *Foeniculum vulgarae* (2 parts), barks of *Cinnamomum zeylanicum* (1 part) and fruits of *Trachyspermum ammi* (1 part) were used for the preparation of the formulation. The raw materials used for this formulation were purchased from the market and authenticated in the Pharmacognosy department of Sri Ramachandra College of Pharmacy. The authentication is

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carried out based on the microscopic characteristics of powdered drug. The finely powdered raw materials were passed through sieve number 60 and mixed in appropriate ratios (16.7g of *Zingiber officinale* and *Foeniculum vulgarae*, 8.7g of *Cinnamomum zeylanicum* and 8.7g of *Trachyspermum ammi*). The churna was packed in an air tight glass container, [11]

EVALUATION OF PHYSICAL PARAMETERS:

1) Determination of pH [13]

The pH of 1% solution of formulated churna was determined using pH meter (Elico pH meter).

2) Determination of Moisture content [13]

The moisture content of churna was found using halogen moisture determining apparatus (Mettler Toledo).

3) Determination of Ash Values [13]

I. Total Ash Value

2gms of churna was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600° C until, it appeared white indicating absence of carbon. It is then cooled in a dessicator and total ash in mg per gm of air dried material is calculated.

II. Acid Insoluble Ash Value

To the crucible containing total ash, 25ml of Hcl was added and boiled gently for 5minutes, then about 5ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ashless filter paper. This was then washed with hot water until filterate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed.

4) Determination of Extractive Values [13]

I. Water Soluble Extractive Value

5gms of churna was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100ml of chloroform water for 18hours. It was then filtered and about 25ml of filterate was transferred into a chinadish and was evaporated to dryness on a waterbath. It was then dried to 105° C for 6hours, cooled and finally weighed.

II. Alcohol Soluble Extractive Values

Ethanol was used as solvent in place of chloroform water and remaining procedure was the same as that of water-soluble extractive value.

5) Determination Of Crude Fibre Content [14]

2gms of accurately weighed churna was placed in a round bottom flask and then 100ml of 0.128 M sulphuric acid was added and refluxed for 1 hour then filtered through ashless filter paper and the residue was washed with water until filterate becomes neutral. The residue was then weighed (a), ignited to ash and finally the weight of ash (b) was determined. The difference between a and b represented the crude fibre content and was calculated on dry weight basis.

6) Determination of Heavy Metal Contamination

I. Arsenic Content [14]

Preparation of Standard Solution (10PPM)

0.33gms of arsenic trioxide was dissolved in 5ml of 2M Sodium hydroxide solution and then diluted to 250ml with water. One volume of this was then diluted to 100 volume with water.

PREPARATION OF SAMPLE

Preparation of Churna solution

The churna solution was prepared by means of diluting 1gm of churna to 100ml using distilled water. This is used to carryout limit test for iron and lead and also to perform qualitative test for mercury.

10ml of churna solution was pipetted out into a flask and about 10ml of concentrated nitric acid was added and evaporated to dryness on a waterbath. The residue was then dried at 130° C for 30minutes then about 10ml of hydrazine molybdate reagent was added and refluxed for 20minutes. The solution was then cooled and absorbance of both standard and test solution was measured at 800nm using Perkin Elmer UV spectrophotometer.

Ii. Limit test for Iron [14]

Preparation of Standard Solution (20 PPM)

One volume of 0.1726% w/v solution of ferric ammonium sulphate solution was diluted in 0.05 M sulphuric acid to ten volume using distilled water.

PROCEDURE

Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for 5minutes and colour obtained in sample was compared with that of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs.

III. Limit Test For Lead [14]

Preparation of Standard (20 PPM)

0.4 gm of lead nitrate was dissolved in water containing 2ml of nitric acid and sufficient water to produce 250ml. About 1volume of above solution was diluted to 10 volume using distilled water.

PROCEDURE

Limit test was performed in Nessler's cylinder. 1ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25ml using distilled water

and then pH was adjusted to value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35ml using distilled water. To both the solutions 10ml freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50ml. It was then allowed to stand for 5minutes and viewed downwards over white surface. The colour produced in test solution should not be more intense than that of standard solution, if so then the sample is said to pass the limit test for lead.

IV. Test for Mercury

To 10 drops of test solution 6M Hcl was added to get a white precipitate. The precipitate was then treated with 6M ammonia solution. If the colour of precipitate changes to grey or black colour then it indicates the presence of mercury.

7) DETERMINATION OF MICROBIAL CONTENT

1gm of churna was dissolved in lactose broth and volume adjusted to 100ml with the same medium. About 10ml of sample was transferred into 100ml of Macconkey broth and incubated for 18-24 hours at 43-45°C. A subculture was prepared on a plate with Macconkey agar and incubated at 43-45°C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram negative rods appearing as reddish zones indicates the presence of *E.coli* if not then it indicates the absence of *E.coli*.

Determination of Digestive Property

Preparation of Extract

About 100mg of accurately weighed quantity of churna was extracted with 20% aqueous glycerol and phosphate buffer (pH7.8) in 1:4 ratio and filtered and the filtrate was used as enzyme source. [15,16]. The standard sample was prepared similar to the test sample.

i. Amylolytic activity

Extract (1ml) of churna and GASTRAP were incubated separately for 15minutes at 27°C and added to 1ml of the substrate (soluble starch1% in phosphate buffer). The enzyme reaction was interrupted by the addition of 2ml of DNS reagent and heated for 5minutes. The absorbance was measured at 520nm. [17, 18].

ii. Lipolytic activity [19,20]

Preparation of Substrate Solution

2ml of castor oil was, neutralized to pH 7 and stirred well with 25ml of water in the presence of 100mg of bile salts (sodium taurocholate) till an emulsion was formed.

PROCEDURE:

Taken 20ml substrate and added 5ml phosphate buffer at pH 7. The contents were stirred slowly in magnetic stirrer and the temperature was maintained at 35°C. The electrodes of the pH meter were dipped in reaction mixture and the pH was adjusted to 7. The enzyme extract (0.5ml) was added immediately and pH recorded. The timer was set such that at zero time the pH was observed as 7. Then pH dropped by 0.2 unit with addition of N/10 NaOH was noted. The pH was brought to initial value and was continued for 30 to 60 minutes. The volume of alkali consumed at each time was noted.

$$\text{LIPOLYTIC ACTIVITY} = \frac{\text{Volume of alkali x Strength of alkali}}{\text{Weight of sample x Time in minutes}}$$

i. Proteolytic activity [22]

Preparation of Substrate Solution

200 ml of boiled milk was treated with acetic acid till caesin precipitates out. The precipitate was then removed, dried and powdered. One gram of prepared caesin was diluted to 100ml using distilled water.

PROCEDURE

Taken 1ml of substrate solution added 1ml of 0.1M phosphate buffer (pH 7.6) and 1ml calcium chloride. To this 1ml crude enzyme extract was added and digestion stopped after 1hour of incubation with 3ml of 5% trichloroacetic acid solution. After 10 minutes precipitate was removed by centrifugation and one portion of supernatant was mixed with 5ml Lowry's reagent. The mixture was then stained with dilute Folin- Ciocalteau reagent (1:2) and optical density measured at a wavelength of 650 nm. The proteolytic activity was then calculated from standard curve in milligrams of tyrosine. Protien estimated by standard method and results were given in milligrams of liberated tyrosine per milligram of dissolved protien per hour at 37° C as specific activity.

RESULTS:

The results of the physical parameter evaluation such as heavy metals, moisture content, ash values including total ash value, acid insoluble ash value, extractive values such as water soluble & alcohol soluble extractive values and crude fibre content were given in table 1 and detection of heavy metals such as arsenic, iron, lead and mercury in table 2. Finally the result of microbial detection was given in table 3.

TABLE 1

EVALUATION OF PHYSICAL PARAMETERS OF CHURNA

S.No	Physical Parameters	Values
1	pH	5.357
2	Moisture content	10.8 % w/w
3	Ash Values	
	I. Total ash II. Acid insoluble ash	10% w/w 5% w/w
4	Extractive values	
	I. Water soluble extractive value II. Alcohol soluble extractive value	0.12% w/w 2% w/w
5	Crude fibre content	9.75% w/w

TABLE 2
DETECTION OF HEAVY METALS IN CHURNA

S.No	HEAVY METAL	Values
1	Arsenic (Spectrophotometry)	0.205 ppm
2	Iron (Limit test)	Within the limit
3	Lead (Limit test)	Within the limit
4	Mercury (Qualitative analysis)	Absent

TABLE 3
DETECTION OF MICROBES IN CHURNA

S.No	MICROORGANISM	Present / Absent
1	*Escherichia coli*	Absent

The amylolytic activity of the churna was found to be 0.294mg/ml while that of GASTRAP was found to be 0.28 mg/ml. The values are represented in fig 1.

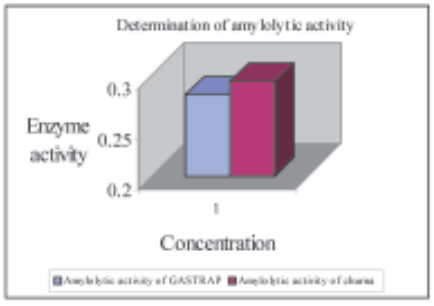

Fig 1.

The lipolytic activity of churna was found to be 0.01633 while that of GASTRAP was found to be 0.02294.The values are represented in fig 2.

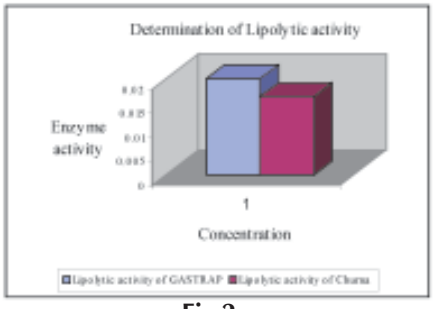

Fig 2.

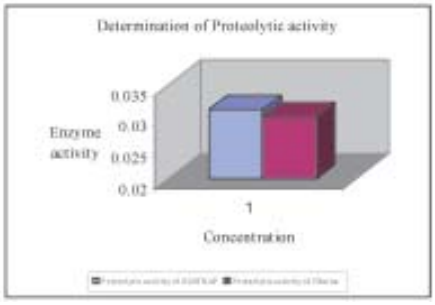

Fig 3.

The proteolytic activity of churna was found to be 0.030 mg/ml while that of GASTRAP was found to be 0.031 mg/ml.

DISCUSSION:

The churna consisting of fine powder of herbs in appropriate ratio was subjected to standardisation by means of various physical, chemical and microbiological methods. The physical parameters such as pH was determined to avoid gastric irritation and the moisture content was determined to find out any increase in weight caused by moisture absorption. The value obtained was found to be within the standards. Since ashing process involves oxidation of components of product, an increase in ash value indicates contamination, substitution and adulteration. The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value obtained is an indicative of silicate impurities, which might have arised due to improper washing of crude drugs. Both the ash values obtained were found to be within the standard limits. The extractive values namely water-soluble and alcohol soluble indicates the amount of active constituent in given amount of plant material when extracted with respective solvents, a lower value compared to standard value indicates presence of exhausted material. In the present study both the extractive values were found to be more than the standard values. The determination of crude fibre content is an indicative of fibre content in formulation and was found to comply with the standard value. Heavy metals if present in formulations will have a deleterious effect on different organs of body in particular kidneys and leads to renal toxicity. Hence evaluation of heavy metals is an important role. Heavy metals include arsenic, iron, lead and mercury. In the present study arsenic was evaluated by means of spectrophotometry, iron & lead by means of limit test where the allowed maximum limit were 20ppm respectively and were found to be within the limits. The presence of mercury was determined qualitatively and found to be absent. The formulated churna was finally subjected to microbiological evaluation namely for *E.coli* and was found to be absent hence the formulated churna complied with the WHO requirements.

The biological activity of churna was evaluated by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with the standard marketed formulation GASTRAP. The amylolytic activity involves the break down of starch into maltose by the action of amylase enzyme. Determination of amylolytic activity brings out the ability of churna to digest starch. In the present study the amylolytic activity of formulated churna was found to be 1.4% greater than that of marketed formulation GASTRAP. Hence the formulated churna was considered to possess the activity of digesting starch. Lipolytic activity is another enzymatic activity that involves the break down of lipids into fatty acids by the action of lipase enzyme. Determination of lipolytic activity brings out the ability of digesting lipids by particular substance. In the present study the lipolytic activity of formulated churna was found to be slightly lesser than that of

GASTRAP. Proteolytic activity is an enzymatic activity that involves break down of proteins into aminoacids by the action of protease enzyme. Determination of proteolytic activity brings out the ability of digesting proteins by a particular substance. In the present study it was determined by means of using folin-ciocalteau method where the phenolic group present in the liberated aminoacid namely tyrosine forms a complex with the reagents added and found to absorb in a wavelength of 660nm. The intensity of colour depends on the amount of aromatic aminoacids present and hence gives the proteolytic activity of churna. In the present study the proteolytic activity of formulated churna was found to be almost equal to that of marketed formulation GASTRAP.

CONCLUSION:

The physical parameters evaluated confirm the standard of the formulated churna. The invitro study of enzymatic activity carried out by above methods brings out the fact that the formulated churna possess the property of digesting starch, lipids and proteins similar to that of marketed formulation GASTRAP.

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COMMUNICATIVE AND COGNITIVE PERFORMANCE OF AN INDIVIDUAL WITH RIGHT HEMISPHERE DAMAGE : A CASE REPORT

Perumal R.C [a], Sundeepkumar.V [a], Reethee A.M.[a]

ABSTRACT

The right and left hemispheres specialize in different functions. The damage in the right hemisphere caused due to stroke, TBI, surgery, infection/illness and tumor are termed as Right Hemisphere Damage (RHD). This damage can lead to cognitive-communication problem such as impaired memory, attention problems, poor reasoning and dysprosodia. This case report presents a thorough analysis of communicative-cognitive performance of an individual with right hemisphere damage consequent to Road Traffic Accident and effect on contra-coup injury on communication and recovery of communicative- cognitive functions.

Key Words: *Traumatic Brain Injuries, cognition, case report*

INTRODUCTION

The functions of the right cerebral hemisphere are complex and diverse and can be regarded as non-dominant or minor only with regard to the linguistic abilities of the left hemisphere. Spatial and affective functions dominate the activities of right hemisphere. These functions may have originally occupied both hemispheres but became lateralized to the right by the asymmetrical acquisition of language abilities by the left hemisphere (1). Whatever the evolutionary background, damage to the right cerebral hemisphere gives rise to complex neuropsychiatric, neurobehavioral deficits (1); linguistic and extra linguistic deficits **(2)**.The causes of right hemisphere damage (RHD) include stroke, traumatic brain injury, surgery, infection/ illness and tumor.

Traumatic Brain Injury (TBI), also called as simple head injury, occurs when a sudden trauma causes damage to the brain (3). TBI can result from a closed head injury (non-penetrating head injury) or an open head injury (penetrating head injury). The leading causes of TBI are falls (28%), motor vehicle-traffic crashes (20%) and assaults (11%) (4). The complications of TBI are abrasions (scrapes), lacerations (cuts), contusions (bruises), coup injuries (trauma at the point of impact) and contra-coup injuries (trauma at the opposite side of the impact). TBI can cause changes in one or more areas such as thinking, reasoning, understanding words, remembering things, paying attention, solving problems, talking, behaving, walking, seeing and/or hearing and learning (5).

People with RHD experience communication problems that are more subtle in nature than those occur from left hemisphere damage. This is due to the fact that, in most of the population, the language centers are in left hemisphere, while cognitive functioning is often housed in the right hemisphere. Because of this, patients were not routinely treated by speech language pathologists until recently. It is currently recognized that they frequently have both communicative and cognitive deficits which can be addressed by speech therapy (6).

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CASE REPORT:

A 60-year old right-handed male was presented to the Department of Speech, Language and Hearing Sciences in June 2006 with RHD consequent to Road Traffic Accident (RTA), which occurred four years ago. He was brought to the department with the complaint of poor communicative and cognitive performance. The client's mother tongue is Telugu and other languages exposed were Tamil and English. History revealed right-sided fall resulting in closed head injury consequent to RTA (Road Traffic Accident). Details of the accident are not known.

Pre-surgical Investigations:

CT scan of brain done in a private hospital on January 2002 revealed right temporal contusion and left parietal acute sub-dural haematoma.

Surgery Details:

The client underwent i) emergency right temporal craniectomy and removal of contused parts, ii) left parietal burr hole and evacuation of acute sub-dural haematomas (Fig 1 and Fig 2)

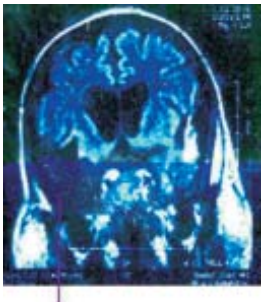

Fig - 1: MRI showing right tempo craniectomy

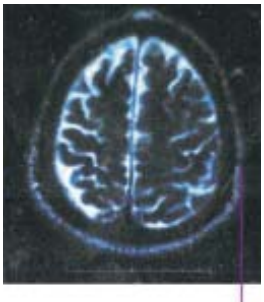

Fig -2 : MRI showing left parietal burr hole

Post-surgical investigations:

CT scan of the brain done on January 2002 showed resolving contusion with resolving edema resolved sub-dural haematoma and right internal capsule infarct. MRI of the brain

in July 2002 (Fig 3 and 4) revealed cystic encephalomalacia in right temporal region, gliotic changes in left temporo-parietal region, old cystic lacunaes in right centrum semiovale and wallerian degeneration of midbrain in right side.

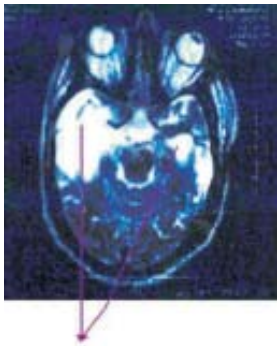

Fig - 3 : MRI showing cystic encephalomalacia in right temporo - parietal and left temporal regions

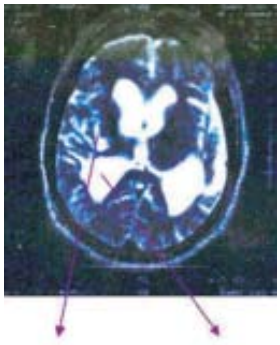

Fig - 4 : MRI showing chronic infarct on right side

Neuropsychological assessment in July 2002 revealed very poor attention and eye contact, anasognosia, proposagnosia, poor facial expression, dysprosodia which are typical features of RHD. The client was advised to attend cognitive therapy for improvement of cognitive functioning of the client and the client has been attending the same since July 2002. Neurological evaluation in July 2002 revealed right third cranial nerve paresis. Right-sided hemiplegia was present. The client was advised for physiotherapy to improve the motor skills.

No speech and language evaluation had been done then. But his speech reported to be fluent and meaningless by the spouse. He was also reported to obey simple commands with gestures inconsistently. The client was brought to our clinic in May 2006 for detailed assessment of communicative abilities and for further management.

Detailed speech and language examination revealed the presence of right and left hemisphere symptoms. The right hemisphere symptoms noticed were poor attention and eye contact (7), impaired visuo-spatial functions and self-orientation (8), poor judgement, reasoning, short-term memory and dysprosodia (6), anosognosia (9), pragmatic and extra linguistic deficits of communication (deficits in perceptual and attentional aspects) (10). This was due to direct trauma on the right side of the brain. Left hemisphere symptoms noticed were poor auditory comprehension (6), paragrammatism (deficits in grammatical structure) (11), semantic paraphasia (substitution of one word by another within the same lexical category), fluent but meaningless speech and perseveration, naming and repetition were fair (12). Presence of left hemisphere language symptoms could be attributed to contra-coup injury. The client had undergone the following tests at the department:

WAB (Western Aphasia Battery) (13) was assessed to qualitatively determine the type of aphasia noticed and the areas assessed were spontaneous speech (including information content and fluency), comprehension, repetition and naming. Table-1 elaborates WAB scores of the client. The client had an Aphasia Quotient of 50, indicative of Wernicke's aphasia. He was provisionally diagnosed to have '*Wernicke's aphasia*'. Diagnostic formulation also included dysprosodia. The family members were counselled regarding the communicative deficits and the importance of Speech and language therapy and were advised to attend therapy in the department. The client is being provided with the same since May 2006 focussing on improving the communicative-cognitive performance of the client.

Table-1: Pre and Post-Therapy WAB scores of the client

S. No	Areas assessed	Scores	
		Pre-therapy	**Post-therapy**
1.	Spontaneous speech		
	i) Information content	5	6
	ii) Fluency	6	6
	Total	**11/20**	**12/20**
2.	Comprehension		
	i) Yes/No questions	0	10
	ii) Auditory word recognition	50	50
	iii) Sequential commands	0	10
	Total	**2.5/10**	**3.5/10**
3.	Repetition	44	80
	Total	**4.4/10**	**8.0/10**
4.	Naming	54	56
	i) Object naming	6	11
	ii) Word fluency	9	9
	iii) Sentence completion	2	5
	iv) Responsive speech	71	81
	Total	**7.1/10**	**8.1/10**
	Type of Aphasia	**Wernicke's Aphasia**	**Transcortical sensory aphasia**

Table-2: Pre and Post-Therapy RICE scores of the client

S.No	Areas Assessed	Scores	
		Pre-therapy	**Post-therapy**
1.	Attention	3	3
2.	Eye contact	3	3
3.	Awareness of illness	3	3
4.	Orientation to space	1	3
5.	Orientation to time	1	3
6.	Orientation to person	2	3
7.	Facial expression	2	3
8.	Intonation	1	1
9.	Topic maintenance	3	3
	Total score	**19**	**24**
	Degree of impairment	**Mod. severe**	**Moderate**

Note: Lesser the RICE score, greater the impairment.

FOLLOW-UP EVALUATION/INVESTIGATIONS:

MRI of the brain repeated on July 2006 revealed cystic encephalomalacia in right temporo-parietal and left temporal regions, secondary wallerian degeneration of midbrain on right side, chronic infarct of corona radiata and centrum semiovale on right side. Neurological assessment on July 2006 reveals resolved III CN paresis. Left sided hemiplegia is still present for which the client is undergoing physiotherapy. Neuropsychological assessment done on September 2006 revealed significant impairment of verbal comprehension and visuo-spatial functions. However improvements were noticed in terms of eye contact, attention, facial expression. Reading comprehension and simple arithmetic calculation were relatively intact. Retrograde Amnesia was present. The client was provisionally diagnosed to have *'Retrograde Amnesia with impaired cognitive functioning'* and was recommended for neuropsychological rehabilitation. Speech and Language evaluation was carried out after four months of therapy in September 2006. WAB and RICE assessments were repeated. On administering WAB, the aphasia quotient was 63.2 indicative of *'Transcortical Sensory Aphasia'* (Table 1). On administering RICE, the client had a total score of 24 indicative of '*Moderate level of impairment*' (Table 2).

DISCUSSION:

After four months of therapy, the client's cognitive functions and overall communicative effectiveness have improved. Pre therapy and post therapy Cognitive and Communicative performances of the client have been explained in detail in Table-3. Significant improvements

Table-3: Pre and Post – therapy communicative and cognitive performance of the client

S.No.	Parameter	Pre-therapy		Post-therapy	
		Baseline	% (Percentage of response)	Progress	% (Percentage of response)
I	**Communicative functions**				
	Comprehension				
1	i) Yes/No questions				
		Absent	0	Responds to Yes questions	50
				Responds to No questions	10
	ii) Auditory word recognition	Present	50	Remains the same	50
	iii) Sequential commands	Absent	0	Comprehends and executes simple commands	10
	iv) Reading comprehension	Absent	0	Comprehends what he reads	40-50
2.	Spontaneous speech			Appropriate response present for	
	i) Information content	Poor	10	questions presented orthographically	70
	ii) Fluency	Fair	60	Remains the same	60
3.	Repetition	Poor	40	Ability to repeat phrases and sentences has improved	80
4.	Naming	Fair	70	Object naming has improved	80
II	**Cognitive Functions:**				
1.	Eye contact	Present	25	Improved	50
2.	Attention span	10 minutes	0-10	30-60 minutes	30-60
3.	Orientation to space				
	i) Person	Poor	0	Able to recognize family members	50
	ii) Place	Poor	0	Orientation of 'where he is' is present	80
	iii) Time	Poor	0	Able to express day, date and time	50
4.	Facial expression	Expressionless	0	Improved (Able to express joy & frustration)	10
5.	Intonation	Flat	0	Expressess question in raising intonation pattern	10
6.	Topic maintenance	Absent	0	Able to sustain a topic for 5-10 minutes	20
7.	Greeting skills	Present but limited	20	Improved (able to greet bye, good morning / afternoon / night	50

were noticed in the areas of comprehension, repetition and orientation to space. The client has *progressed from Wernicke's aphasia to Transcortical Sensory Aphasia and the severity of the cognitive impairment has also reduced from moderately severe to moderate degree of impairment*. Slow rate of progress could be attributed to delayed onset of intervention.

It is learnt that adequate care has to be taken while evaluating individuals with RHD. The person with RHD might demonstrate linguistic symptoms consequent to contra-coup injury. It is also evident that early intervention, intensive cognitive therapy and speech & language therapy could considerably improve communication and cognitive performance of individuals with RHD.

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BLUNT TRAUMA LIVER –CASE REPORTS AND REVIEW

A.C. Senthil Kumar [a], R. Ramya [a], Naveen Alexander [a]

ABSTRACT

Liver is the second most frequently injured organ in blunt trauma patient. Recently there is a shift from surgery to non-operative management due to the improvement of the imaging techniques and to the new concepts in the angiographic management of intra-hepatic vascular injuries. We present two cases of Poly-trauma with blunt liver injury which were managed at Sri Ramachandra Medical Centre surgically. Management of liver injuries is either surgically or non surgically depending on a variety of factors, the most important ones being the hemodynamic status and associated injuries. The operative techniques include perihepatic packing, omental packing, absorbable meshes, liver resection and even liver transplantation.

The characterization of blunt liver trauma is performed using a CT-based grading system, adopted from the American Association for the Surgery of Trauma (AAST) and adapted for CT by SE Mirvis in 1989. This 6-grades classification reflects the extent of parenchymal liver damage, but cannot reliably predict the probable clinical outcome of attempted nonsurgical management. Arterial contrast media extravasation has been reported as an helpful sign for improving the success of nonsurgical management, because it allows arterial embolization to be performed before the patient become hemodynamically unstable.

Reports suggest that an algorithm based on CT criteria, including the CT-grade of hepatic injuries (using the AAST injury severity scale classification), the presence of a contrast blush on the arterial phase and the involvement of a major hepatic vein, could help select high risk patients to angiographic procedure.

Angiographic embolization of arterial blush is not only used to improve the success of non-operative management but also as a precious adjunct to surgery to help stop hemorrhage in extended fracture of the liver. The main delayed complications of liver trauma consist in super-infection, abscess, bile leak, biloma and arterio-venous fistula and a remote post-traumatic biliary duct stenosis are generally encountered in high grade injuries. Abscess and intra-peritoneal bile leaks are most often treated by percutaneous drainage; resolution of a bile duct tear is usually a long term process. Scintigraphy and, more recently, MRI can be used to confirm and localize a bile leak. The percentage of successful non- operative management will be increased if the radiologist could help the surgeon to identify patients at risk of bleeding, to select them for angiographic embolization.

Key words: *Blunt injury, Liver, Surgical procedure*

INTRODUCTION:

The relatively fixed position of the liver and its large size makes it more prone for injury in blunt trauma of the abdomen. Liver and spleen together, account for 75% of injuries in blunt abdominal trauma. (1) Though liver is the second most commonly injured organ in abdominal trauma, it is the most common cause of death following abdominal injury. Compared to splenic injuries, management of liver trauma still remains a challenge in the best of trauma centers.

In the past, most liver injuries were treated surgically. However evidence confirms that about 86% of liver injuries have stopped bleeding by the time surgical exploration is performed and 67% of laparotomies done for blunt trauma abdomen are non-theraupetic(2) Imaging techniques especially Computerised Tomographic Scan(CT) has created remarkable impact in managing liver trauma patients by reducing the number of laparotomies.(3,4) About 80% of adults and 97% of children are presently managed conservatively world wide at high volume trauma centres.

The large size of the liver, the friable parenchyma, its thin capsule and its relatively fixed position make it prone to blunt injury. Right lobe is more often involved, owing to its larger size and proximity to the ribs. Compression against the fixed ribs, spine or posterior abdominal wall result in predominant damage to segments 6, 7 and 8 of the liver (>85%). Pressure on right hemithorax may propagate through the diaphragm producing contusion of dome of right lobe of liver. Liver's ligamentous attachments to diaphragm and posterior abdominal wall act as sites of shearing forces during deceleration injury. Liver injury can also occur as a result of transmission of excessively high venous pressure to remote body sites at the time of impact. Weaker connective tissue framework, relatively large size and incomplete maturation and more flexible ribs account for higher chance of liver injury in children compared to adults. Deceleration injuries producing shearing forces may tear hepatic lobes and often involve the inferior vena cava and hepatic veins. A steering column injury can damage an entire lobe. Liver trauma may result in subcapsular/intrahepatic hematomas, lacerations, contusions, hepatic vascular injury and bile duct injury. Most blunt trauma liver (80% in adults

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and 97% in children) are treated conservatively (2). Conservative treatment mandates repeated clinical monitoring and surgical intervention if conservative treatment fails. A comparison of patients receiving operative and non-operative treatment of liver injuries has revealed no difference in the length of hospital stay, but requirements for blood transfusion and intraabdominal complications were significantly lower in those managed conservatively. In this article, we have reviewed two challenging cases of blunt liver trauma in poly-trauma patients managed in our surgical unit of Sri Ramachandra Medical centre during the time period June – August 2007.

CASE REPORTS OF BLUNT HEPATIC TRAUMA IN POLYTRAUMA PATIENTS:

Case1.

A 27 year old male was brought to Emergency Room(ER) with history of Road traffic accident(RTA). On examination he was conscious, oriented, tachypnoeic with pulse rate of 110/min and BP of 80/60 mm Hg. Examination of abdomen revealed diffuse tenderness. Guarding was present in the entire abdomen. He also had fracture of right lower end of radius with tenderness over the lower back. Neurological examination was normal. Ultrasound revealed free fluid in abdomen and solid viscerae were normal. X ray of lumbar spine revealed multiple transverse process fractures in L1 – L5 level. Despite adequate resuscitation with crystalloids and whole blood, patient did not improve. Emergency laparotomy was done when liver laceration 5 x 3x 2 cm on anterolateral surface with active hemorrhage was noted. Hepatic packing did not stop the bleeding and so hepatorraphy with omental packing was done to achieve hemostasis. A contusion in the neck of pancreas 2 x 2 cm was found which was left alone. The lumbar spine and radial injuries were managed conservatively. Patient was in the hospital for 2 weeks in all. He was started on oral liquids from 4th postoperative day(POD), drain removed on 6th POD and suture removal done on 10th POD. Patient did well postoperatively and was discharged on the 14th POD.

Case 2:

A 28 years old male patient was brought to ER following a RTA with BP of 80/60 mm Hg. Patient was conscious, oriented and had pallor. Urinary catheterisation revealed frank hematuria. Abdomen was distended. Guarding, rigidity and diffuse tenderness were present over the entire abdomen. Patient was resuscitated with crystalloids and whole blood when BP improved to 130/80 mm Hg and pulse rate of 100/min. Initial Ultrasound revealed grade 2 renal injury with moderate hemoperitoneum, liver and other viscerae were normal. As CT was nonfunctional on the day, urgent Intravenous Pyelogram (IVP) was done which showed a non-enhancing Right Kidney. Patient was shifted to Intensive care unit(ICU) and managed conservatively. Due to progressive drop in Hemoglobin despite adequate blood transfusion, a repeat Ultrasonogram(USG) was done which picked up an additional injury, a liver laceration of 3 x 2 cm. Contrast CT Abdomen was done (18 hours after admission) which showed liver laceration and grade 5 renal injury(Figure 1).

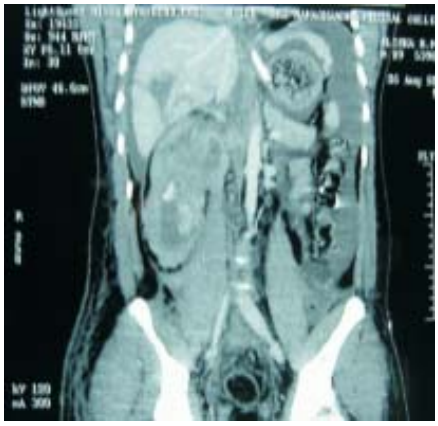

Figure 1. CT picture of case 2, showing extensive liver injury with Right Kidney injury

In view of increasing tranfusion requirements, emergency laparotomy through a midline abdominal incision was done. Right Kidney was shattered with renal vein injury. Right Nephrectomy was done. The laceration in segment 5 of liver on anterolateral surface of right lobe was packed with greater omentum and hemostasis was secured. Postoperatively, a right chest drain was placed for right hemothorax. On 1st Post – operative day, abdominal drain revealed 200 ml of fresh blood with elevated INR and PT. Despite adequate resuscitation with whole blood and FFP, patient did not improve. Emergency CT Angiogram was done which showed hemoperitoneum with liver laceration in the anterior and posterior surfaces(Figure 2).

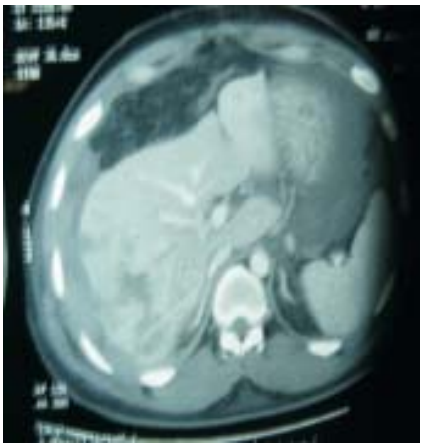

Figure 2. CT angiogram showing extensive liver damage with fresh bleeding from liver laceration

Patient was reexplored. After making a T shaped incision with horizontal limb through the right coastal margin, liver laceration in posterior surface was found actively bleeding. Greater omentum was fixed to the laceration and tagged to the diaphragm. A perihepatic pack was kept and the same brought through a separate stab incision in right hypochondrium. Post-operatively left chest drain was also

placed in view of left hemothorax. He was on ventilatory support and coagulation abnormalities were corrected. Patient was reexplored on 4th day after packing and pack removal was done. Postoperatively patient developed pneumonia, anasarca with hypoalbuminemia. These were aggressively treated with infusion of IV albumin, intravenous antibiotics, chest physiotherapy and nebulisation. Patient was extubated. The chest drains were removed after the drain volume decreased and lung expansion improved. The overall transfusion requirement for the patient including all blood components was over a hundred units. The patient was in the hospital for about 5 weeks prior to discharge. Patient is doing well presently.

Predominant cause of blunt hepatic trauma is by road traffic accidents. A patient with a history of blunt trauma to abdomen should arise suspicion of liver injury especially if is in the right lower chest wall or right upper abdomen. Signs and symptoms of liver injury depends on the level of blood loss, peritoneal irritation and presence of associated injuries. Right upper quadrant tenderness, guarding and rebound abdominal tenderness is common, but nonspecific signs may dominate. Peritonism may be severe in case of bile leaks. Elevation of serum liver enzymes in blunt trauma abdomen suggest liver injury although pre-existing causes like fatty liver may also be responsible . Following blunt trauma of the abdomen, a conscious patient who is hemodynamically unstable and has generalised peritonitis should undergo immediate laparotomy. In the presence of neurological impairment or with equivocal physical signs, traditionally a diagnostic peritoneal lavage (DPL) used to be done. DPL being invasive, time consuming, non-specific and oversensitive to presence of blood results in higher rate of nontheraupetic laparotomies(3). DPL has largely been replaced by ultrasonography. The Focused Abdominal Sonography in Trauma(FAST) is non-invasive, repeatable and can be performed in resuscitation area/ emergency room while other assessments are carried out. Utility of FAST in high volume trauma centres is proven unlike in lower volume centres because it remains operator dependent.

If patient is hemodynamically stable following blunt injury, a thorough radiological assessment is possible. Despite the advances in radiology, plain radiography still has a role. Though non-specific, plain radiography is useful in evaluating rib and spinal injuries in patients with blunt abdominal trauma. Fractures of right lower ribs, pneumoperitoneum, major diaphragmatic injury and gross organ displacement can be identified. Ultrasound can demonstrate lesions like hematoma, contusion, bilioma and hemoperitoneum. Subcapsular hematomas appear as curvilinear fluid collections with echogenicity varying with age. Initially hematomas are anechoic becoming echogenic by 24 hours. By 4-5 days, hematoma again becomes hypoechoic or anechoic. Septa and internal echoes develop within hemorrhagic collections by 1-4 weeks. Predominant drawbacks with ultrasound are that it is an operator dependent tool and its inability to pick dome or lateral segment of left lobe injuries. Sensitivity in detecting free abdominal fluid by USG is reported to be only 44% when associated with bowel or mesentric injury (2). Associated organ injury may be missed especially of the bowel, mesentry, pancreas, adrenal gland and bone.

CT Scan has become the gold standard in diagnosis of solid organ injury. Importantly, it can assess other visceral injuries that may require operative management like an associated transection of pancreas. Contrast enhanced CT Scan is accurate in localising the site and extent of liver and associated injuries. Spiral CT is the preferred technique. Multidetector-row CT offers further advantage of faster scanning time and thinner sections. American Association for Surgery of trauma (AAST) has devised a CT criteria for severity of liver injury. (Table 1).

Table 1.

CT Criteria for Liver Injury scale (AAST)

Grade 1	Subcapsular hematoma less than 1 cm in maximal thickness, capsular avulsion, superficial parenchymal laceration less than 1 cm deep, and isolated periportal blood tracking
Grade 2	Parenchymal laceration 1-3 cm deep and parenchymal/subcapsular hematomas 1-3 cm thick
Grade 3	Parenchymal laceration more than 3 cm deep and parenchymal or subcapsular hematoma more than 3 cm in diameter
Grade 4	Parenchymal/subcapsular hematoma more than 10 cm in diameter, lobar destruction, or devascularization
Grade 5	Global destruction or devascularization of the liver
Grade 6	Hepatic avulsion

Major features of blunt liver trauma include lacerations, subcapsular and parenchyma hematomas, active hemorrhage and juxtra-hepatic venous injuries. Minor CT features include periportal low attenuation and a flat IVC. Active hemorrhage following blunt trauma is typically identified at early phase contrast enhanced CT as focal attenuation areas that represent a collection of extravasated contrast material secondary to arterial bleeding. Willman et al(5)reported that attenuation of active arterial extravasation at multi-detector row CT range from 91-274 HU (mean 155 HU) whereas clotted blood ranged from 28-82 HU (mean 54 HU). Active hemorrhage can manifest as extravasation of contrast material either locally into a parenchymal hematoma or freely into the peritoneal space as a jet. Major hepatic venous injury seen at CT should considered as indicator of severe injury. Poletti et al (6)

reported that liver related surgery was 6.5 times more frequently required when laceration extended into one or more hepatic veins than when it did not. As more and more patients with complex grade IV and V liver injuries have been treated nonsurgically, prevalence of delayed complications picked up by follow up CT has increased, which can arise weeks to months after injury.(7) Prevalance of such complication in blunt liver trauma range from 5% to 23%(8, 9 &10). These include delayed hemorrhage, abscess, post-traumatic pseudoanerysm, hemobilia and bile peritonitis. False positives in CT diagnosis of liver injury may occur as a result of beam holding artefacts from adjacent ribs mimicking a contusion or hematoma. Nasogastric tube with air fluid levels may produce streak artefacts through out the left lobe of liver. False negative finding may occur in patients with fatty liver.

Magnetic Resonance Imaging(MRI) has limited role in evaluating blunt abdominal trauma and no added advantages over CT though theoretically it can be used in follow up monitoring without radiation exposure especially in young and pregnant women.

Radio-nucleotide Scanning in Tc99m sulfur colloid or Tc99m labeled denatured red blood cell studies were widely used in evaluation prior to widespread availability of CT scanning. Despite the disadvantages in the form of nonspecific findings and inability to evaluate intraperitoneal and retroperitoneal organs, it is useful especially when contrast use is contraindicated, in patients who cannot hold their breath or in patients with metallic objects or surgical clips in the abdominal cavity. Tc99m IDA uptake imaging scan is especially useful when a bile leak or bilioma is suspected.

Dynamic angiography has no role in patients presenting with hemodynamic instability. It is useful in demonstrating the site of active bleeding providing an opportunity for trans-catheter embolisation.

The decision to manage a patient with blunt trauma liver surgically or conservatively is made based on hemodynamic status and not on CT severity scoring. Yet, it is seen that CT is occasionally helpful in predicting success of non-operative management and utility of angiographic embolisation(6,11). CT may influence decision for surgical exploration in case of associated finding like bowel perforation and major vascular injury.

Non-operative Management of blunt hepatic trauma:

There has been a significant increase in the number of non-operative management following the publication of the first retrospective series of adult liver patients managed conservatively by Knudson and colleagues(4). Several prospective randomized trials have confirmed the same.(12 ,13). The results have showed that conservative management is associated with fewer liver related and intraabdominal complications and lesser mortality compared to operative management. (14) Conservative management does not result in a greater need for blood transfusion. Overall success of non-operative treatment in appropriately selected patients exceeds 95%(15,16). If conservatively managed, it should be borne in mind that risk of hollow organ injury, though small, is increased. There is a significant risk of increase in delayed hemorrhage. Patients who fail with an initial conservative approach despite close supervision should be detected and treated accordingly.

Operative management of blunt liver trauma:

Primary operative intervention is indicated for liver injury if patient is hemodynamically unstable despite adequate initial resuscitation. Prefered incision in emergency is a long midline, although access can be improved by converting into a "T" by adding a right transverse component. Subcostal incision can be used in patients operated after initial conservative management.

Laparotomy for liver injuries is no different from any other trauma laparotomy. Liver hemorrhage can usually be initially controlled by direct pressure using packs. Additional techniques include the Pringle manoeuvre, bimanual compression of liver or manual compression of aorta above the coeliac trunk. Intravascular volume replenishment and coagulopathy correction with packed cells, platelets, fresh frozen plasma and cryoprecipitate is crucial. After adequate resuscitation and adequate mobilization of liver a useful assessment of the injury if necessary after a Pringle manoeuvre by applying a vascular clamp should be done (Figure 3). Depending on the injury and experience of surgeon following methods may be used.

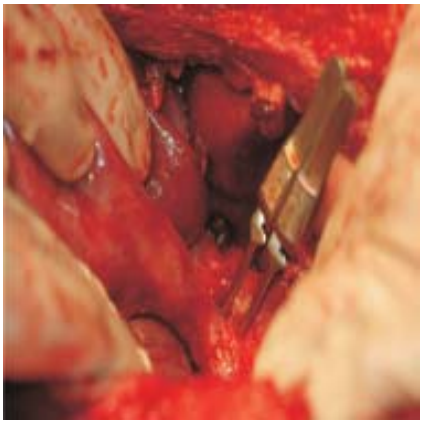

Figure 3. Pringle s manoeuvre done with bull dog vascular clamps applied over the epiploic foramen

Perihepatic packing:

When definitive control of hemorrhage cannot be obtained, liver injury should be packed, incision closed and patient referred to a specialized centre for definite treatment. Even in high volume centres, packing can be employed as a damage control strategy in patients who are critically unstable, coagulopathic or acidotic. Packing is effective at controlling major hemorrhage from liver injuries even in patients with caval or hepatic venous injuries.

Finger fracture method:

Hepatotomy by finger fracture with direct suture ligation to achieve hemostasis is a useful technique. Diathermy coagulation or Argon beam coagulator use is invaluable.

Omental Packing:

Stone and Lamb(17) in 1975 reported using greater omentum as a pedicled flap to fill the defect in liver parenchyma. This fills the dead space and stops venous oozing.(Figure 4)

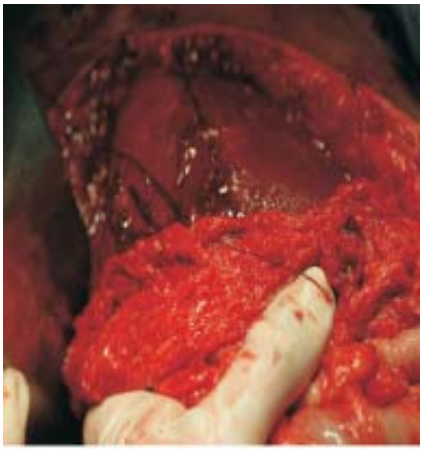

Figure 4. Liver laceration being packed with greater omentum

Use of absorbable polyglactin mesh to wrap the parenchyma disruption:

Advantages include need for a second laparotomy is almost nil and ease of abdominal closure. Disadvantage is that it is a time consuming procedure.

Resectional Debridement:

It involves removal of devitalised liver tissue using lines of injury as boundaries of resection. It is usually done at the time of removal of pack, as necrotic tissue will be well demarcated by 48 hours after injury.

Anatomical Resection:

It is practically difficult due to associated shock, coagulopathy and organ injuries. Excellent results are reported from experienced centres.(18) Selective ligation of hepatic artery is useful when other measures have failed, especially so when pedicle clamping has demonstrated to arrest hemorrhage. To prevent acute gangrenous cholecystitis, a cholecystectomy must be performed.

Suspicion of hepatic venous and retrohepatic caval injuries should arise whenever Pringle manoeuvre fails to arrest hemorrhage. Options available are total vascular exclusion clamping IVC, suprahepatic cava in addition to Pringle manoeuvre), venovenous bypass or atriocaval shunting via a chest tube through the right atrial appendage into the IVC(Shrock in 1968) (19). Packing can effectively control bleeding from retrohepatic caval injuries (20), only using vascular exclusion as a last resort.

Liver transplantation may be considered as an option for very severe hepatic trauma only in very high volume centres with expertise for liver transplantation.(21 & 22).

Interventional Radiology in blunt hepatic trauma:

The development of non-surgical interventional techniques to treat the complications of liver trauma (i.e. CT-guided drainage, angiographic embolization, percutaneous balloon dilatation of a biliary duct stenosis) have fostered the trend toward non-operative management. Angiography and embolisation are the most traditionally used adjuncts to nonoperative management of liver trauma. Angiography is recommended in hemodynamically stable patients where initial CT Scan show a "blush". Pooling of contrast within the liver parenchymal is amenable to angioembolisation unlike those pooling into the peritoneal cavity. Trans-catheter embolisation is technically successful in about 80% of appropriately selected cases(23&24). Angiographic embolization of arterial blush is not only used to improve the success of non-operative management but also as a precious adjunct to surgery to help stop hemorrhage in extended fracture of the liver. Abscess and intra-peritoneal bile leaks are most often treated by percutaneous drainage.

Complications of Management of hepatic trauma:

Coexisting intraabdominal complications may be missed at initial presentation and may become apparent after initial delay. Septic complications such as intraabdominal abscess and bile leak are recognized late complications and may require radiological, surgical or endoscopic interventions. Hemorrhage, coagulopathy including DIC, hemobilia, arterioportal fistulas and sepsis due to infection of bile collection, blood or devitalised liver tissue are other late complications.

CONCLUSION:

Blunt hepatic injuries even though statistically less common than splenic blunt injuries, account for more than 50% of morbidity and mortality The advancements in radiology both as a diagnostic and as an interventional tool, together with the improvement in intensive care mean that more and more hepatic blunt trauma are now managed conservatively. Surgical exploration in hemodynamically stable patients is more of an exception than the rule. It does not mean the end of the road for the surgeon though, surgeries ranging from very simple perihepatic packing to liver transplantation are required in selected cases especially in unstable patients where one cannot depend on the radiology tools. This paper is meant to revisit the older techniques as well as look to the future in the management of blunt hepatic trauma.

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UNUSUAL CLINICAL PRESENTATION OF CAT SCRATCH DISEASE – A PROFILE

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ABSTRACT:

Cat scratch disease (CSD) is a syndrome that is characterized by regional lymphadenopathy after a cat scratch or bite .The causative agent of CSD has been controversial. Bartonella species,most commonly B.Henselae and sometimes B.Clarridgeiae is implicated as the cause. A few patients have developed a serologic reaction to Afipia Felix Bacilli. CSD manifests as painful regional lymphadenopathy presenting for several weeks to months after a cat scratch. Occasionally, infection may disseminate to produce more generalized lymphadenopathy and systemic manifestations of lymphoma.

CSD occurs worldwide and the animal implicated is usually a kitten. In United States approximately 24,000 cases occur annually, resulting in over 2,000 hospitalizations. About 90% of the patients have a history of exposure to cats and a cat scratch or bite have occurred in 75% of these individuals. There is no evidence that the agents of CSD produce illness or infections in cat. CSD occurs only in humans. Children are more commonly affected than adults. Mostly occur in summer when fleas are active. The flea may serve to transmit infection between cats, it is not known whether humans can be infected through the bite of an infected flea.

In this report we describe a rare case of Cat Scratch Disease with its clinico pathological manifestations and a brief summary of its management.

Key words: *Cat-Scratch Disease, Bartonella, case report*

INTRODUCTION:

CSD was first recognized as a clinical entity in the U.S by Foshay in early 1930s, during the course of the studies in Tularemia(1). An antigen he prepared from the pus removed from affected Lymph Node produced a positive reaction on intradermal injection into patients with CSD. At the same time Debre in France, recognized the occurrence of suppurative adenitis in children with negative tuberculin test but with numerous cat scratches. Debre was able to study patients with positive skin tests for CSD and this led to the first clinical description of this disease in 1950. In 1983, Wear and associates demonstrated the pleomorphic Gram negative bacilli in lymphnode obtained from children suspected to suffer from CSD by histopathological examination using Warthin-Starry silver impregnation stain(2). The incidence of the disease is unknown since there is neither a commonly available means of diagnosis nor a systematic reporting. CSD should be suspected if the patient has a positive history of exposure to cats and develops lymphadenopathy along with skin lesions.

CASE REPORT:-

An 18 year old male from Chennai, India presented with complaints of painful swelling of the right side of neck for past 30 days. The swelling was sudden in onset, gradually progressive in nature, and was associated with evening rise in temperature.

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On examination, a swelling was seen on the right side of the neck 2x3cm, smooth, soft, tender mobile and deep to the Sternocleidomastoid muscle (SCM), extending between the junction of middle one third and lower one third of SCM, to the region about 2 finger breadths above the medial one third of right clavicle The swelling was non pulsatile, non translucent, with no impulse on coughing and did not move with deglutition or on protrusion of tongue.

There was no past history of recurrent cough, loss of appetite, or decrease in weight or contact with cat or any other pet animals.

All blood investigations were normal. Mantoux test and chest X-ray were negative for tuberculosis. Fine Needle Aspiration Cytology (FNAC) of the lymphnode showed a granulomatous inflammation probably of tuberculous origin. At this point, with a working provisional diagnosis of tuberculous lymphadenitis, patient was started on empirical Anti Tuberculous Treatment.

Excisional biopsy of the mass was done. Intraoperatively, a suppurative lymphnode which was seen adherent to the internal jugular vein was peeled off and sent for histopathological examination. Cheesy material was seen inside the mass which was aspirated and sent for culture and sensitivity including Acid Fast Bacilli culture.

Culture showed no growth. Acid Fast Bacilli and Fungal stains were Negative.

Histopathological Examinations showed partial distortion of lymph node architecture. **Stellate Abscesses** were conspicuously seen in some fields while in other areas there was encirclement by epitheloid histiocytes. (Figure 1 and 2) Also, granulomas consisting of epitheloid cells and giant cells were noticed infrequently.With these histopathological features a diagnosis suggestive of a CSD was made after a lot of deliberation and consultations with experts in the field of histopathology.

Tissue Sections Stained With Haematoxylin & Eosin Stain Showing Stellate Abscesses

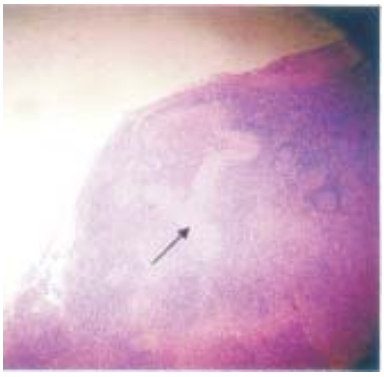

Fig: 1 (magnification 100x)

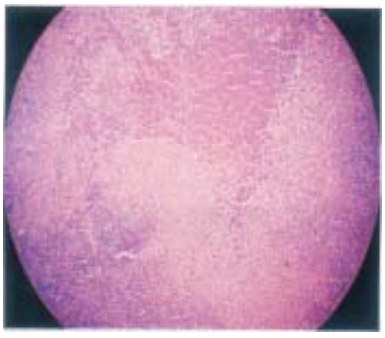

Fig: 2 (magnification 400x)

Warthin-Starry silver stain- a non-specific silver stain was done-but was not able to identify any microorganisms.

Blood was sent to the Centre for Diseases Control and Prevention, or CDC, along with a presumed diagnosis of CSD. The CDC identified antibodies to B henselae with a titer of 1: 130 (normal < 1:64).

With this, the diagnosis of CSD was confirmed and, anti tuberculous treatment was discontinued and Doxycycline 200 mg 1 bid was started for a period of 2 weeks. Patient is being followed up regularly and there has been a significant improvement in the patient's general condition.

DISCUSSION:

Chronic regional lymphadenopathy is the most common clinical feature of CSD and usually develops about 2 weeks after the scratch or contact with cat. An inoculation site may be detected in more than two thirds of patients when actively sought. Primary skin papule or pustule occurs within 3-10 days from the time of scratch or contact. Most primary lesions persist for about 1-3 weeks. Low grade fever lasting for several days occurs in about 30% of patients. Malaise or fatigues is noted in 25% and headache and sore throat in about 10% of patients.

Lymphadenitis is the major manifestation of CSD. The enlarged tender lymphnodes are most commonly found in the head or neck areas. The axillary nodes are frequently involved, less commonly epitrochlear, inguinal, femoral and rarely supraclavicular nodes may be enlarged. Single node involvement occurs in almost half of the patients, involvement of multiple lymphnodes in the same site occur in about 20% of patients.

About one third of patients have lymphnode enlargement involving several sites. Node enlargement persists for 2-4 months, but has been known to last for two years. Suppuration of involved lymphnode occurs in 10% of patients(3).

Atypical manifestation of CSD may be seen in some patients (Table 1). Symptoms and signs of Oculoglandular syndrome or Parinaud Syndrome presents as an ocular granuloma or conjunctivitis with preauricular lymphadenopathy.

Table 1 : Extra –lymphatic manifestations of CSD

Symptoms and signs	Frequency	Symptoms and signs	Frequency
Fever (38.3^0 to 41.2^0 C)	28%	Sore throat	7%
Malaise Fatigue	30%	Exanthem Conjunctivitis	5% 3.3%
Headache	13%	Arthralgia	2.5%
Anorexia, emesis	14%	Seizures, coma	3%
Splenomegaly	9.5%	Blindness	2%

Pathology: Infections by B.Henselae can produce two entirely different pathological reactions depending on the immune status of the host

1. CSD which is a granulomatous inflammation with stellate necrosis.
2. Bacillary angiomatosis

Initially, in CSD, lymph node shows lymphoid hyperplasia. Later, scattered granulomas appear and some may contain central areas of necrosis with rare multinucleated giant cells. As the disease progresses, stellate areas necrose and coalesce to form one or more abscesses. If the lymph node capsule ruptures pus extends to the contiguous areas.

Bartonella Henselae in Warthin Starry Silver stain

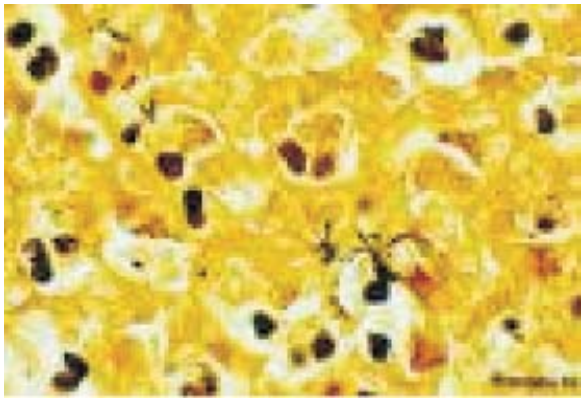

Fig 3 :Magnification 400x

Diagnosis CSD should be suspected if the patient has a history of exposure to cats and develops lymphadenopathy and a skin lesion. The diagnosis can be confirmed by pathological examination of the involved nodes. Sometimes, tiny bacilli in clusters can be seen in biopsy samples stained with Warthin Starry silver stain (figure 3). The gold standard for diagnosing CSD is by an indirect immunofluorescent antibody assay or IFA (4). If CSD is present, high titers of antibody will react to the B. henselae antigen. Titer of 1:64 or higher is considered positive. The identification of B Henselae 16 S ribosomal Ribo Nucleic Acid genes in biopsy material by Polymerase Chain Reaction Amplification with specific oligonucleotide primers can also be diagnostically useful, but these methods are not commercially available(5).

Treatment: This disease is generally self limiting. However tender regional lymphadenopathy and systemic symptoms may be debilitating to the patient. Hence early detection and treatment need to be started before any systemic complications occur. The oral antimicrobial agents that can be used include ciprofloxacin, Gentamicin sulfate, Rifampin, TMP – SMX, Doxycycline is shown in table 2.

Table 2 : Antibiotic Therapy for CSD (6)

Antibiotic	Route	Dosage	Frequency	Duration
Ciprofloxacin	PO	20-30 mg/kg	Q12h	10-21 days or more
Gentamicin sulfate	IM or IV	5 mg/kg	Q8h	5-10 days
Rifampin	PO	10-20 mg/kg (max 600 mg/kg daily)	Q8-12 h	10-21 days
TMP – SMX	PO	10-20 mg/kg TMP50-100 mg/kg SMX	Q8-12 h	10-14 days
Doxycycline	PO	3-4mg/kg	BD	10-14 days

TMP - SMX : Trimethoprim - Sulfamethaxazole

Differential diagnosis: Lympadenitis occurs in cases like Koch's Adenitis, Atypical Mycobacterial Infection, Tularemia, Toxoplasmosis, Infectiousmononucleoses, Lymphogranulomavenerum, Coccidioidomycoses and tumors.

CONCLUSION:

Cat-Scratch Disease is a self limiting chronic regional lymphadenitis which can occur in those who have contact with cats. So it should be borne in mind as a differential diagnosis, while evaluating chronic lymphadenitis.

The following conclusions can be inferred with this clinical experience-

- This unusual case suggests a need to create an awareness regarding this potentially dangerous condition if left untreated.
- All patients with cervical lymphadenopathy need focused histopathological diagnosis.
- There may be varied clinical presentation of the same pathological entity.
- Almost all the chronic granulomatous conditions behave similarly in the modes of presentation.
- Empirical treatment with Antituberculous drugs should be instituted only for a selected group of patients without any definitive histopathological diagnosis.
- A proper insight into the history of "Cat Scratch" preceding the initial presentation to the doctor can help in diagnosis especially when all corroborative radiological & laboratory analysis for Tuberculosis is negative.
- Sometimes, unusually (10% of cases), even without history of contact with cats, diagnosis can be clinched based on Histopathology.

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A CASE OF LEPTOSPIROSIS WITH OCULAR MANIFESTATIONS

S. Viswanathan [a], V.Akila Ramkumar [a], Elfride Sanjana [a], Ramya Sampath [a]

ABSTRACT

Leptospirosis, a zoonotic disease caused by the water-borne spirochete Leptospira. Although it is one of the world's most widespread febrile diseases, it remain underdiagnosed, mainly because of protean manifestations, lack of awareness, and nonavailability of laboratory support. Ocular manifestations are noted in the second phase of illness, but these remain latent mainly because of the prolonged symptom-free period that separates the systemic manifestations from detection of ocular manifestations.

A case of leptospirosis with ocular manifestations notably retinal haemorrhages, roth spot and accumulations of sub retinal fluid is presented here.

Key words: *leptospirosis, eye manifestation, case report*

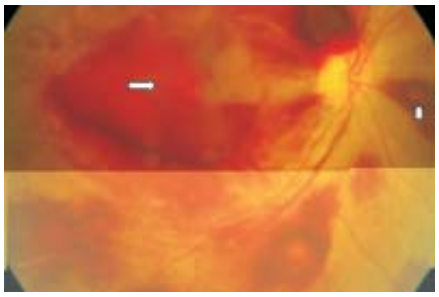
INTRODUCTION

Leptospirosis, a zoonotic disease caused by the water-borne spirochete Leptospira. Although it is one of the world's most widespread febrile diseases, it remain underdiagnosed, mainly because of protean manifestations, lack of awareness, and nonavailability of laboratory support. The classical presentation of the disease is an acute biphasic febrile illness.Ocular manifestations are noted in the second phase of illness, but these remain latent mainly because of the prolonged symptom-free period that separates the systemic manifestations from detection of ocular manifestations.

CASE REPORT

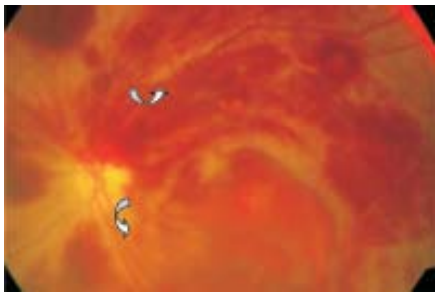
A 23 year old female presented to the Emergency department with complaints of fever for the past one month, with sudden onset of diminished vision for one week. Investigations for fever done elsewhere revealed the following laboratory results: S.typhi and Leptospirosis positive; Hb- 4.8g%; she had received treatment with Packed cell transfusion for her anaemia and Ampicillin 500mg 6th hourly for 10 days. Clinical findings: On general examination patient was febrile and anaemic. Ophthalmic findings revealed visual acuity(Bedside vision) **RIGHT EYE:** 2/60 and **LEFT EYE** :2/60. Anterior segment :**RIGHT EYE**- Eyelids normal, Conjunctival Suffusion +, Cornea clear, AC normal, Pupil 3mm reacting to light(direct and consensual) Lens clear. INTRA OCULAR PRESSURE at 11 am (Applanation Tonometry) 14mmhg**. LEFT EYE**- Eyelids Normal, Conjunctival Suffusion +, Cornea clear, AC Normal, Pupil 3mm reacting to light(direct and consensual) Lens clear. INTRA OCULAR PRESSURE at 11am (Applanation Tonometry)16mmhg. Fundus**: RIGHT EYE**-Media clear, Disc size; shape; margins are normal, Venous dilatation + Superficial haemorrhages + Plenty of pre-retinal haemorrhages + Roth spots + Subretinal fluid + (Fig. 1) **LEFT EYE**- Media clear, Disc size;shape;margins are normal, Venous dilatation + Superficial haemorhages + Plenty of pre-retinal haemorrhages + Roth spots + Sub-hyaloid haemorrhages + Subretinal fluid + (Fig. 2)

Fig 1



ROTH SPOT PRE-RETINAL HAEMORRHAGE

Fig 2



VENOUS DILATATION SPLINTER HAEMORRHAGES

INVESTIGATIONS

Hb-10.2g%(following PC transfusion) ESR – 59 mm/hr
PLATELETS – 39000cells/cu.mm
RBC – 3.26million/cu.mm
PT – 19.9, PTT – 38.4, INR – 1.61
BUN – 19, Cr – 0.6

LFT:	TOTAL BILIRUBIN	- 1.8
	DIRECT BILIRUBIN	- 0.6
	SGPT	- 1744
	SGOT	- 255
	TOTAL PROTEINS	- 6.7
	ALBUMIN	- 3.5
	GLOBULIN	- 3.5
	ALKALINE PHOSPHATASE	- 114

USG abdomen was normal.
IgM Titres for Leptospirosis was raised
QBC- MP was found to be Negative

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TREATMENT GIVEN

Nil Ophthalmic intervention warranted, as the haemorrhages are said to resolve during course of time(1). Patient was advised regular follow up once in two weeks.

DISCUSSION

Leptospirosis is a world-wide disease with higher incidence and prevalence in the tropical and subtropical region. The natural reservoirs are rodents, domestic animals-livestock/dogs. Humans are accidental hosts and infection is due to contact with infected urine, tissue and water. Pathological leptospira belongs to the species Leptospira interrogans. The disease is often biphasic – early leptospiremic and late immune phase.

Weil's disease is a severe form associated with jaundice, hepatosplenomegaly, azotemia, haemorrhages, anaemia, persistent fever, and altered mental status. Autoimmunity is believed to be the underlying pathologenic mechanism in ocular pathogenesis(2). All forms of leptospires can damage the wall of small blood vessels; this damage leads to vasculitis with leakage and extravasation of cells, including haemorrhages. It causes direct cell death and toxicity(3). After 4 to 7 days of the initial bacteremia the leptospirosis are eliminated by the immune system from all host tissues except from immunologically privileged places like the brain/eyes resulting in immunological pathology in the eyes like uveitis(2days to 4weeks)(4). Ocular involvement occurs during the immunological phase typically with panuveitis often accompanied with retinal periphlebitis and hypopyon.(5)

Ocular complications occur from 2weeks to 6months after the febrile stage and can lead to decrease in vision and blindness. Manifestations include congestion, subconjuctival haemorrhage, icterus, iridocyclitis in the anterior segment. Posterior segment manifestations are: vitritis, pars planitis, periphlebitis, choroiditis, papillitis, macular edema, retinal haemorrhages, retinal exudates, and arteritis. Two recent studies(6&7) from South India have identified non-granulomatous uveitis hypopyon, cataract, vitreous inflammatory reaction, retinal vasculitis and papillitis as more common ocular manifestations. Retinal vasculitis was seen in 4-8% to 51% of patients with leptospiral uveitis. (8) Venous involvement is more common and only 1% of them had arteritis. Retinal vasculitis is of the perivasculitis type.

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PRIMARY TUBERCULAR MASTOIDITIS – A RARE PRESENTATION

A. Ravi kumar [a], Senthil .K [a], Prasanna Kumar. S [a], Gopinath [a], Gaurav Bambha [a]

ABSTRACT:

Tuberculosis can masquerade any disease; primary tubercular mastoiditis is a rare clinical entity. We report a case of tubercular mastoiditis which presented as a postaural swelling with intact tympanic membrane, no hearing loss. The surgical findings revealed mastoid and extradural extension of disease. Histopathological examination of excised tissue clinched the diagnosis. There was no evidence of pulmonary or any other tubercular foci.

***Key words**: Primary tubercular mastoiditis, Silent mastoiditis.*

INTRODUCTION:

Tubercular mastoiditis was first described by Jean Louis Petit in the 18th century; Wilde in 1853 discussed the classical picture of tuberculosis otitis media as a disease characterised by painless, insidious onset of ear discharge, multiple perforations in the tympanic membrane, pale granulations in middle ear cleft. Politzer discussed the destructive nature of this disease in 1882; it was in 1892 that Koch demonstrated the tubercle bacilli.[1] The incidence of tuberculosis otitis media has been reported to be 0.04% to 0.9% of all Chronic suppurative otitis media (CSOM) in the developed countries. [3,4] Tuberculosis affects the middle ear through three routes either through the Eustachian tube, blood borne dissemination or direct implantation through the external auditory canal and tympanic membrane perforation. The incidence is thought to be more and is on the rise in the developing countries[10], In recent years extra pulmonary tuberculosis has more frequently been associated with mastoiditis in patients with immunodeficiency state.

Primary tubercular mastoiditis is a silent tubercular mastoiditis i.e. there is no history of ear discharge, normal tympanic membrane and hearing and no evidence of tubercular foci in the lungs or any where else in the body.[2] Silent mastoiditis refers to clinically undetected or undetectable middle ear pathology.

CASE REPORT:

A 12yr, male child presented to our OPD with left postaural swelling for the past 2 months, which was insidious in onset progressive and painless. There was no history of ear discharge, trauma, ear surgery in the past. There was no history of fever. However patient complained of dull global headache.

Examination showed a smooth 2x4 cm left postaural swelling, the skin over the swelling was normal (Fig.1), no local rise in temperature, nontender, soft in consistency, with no fluctuation, and not reducible. The postaural sulcus was normal. Otoscopy showed a normal mobile tympanic membrane. Tunning fork tests showed normal response. Pure tone audiogram showed normal hearing. A provisional diagnosis of chronic postaural lymphadenitis was made. Routine blood test were normal except ESR which was 12mm in 1st hr. Chest X-ray was normal, Mantoux test was negative FNAC was suggestive of reactive lymphadenitis. Incisional biopsy was done under local anaesthesia. During the procedure pale granulation was noted, which was extending into the mastoid cortex; Histopathological examination (HPE) showed features of chronic granulomatous disease suggestive of tubercular or fungal granuloma.

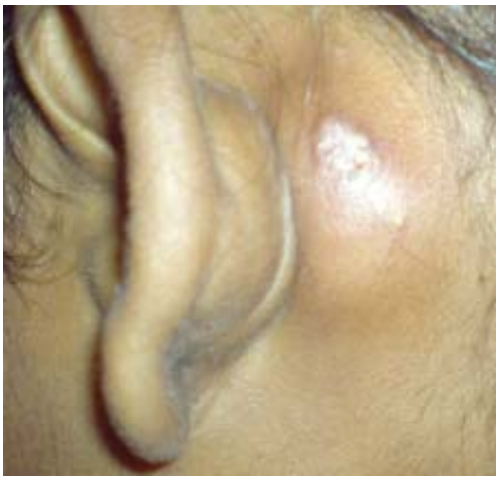

Fig. 1 Post Auricular Swelling on the left side

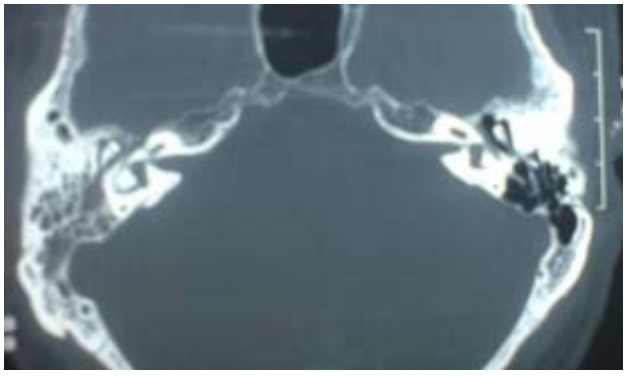

Fig.2. HRCT temporal bone suggestive of left mastoiditis

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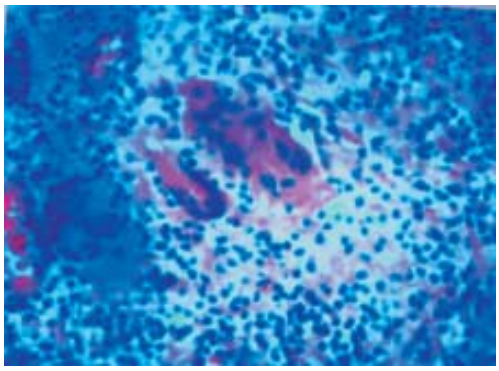

Fig .3. Microscopic view of mastoid granulation tissue showing giant cell in an ill defined epitheloid granuloma (Hematoxylin and eosin stain x 200)

In consultation with pulmonologist antitubercular therapy was started (ATT) (2HRZE/ 4HR) based on the histopathological report and clinical suspicion and was discharged. 2 weeks later the patient came back with a discharging sinus in the post aural region and persistent global head ache. HRCT scan of the temporal bone and brain showed soft tissue opacity in the mastoid suggestive of mastoiditis. (Fig.2). A differential diagnosis of congenital cholesteatoma, tuberculosis otitis media (TOM), or fungal granuloma was considered. He was taken up for surgery. Through a postaural incision the fistula and granulations were excised. The mastoid cortex was found to be filled with pale granulations which were extending up to the tip cells below. The sinus plate was found to be eroded and granulations were found on the sinus and the dura. Granulations were carefully removed. The mastoid antrum was found to be free of disease, tympanum was normal with normal ossicular chain. Granulation tissue was sent for histopathological examination (HPE), acid fast bacilli (AFB) staining and culture. AFB staining and culture were negative, HPE showed chronic inflammatory granulation tissue with ill defined epitheloid granulomas having multinucleated giant cells and caseating necrosis suggestive of tuberculous granuloma [fig. 3]. He was continued on ATT and after follow up of 6 months he is asymptomatic with normal hearing (fig. 4).

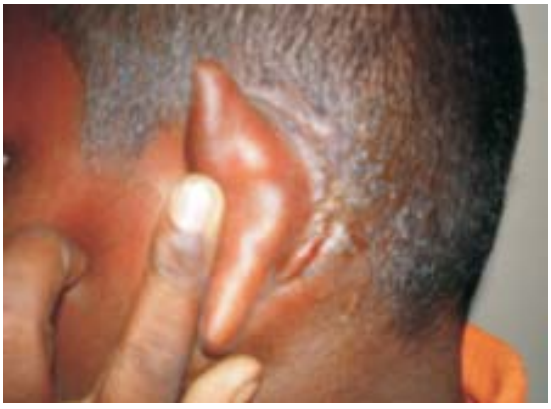

Fig.4. Post aural wound after 6 months after surgery

DISCUSSION:

The classical description of tubercular otitis media is a painless, odourless otorrhea, insidious in onset with multiple perforation of the tympanic membrane, with abundant granulation and hearing loss which is out of proportion to the clinical finding. Contrary to the classical description, in our case there was no evidence of middle ear involvement with an intact ossicular chain and no hearing loss.

The most common mode of infection is secondary to spread of infection from the lungs; the route of infection is thought to be via the Eustachian tube, haematogenous route or via the perforated tympanic membrane. Kim et al[8] have reported a case in which Tympanostomy tube insertion was thought to be the cause of tubercular otitis media; A review of literature in showed very few cases in which there was no involvement of the tympanum. Similarly there was no involvement of the tympanum in the case we report here and the disease was only confined to the mastoid. In our case there was no evidence of pulmonary tuberculosis and since the tympanum was free of any disease, the route of transmission could not have been through the Eustachian tube, and could have been only by haematogenous route.

The diagnosis of tubercular otitis media requires a high index of suspicion even in the absence of pulmonary Koch's, demonstration of AFB in the ear discharge is difficult. The positivity for AFB in ear discharge varies from 5 to 35% and on repeated examinations it improves to 50% [7], diagnosis of extra pulmonary tuberculosis is essentially clinical, [10] and antitubercular therapy can be started only on clinical or histopathological suspicion.[9] Early institution of ATT is mandatory and is the main stay of treatment especially to avoid serious complication. The role of surgery is limited and indications for surgical intervention include cases unresponsive to medical therapy, extensive disease with bone sequestrae.

Untreated TOM can result in permanent, severe sequel, such as facial paralysis, hearing impairment, and intracranial dissemination of infection. Therefore, early suspicion and timely diagnosis are of paramount importance.

CONCLUSION:

Primary tubercular mastoiditis is a rare clinical entity, the diagnosis of which requires a high index of suspicion. Early institution of treatment results in resolution of disease and prevention of serious complications [5]. In this case we have reported a rare presentation of tubercular mastoiditis without middle ear involvement. This case where disease extended to dura shows that silent mastoiditis can be a potential cause of complication such as meningitis inspite of presence of intact tympanic membrane.

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THREE CASES OF BLUNT RENAL TRAUMA IN CHILDREN

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ABSTRACT

Traumatic injury to the kidney accounts for greater than 60 % of the pediatric genitourinary injuries. This case report highlights the various presentations of renal trauma in pediatric population and the management of children with blunt renal trauma, both conservative and operative approaches. Three cases of pediatric blunt renal injuries were managed by department of pediatric surgery at SRMC, Chennai from Oct 2005 to June 2007. Age of the patients ranged from 12 to 14 years (Mean – 13 Yrs). Mode of injury varied among the three cases. Two cases of grade III renal injury were managed conservatively. One patient with grade V injury with transected kidney underwent nephrectomy. Conservative management including close observation with periodic assessment of blood hemoglobin, renal parameters, and ultrasound abdomen is effective even in grade III renal injury. Indications for surgery include expanding abdomen mass, fall in hematocrit or hemoglobin, deterioration of general condition of the patient.

MesH words: *Blunt injury, child, kidney*

INTRODUCTION:

Blunt trauma is responsible for 90% of the genitourinary injuries in childhood, with approximately 90% having coexisting injuries to the thorax, spine, pelvis, femur or intra abdominal organs (1, 2). Traumatic injury to the kidney accounts for greater than 60 % of the pediatric genitourinary injuries (3,4,5,6.)

The pediatric kidney is believed to be more susceptible to trauma because it is protected by an immature, more pliable thoracic cage and weaker abdominal musculature, has less perirenal fat and sits in a lower position in the abdomen than its adult counterpart. In contrast to adults, in children, hematuria is very unreliable sign in determining the need to screen for renal injures. In some studies there is no evidence of gross or microscopic hematuria in up to 70% children sustaining grade 2 or higher renal injury(1).

DESCRIPTION OF CASES

During the period from October 2005 to June 2007, 3 cases of pediatric blunt renal trauma were managed by the Dept of Pediatric Surgery, SRMC, Chennai. We have reviewed the records retrospectively to study and analyze the various presentations and different modalities of management.

Case - 1

A twelve year old boy who sustained blunt abdominal injury following fall from the bicycle 2 days prior, presented to casualty with history of passing red coloured urine. On examination, right iliac and lumbar region tenderness with a diffuse swelling 2cm x 3 cm was found. Blood was seen at the urethral meatus. Patient had associated forehead laceration and ecchymosis around the right eye. CT scan abdomen revealed contusion of lower pole of right kidney, with perinephric hematoma and renal parenchymal laceration of 1.5cm (grade-3 renal injury). At admission Hemoglobin was 10.5 gm%, blood counts, urine analysis, renal function tests and serum electrolytes were normal. On day 2 and day 4 of admission hemoglobin levels, RFT and serum electrolytes were normal. Patient was managed conservatively with IV antibiotics, analgesics and close monitoring of vitals. The mass and tenderness decreased significantly and vitals were stable. Ultrasound abdomen on day 10 showed resolution of the mass, but with residual perinephric hematoma. Patient was discharged on day 11 with oral antibiotics for 5 days. During follow up at 1 month as outpatient, child was afebrile; BP recording was 106/70 mm of Hg, USG abdomen showed near complete resolution of perinephric hematoma. During further follow up at 3,6 and 9 months the child was asymptomatic, BP recording was normal and USG abdomen normal.

Case – 2

Thirteen year old boy with history of slip and fall on the ground while playing a day back, presented to emergency room with complaints of lower abdomen pain and back pain since the time of injury, history of hematuria 4-5 episodes and 2 episodes of non bilious vomiting. On examination vitals were stable, tenderness present in the left lumbar region with mild guarding. At admission USG abdomen showed features suggestive of renal injury. CT scan with contrast done on the same day revealed laceration of renal parenchyma with perinephric collection suggestive of grade III renal injury. Urine microscopy showed granular casts, plenty of RBCs and pus cells, urine protein positive. Hemoglobin level, RFT and serum electrolytes were normal. On day 9 USG abdomen showed resolving renal contusion and perinephric hematoma.

Patient was managed conservatively with IV fluids, IV antibiotics, analgesics and absolute bed rest. Tenderness decreased over a period of 1week. Patient was discharged

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on day 10 with oral cotrimoxazole for 5 days. On follow up at 1 month child was afebrile, BP recording was 112/70 mm of Hg. USG abdomen showed resolution of renal contusion and perinephric hematoma. During further follow up at 3 and 6 months child was normotensive and USG abdomen was normal.

Case - 3

Fourteen year old boy referred from Andaman and Nicobar islands, with history of right loin pain following fall from a height, 2 days back. No history of hematuria. On examination vitals were stable, tenderness and diffuse mass in the right loin. Patient was admitted, CT scan done on day 1 shows, total disruption of the lower pole along with the pelviureteric junction (Figure-1, 2). Hemoglobin was 8.9 gm%, urine microscopy, RFT and LFT were normal. Two units of packed cells were transfused in the ward. Child was taken up for exploratory laparotomy on day 3 in view of gross hematuria, expanding abdominal mass and increasing biliary aspirate.

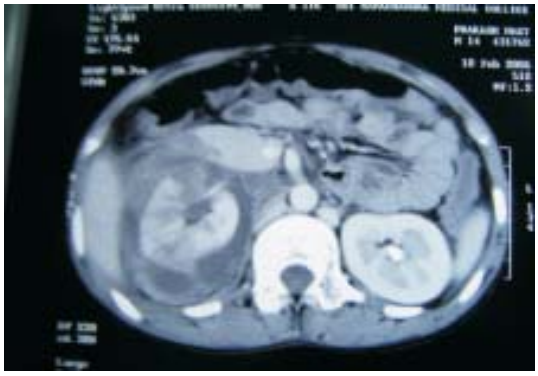

Figure-1 Contrast CT scan showing laceration of right renal parenchyma with perinephric collection.

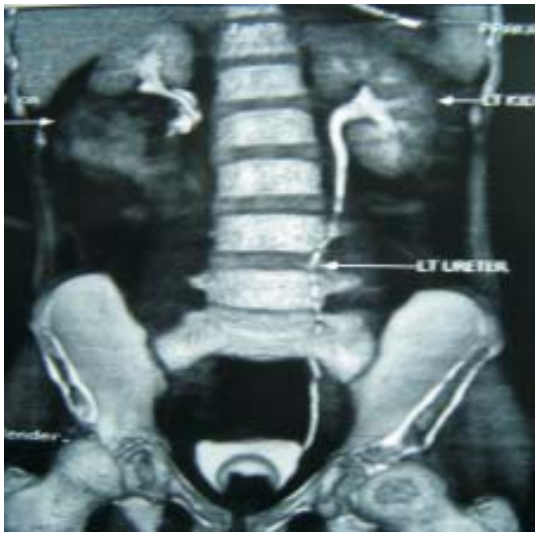

Figure-2 Three dimensional reconstruction of contrast CT showing right kidney laceration and contrast extravasation with non visualization of right ureter

Operative findings include full thickness total laceration of right kidney transversely into two pieces with a perinephric hematoma. (Figure-3) suggestive of grade 5 renal injury. The lower half was separated completely along with renal pelvis and ureter. There was a hematoma in the duodenum and no other bowel injury was present. On evacuating the hematoma blood gushed from the junction of renal vein and inferior vena cava, kidney became pale. Vascular surgeon opinion was sought. As the kidney could not be salvaged right nephrectomy was done after isolating the renal pedicle (Figure-4). Estimated intra operative blood loss was 2 liters. Three units of whole blood and 1 unit of FFP were transfused. Patient made satisfactory recovery in the post operative period.

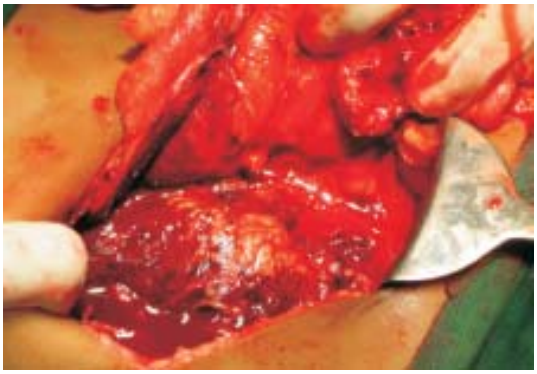

Figure-3 Operative photograph showing perirenal hematoma

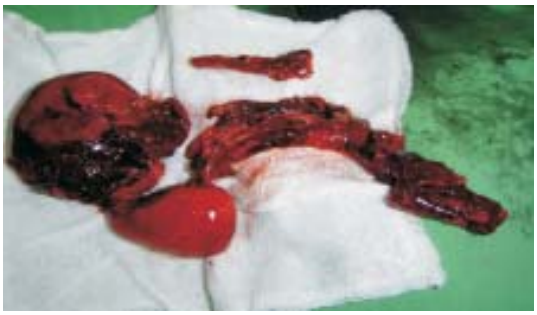

Figure-4 Nephrectomy specimen showing full thickness transverse laceration of kidney with pelvis and ureter attached to lower pole.

Post operative hemoglobin, WBC count, RFT & Electrolytes were normal. Child was discharged on 9th post operative day. During follow up at 1 month child was afebrile and BP recording was 114/72mm of Hg. On further follow up at 3 and 6 months child was normal clinically, BP recording and USG abdomen were normal.

DISCUSSION

Blunt renal trauma in children is the commonest pediatric genitourinary injury. Presenting features in our study group includes hematuria, loin pain, and loin mass.

Focused assessment with sonography for Trauma (FAST) is operator and experience dependent. A FAST scan that is negative for intra abdominal injuries combined with normal serial physical examinations over a 24 hour period of observation will virtually rule out the presence of significant

intra abdominal injuries(7). In the clinically stable patient triphasic abdominal and pelvic CT is the most sensitive method for diagnosis and classification of genitourinary trauma(1,2). Single shot intra venous pyelography taken 10-15 minutes after injection of contrast in the operating room is helpful in detecting a normally functioning contralateral kidney if unilateral nephrectomy is a consideration.

Super selective angiographic embolization of renal artery branches for persistent or secondary hemorrhage has a success rate approaching 80 %. Repeat CT scan 2 to 3 days after the trauma in renal injuries of grade III and above is recommended(2). In our study a follow up ultrasound scan was done before discharge of conservatively managed patients. Classification of renal trauma into 5 grades guides the treatment modality (Table-1)(8).

Table-1 Classification of Renal Injuries

Grade of renal injury		Description
I	Contusion Hematoma	Microscopic or gross hematuria; urologic studies normal Subcapsular, nonexpanding without parenchymal laceration
II	Hematoma Laceration	Nonexpanding perirenal hematoma confined to retroperitoneum < 1cm parenchymal depth of renal cortex without urinary extravasation
III	Laceration	> 1cm parenchymal laceration depth without collecting system rupture or urinary extravasations
IV	Laceration Vascular	Laceration extending into collecting system with urinary extravasations. Injury to renal vasculature with contained hematoma.
V	Laceration Vascular	Completely shattered kidney Renal hilar avulsion that devascularizes kidney

Ideal candidate for non operative management is the hemodynamically stable patient with grade I or II renal injury[2]. Patients with isolated grade 3, 4 and 5 renal injuries are also candidates for non operative treatment(1, 2). Non operative therapy consists of bed rest, close monitoring of vital signs and urine output, serial abdominal examinations, serial hemoglobin determination, transfusion as indicated and intravenous broad spectrum antibiotics. In our study 2 children with grade 3 injuries managed with the above protocol recovered satisfactorily and were discharged from the hospital without any complications.

Absolute indications for renal exploration after trauma include hemodynamic instability due to renal bleeding, expanding or pulsatile retroperitoneal hematoma and inability to stop persistent or delayed hemorrhage via selective vascular embolization. Nephrectomy should be considered in irreparable grade 4 and 5 renal injuries. Only one child underwent nephrectomy for grade 5 renal injury (shattered kidney) among the cases reported here.

Trauma induced renal vascular hypertension occurs after a grade 3 or higher injuries in approximately 5% (2). Common causes of renal hypertension are renal ischemia from segmental arterial occlusion, main renal arterial occlusion with intact peripheral blood flow to the kidney and a trauma induced arteriovenous malformation. In this case report, so far none of the children have developed these complications when examined during follow up. However further follow up is required to detect whether these complications occur**.**

Although no conclusion can be drawn from a series involving 3 patients, it is our observation that renal injury can present with varied symptoms at different age groups. Classification of renal injury and grading helps in deciding whether conservative or operative management is required. Conservative management has a role in grade 3 and higher renal injuries provided patient is hemodynamically stable and closely monitored in a tertiary referral centre like ours.

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