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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 calendar 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 one side only with double spacing throughout. Pages should be numbered consecutively, beginning with title page. Lettering should be 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
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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, Sri Ramachandra University, Porur, Chennai - 600 116. www.smc.edu/srjm
From the Editor’s Desk

It was a moment of joy for the editorial board when the maiden edition of Sri Ramachandra Journal of Medicine was released in September 2006. We had apprehension and fear about the outcome and response from the readers. Fortunately, it was positive and encouraging from every quarter of our university and other major educational institutions. It was delightful experience to get constructive comments, words of encouragement for the first issue.

With the good wishes and encouragement of the management and senior faculty member, we are venturing with the next issue. This issue includes the Sri Ramachandra experience to formalize the informal curriculum using an innovative method to impart “professional values”, the concluding part of the article on interventional radiology, the application of use of fluorescent antibody conjugate in labelling transformed mitotic cells with the possibility of exploitation of these antibodies for cancer detection, review articles and case reports. We are aware of our responsibility and we in the editorial board hope to live up to your expectations.

This second edition is in front of you with untiring work done by all my colleagues under the watchful eyes of the Chief Editor Dr. K.V. Somasundaram. I will be failing in my duty if I don’t highlight the silent, efficient, hard work put up by Mrs. Viji of the Dean’s office. I hope this edition will draw the attention of many more of our readers and give us their suggestions and constructive criticism to enable us to improve further.

J.S.N.MURTHY
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The Editorial Board gratefully acknowledges their contribution.
மாரியூணர் முன்னெச்சல்

சாலையேறியுள்ள சர்வதேச முன்னெச்சல் நூற்றாண்டுகள்

மக்கள் சேர்க்கப்பட்டு, நவீன சேர்க்கப்பட்டு புகழிக்கப்பட்டு

சொருட்டு செய்யப்பட்டு மத்திய வைக்கப்பட்டு முற்பார்வை

எனும் முன்னெச்சல் முன்னெச்சல் என்று எழுதியது

செயல்வாய்ப்பு முன்னெச்சல் முன்னெச்சல் முன்னெச்சல்

முன்னெச்சல் முன்னெச்சல் முன்னெச்சல் முன்னெச்சல்

செயல்வாய்ப்பு முன்னெச்சல் முன்னெச்சல்

நூற்றாண்டு காலத்தில் முன்னெச்சல் என்று எழுதியது

சிற்றுநி காலத்தில் அதிகாரி என்று எழுதியது

ஆர்மோனிக் செயல்வாய்ப்பு என்று எழுதியது

செயல்வாய்ப்பு முன்னெச்சல் என்று எழுதியது

அனுமானத்தை விளக்கவும் என்று எழுதியது

செயல்வாய்ப்பு முன்னெச்சல் என்று எழுதியது

செயல்வாய்ப்பு முன்னெச்சல் என்று எழுதியது

- Dr. D. குருநாதாந்தன்
Education is an inclusive process; is enriching life; formally or informally, every experience is an education. Every event in life, every moment of living is an education.

Accordingly, medical education, at the first instance, should create an individual who will be a medical person. Corollary, that person should continue to be educated as anyone else, as an individual and as a professional. The period spent in the university as Under-Graduate or Post-Graduate should help to evolve this total, complete person.

India’s first national policy on Education was announced in 1968. After periodic minor changes, the next one was announced 18 years later. Concepts on education are evolving at a very high speed and our policies should accommodate them, adapt and develop with a much greater speed and power. For historic reasons, English has spread in our country – this is a blessing, though a legacy of colonial rub. And this is an important base for creating a knowledge power house.

Tracing the history of medical education in India, one becomes aware of what was practised few thousands of years before. Very many of them are in vogue today with English equivalents like personality of the practitioner, character, innovation, learning by roll-model, women’s role etc., As you read and understand these works, you are spurred to greater heights.

Today knowledge proliferates but wisdom languishes. The real education is the education by values.

With enough background material and guidelines as advocated by UNESCO, a system has already been conceptualized. This consists of three layers.

The formation layer aims to build the personality based on values and enables continuous learning. This includes values which are social, economics, wellness, citizenship, humanities and aesthetics, moral and spiritual.

The next layer should help one “Learning to learn” and “Learning to do”. This consists mostly of skills – like learning from different sources, sensory inputs, studying, communication including languages, expression in different situations etc; technology improvement, personality and skills for living life as it should be.

The third layer should facilitate transformation into “Learning to live”. This has a myriad topics like work culture, Total Quality Management, competitiveness, global approaches, entrepreneurship, corporatisation, professionalism, innovation or thinking differently, attitudinal changes and certain autonomy.

To achieve this aim, the faculty should be trained. In the first year, teachers should trained in teaching-learning process and their skills should be credentialled. The present national process for training teachers may need modification before applying to health professionals. Over a period, mentoring is necessary for the teachers also. Research is a big topic in India today. It is an exciting field. There are lots of money for the future which could make this country one of the frontliners in medical innovations like biotechnology or nanotechnology. The assessment of the teacher should be on well laid principles and the concerned individual should be aware of it. Assessment from different sources should be encouraged. Today they also need to be trained in basic administration. A system of education should have a good assessment procedure in place. We need to be more practical. Teachers, particularly assessors should be motivated and supported well financially. Motivation should be altruistic and not for pride or prestige.

Out students are going to be professionals. So the curriculum should include professional development as important segment, atleast in the period of resident training. This should encompass accepted principles like development as an individual, personality, character, skills in
management of time, people, finance, stress and strain of living, active listening, body language, etc., One should not forget gender issues, customer expectations, stake holder’s demands, civil law, ethics and good laboratory practice.

One is reminded again that it is in this profession, globally, that we have maximum number of suicides, alcoholic and depressions. Why is it? Have we failed to train them properly? Obviously emotional development has not been stressed as much as being intelligent. Gandhiji said “Information without formation leads to deformation”. One should also remember what former Prime Minister Mr. Jawaharlal Nehru famously described as “Scientific temperament”.

The student should have interest developed in “learning” as an art, to be practised continuously, curiosity of the childhood should become an “enquiring mind” and “discerning eyes” of the future. One should also practise to unlearn what is obsolete and relearn regularly. For the professional to practice, can we not apply a compulsory periodic assessment to make them really worthy of the trust based on them by the society?

Our training of residents leaves much to be desired. Currently they learn to practice what they have learnt, under supervisors. This is good and entails them for registration. But it is just not enough. They should have diversity or a choice depending on what they want to be next step. And there should be an assessment at the completion of the period, reflected in a comprehensive certificate.

What with WTO and GATS, there is a track in translational higher education, currently a hot issue. Is education still a service, a calling or just another profession? If the students and society become customers, then it becomes a trade.

We should be able to think differently, not be afraid of a change. That is not fundamentalism or extremism. As aptly put by many, we are often prisoners of our own thoughts. The inputs are from many and variable sources, often not tested or challenged. Thought is just a network, made of threads. Not being afraid of the unknown, we should learn to think differently and constructively both as an individual and as a group. This is just as advocated by many as a useful tool, a must for a future – future is not some distance away, it is what we create today.

The curriculum with due checks and assessment process should change to include contemporary issues like related civil law, consumerism, ethics etc.,

There are quite a few lacunae today. Importantly there is very little study or research to assess the utility of what is in the curriculum in the immediate period – internship; during P.G. program or while doing professional work. Does a very high academic record means he will be a highly competent professional, a good individual. It is necessary to be a professional. It is a very comprehensive term. It is essential today especially since expectations and awareness are increasing; it is highly error-prone service. Health care is very expensive. It is a truly global scenario.

Research is an integral part of higher education, big or small, then should be one project at least and scientific writing and speaking should be developed.

A sentence needs a full stop to be meaningful; but education has no full stops.
Prof. S. Rangaswami appointed Vice-Chancellor, being felicitated by the Chancellor

Prof. S. Rangaswami's Inaugural address as Vice-Chancellor
Life Cell and Sri Ramachandra “TRICELL” Inauguration by Chancellor

“Horizons” - Dr. Amit Patel, Director, Cardiac Stem Cell Therapy
Pittsburg University
Photo Gallery

HMI - Sept. 2006 @ Sri Ramachandra

Harvard Medical International - 'Team for Patient Safety'

Active Participants

Globalisation of the Team
CREATING PRODEV – A PROGRAM TO FORMALIZE THE INFORMAL CURRICULUM

Krishna G Seshadri

BACKGROUND

One of the most intriguing aspects of becoming a physician is learning the skills that many educationists and experts club together in a rather unwieldy basket called the “hidden curriculum”. The word intriguing is used deliberately in this context because many aspects of the nature and the acquisition of the hidden curriculum are indeed shrouded in intrigue. Anyone who examines the medical profession from a good objective distance is sure to be amazed that this profession spends an enormous amount of time in instructing its apprentices on the more capricious aspects of its trade, for instance the choice of therapy for Chronic Myeloid Leukemia which has changed at least twice in the last ten years and little or no time at all in the skills that will probably remain unchanged for the rest of the learner’s career for example communication with a patient. From a faculty perspective even more dangerous is the knowledge that “the hidden curriculum – which for the purpose of this article we will call professionalism (although this does include a number of skills that does not strictly fall into this category) was relegated to an ill defined osmotic process called “role modeling”. Unfortunately as Stern elegantly points out, role modeling is in the eye of the beholder – the student and not the teacher. “Individuals who are seen as mentors may not realize that they are teaching professional values and those not seen as mentors may believe they are.”

For a major part of the last century, the acquisition of the hidden curriculum – which for the purpose of this article we will call professionalism (although this does include a number of skills that does not strictly fall into this category) was relegated to an ill defined osmotic process called “role modeling”. Unfortunately as Stern elegantly points out, role modeling is in the eye of the beholder – the student and not the teacher. “Individuals who are seen as mentors may not realize that they are teaching professional values and those not seen as mentors may believe they are.”

Since the 1970s many medical schools around the world have developed modules or content areas that teach as the least, medical ethics. National committees that design curricula have acknowledged the need to formalize the “informal curriculum.” Several excellent examples of attempts by prominent medical schools, to create an environment of acquisition of, and the reflection on these “professional values” by medical students have been published. These innovative methods have in part been spurred on and sustained by a recognition by regulatory and licensing agencies in various nations (including the Medical Council of India) that these skills must be an important part of the core curriculum.

THE SRI RAMACHANDRA EXPERIENCE

The curricular innovation initiative can be traced back to the conduct of the “Status of Progress of Reforms in Undergraduate Curriculum and Education (SPRUCE)” a conference conducted by our university in the year 2000 with the support of the Medical Council of India. The conference highlighted that there was a common aspiration among the many stake holders of education in India in creating a more progressive patient centered problem based curriculum with clear emphasis on professional development. Following this Sri Ramachandra University (SRU) created a curriculum development initiative with a view to create a competency based curriculum by the year 2008.

The curriculum development group developed a unique process of working backwards – first identifying the knowledge, skills and attitudes that a physician needs to possess while he or she practices in a particular domain after graduation and then place them accordingly into the various phases and departments. The cross content areas were marked for vertical or horizontal integration. It was apparent during this process, that a large number of competencies that would in the past be left out in a grey zone to be acquired informally will now have to be formalized into the curriculum. As the curriculum development process proceeded, the group discovered that this basket was rapidly topping up and needed to be addressed in a fashion that will be complementary to the new curriculum. Further direction to the curriculum development process was given by the leadership of the Sri Ramachandra University which expressed a desire to see “students from Sri Ramachandra graduate with real life skills”.

The curriculum group felt that this was an opportunity to develop a unique model to impart professional skills – and PRODEV was born.

DEVELOPING PRODEV

The first step in creating PRODEV was to establish a needs document (table 1). A faculty questionnaire was circulated that asked the respondents to indicate if they felt if formal instruction was desirable in seventeen areas of the informal curriculum. Except for “alternate medicine” for which there was minor disagreement, the overwhelming response for each of these questions was one of strongly agree or agree. Several focus groups were also held with various levels of students to ascertain their views.
Once the need was established, content areas were identified (Table 2) and a debate initiated on who should teach these content areas. There are several reports on content areas in the literature, the most prominent of them being the Pond report which suggested the identification of a group of experts to teach medical ethics. It was clear from the questionnaire submitted to the faculty that a majority of faculty professed no experience or comfort in teaching professionalism formally. However, it was evident that faculty cutting across departmental and hierarchical lines were eager and willing to be part of the process (except one, all the respondents expressed willingness to be part of this process). It was fortuitous that the program chose interested novices than confine this to the hands of a small group of skilled experts as will be evident below.

From the outset, it was clear that the process to implement PRODEV needs to be longitudinal, spread across the five and a half years, must straddle departmental silos and must be taught by any faculty who are willing to teach. Departments willingly donated surplus time for the program. Three hours (one in the morning and two in the afternoon) once in two weeks were made available for PRODEV. Once the time was allocated it was decided to start the program with the students in the 8th semester progressively moving to lower phases each year depending on the success of the program.

**Creating a Structure for PRODEV (Fig. 1)**

A hybrid method of instruction was evolved – the discussion would revolve around a case introduced to a small group by a facilitator; the group would identify learning objectives and do self study and reflect for two weeks. An anchoring lecture would then be delivered by “an expert” on the morning of the discussion. The patient problem would be discussed by the small group in the afternoon and the case resolved.

![Figure 1. Structure of Prodev](image)

After the completion of several blocks of cases – an assessment would be conducted both formative based on a standard form developed by the curriculum group as part of the curriculum development initiative as well as a summative test that consisted of 1) a morning written session consisting of MCQs and a creative writing essay and 2) an afternoon session with an ethics or a communication OSCE.

**Implementing PRODEV**

In January 2005 the first faculty development for PRODEV was held for sixty faculty members. Three faculty members were assigned to each group to be facilitators. The expectation was that the faculty will not only help these groups with PRODEV but also evolve into mentors for the

Table 1. Steps in Developing PRODEV

<table>
<thead>
<tr>
<th>Step</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Establish need</td>
<td>Faculty questionnaire, Student Focus groups</td>
</tr>
<tr>
<td>2. Content identification</td>
<td>Literature review, Discussion with peers from other institutions, Validation by faculty questionnaire</td>
</tr>
<tr>
<td>3. Structure development</td>
<td>Consensus by curriculum group to create a hybrid program (see text)</td>
</tr>
<tr>
<td>4. Faculty development</td>
<td>Formal day long faculty development program</td>
</tr>
<tr>
<td>5. Content development</td>
<td>Case writing, identification of key learning articles, identification of important learning sites on the world wide web</td>
</tr>
<tr>
<td>6. Student assessment</td>
<td>Formative bimonthly assessment, MCQs, Creative writing and Ethics and communications OSCE</td>
</tr>
<tr>
<td>7. System assessment for CQI</td>
<td>Formal bimonthly feed back from faculty and students. Half yearly open house with faculty and students</td>
</tr>
</tbody>
</table>

Table 2. PRODEV content areas

1. What is expected of a doctor in the 21st century
2. Foundations of Medicine and the law
3. Foundations of Medical Ethics
4. Reasoning in Clinical Practice
5. Introduction to Clinical Practice
6. Ethical Aspects of Human Research
7. Taking the bench to the bedside
8. Effective Communication
9. Connecting with Patients
10. Working in a Health Care Team
11. Etiquette for Doctors
12. Health Care Systems
13. Quality in Health Care
14. The Economics of Health Care
15. Alternate Health Care Systems
16. Personal Development for Professional Growth
17. Preparing for Lifelong Learning

students. The program was started with the incoming 8th semester students and extended to the sixth semester students in July 2005. The first PRODEV case was provocatively called “How to buy a kidney”. Two batches of medical students have completed PRODEV.

OUTCOMES

PRODEV has been a gratifying experience for its creators, facilitators and students. An analysis of student and faculty feedback reveals that PRODEV has realized most of its intended outcomes; in addition realized several unintended but desirable outcomes. These are summarized in table3.

Table 3. Benefits from PRODEV – based on responses obtained in written feedback

For the Students
1. “Learnt how to talk to patients”
2. “Learnt to face real life situations”
3. “Were able to interact with faculty in an informal atmosphere”
4. “Were able to learn from our colleagues”

For the faculty
1. “Widened our horizons”
2. “Learnt about topics we were not too comfortable with”
3. “Were able to work with colleagues from other departments”

For the Curriculum process
1. A preview of the integrative curriculum
2. A situation were faculty were able work across silos
3. A test for seeing how students use reflection and introspection
4. A test of collaborative and group learning

For the curriculum group, PRODEV was an interesting test run of the new curriculum – a testing laboratory to find out what will be the consequences of introducing a hybrid program, how faculty work across departmental silos, how students migrate from an environment of emphasis on rote knowledge to an environment where a larger part of the learning responsibility will rest on them. The results emboldened the curriculum development group to roll out the preclinical integrated curriculum a year ahead of schedule.

Student and faculty involvement have been the key to the success of the program and the intensity of their involvement has carried the program forward. It is too premature to ascertain the long term gains in the career and professional lives of students but a random survey of the first batch of students who are currently in their CRRI year reveals that they feel better equipped to handle “every day medicine”.

CHALLENGES THAT REMAIN

While PRODEV has unfettered acceptance in the institution – it still faces the challenge of not being an integral “core of the curriculum”. A decision was made to not extend PRODEV into the 9th semester as students and some faculty perceived that this would be a distraction in a semester that student attention must be focused on their final examination. Unless the content areas championed by PRODEV are an integral part of this examination the attitude towards professional development sessions will remain, and be an issue that will need to be addressed as we gain more experience and more support from regulatory agencies like the Medical Council of India.

CONCLUSION

PRODEV has been a successful attempt at introducing a longitudinal multidisciplinary professional development program for MBBS students. Its success has paved the path for the implementation of an integrated competency based patient centered curriculum in the university.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the contributions of the leadership, the curriculum development group, the PRODEV coordinators, the medical education unit, faculty and all the students who made PRODEV possible.

REFERENCES:
INTRODUCTION:
Radiology remained a diagnostic science ever since its inception following the discovery of X-rays by Roentgen in 1895. The speciality has grown with contrast studies, fluoroscopy and invasive access to blood vessels by Seldinger’s technique which have paved a new way to approach clinical diagnosis. The term “interventional radiology” was coined by Alexander Margulis in 1960 to denote a group of procedures done with the help of imaging. Initially it began with simple image guided biopsy or abscess drainage with the help of fluoroscopy. The addition of cross sectional imaging in the last two decades such as CT, Ultrasound and MRI has provided a new dimension in understanding disease process. The innovation of angioplasty (PTA) by Charles Dotter in 1964 was a break through in interventional radiology. Slowly but inevitably procedures that once required surgeons and surgical incisions have been replaced by percutaneous image guided techniques. With the addition of technological innovation and micro devices, the scope, and the number of procedures have increased with a separate speciality being started for each organ/system of the body. The addition of 3D rotational angiography was a boon to the interventional neuroradiologist. Basically interventional procedures aim at opening or closing, i.e., opening of a blocked duct or blood vessel and closing of an abnormal or leaking duct or vessel by introduction of various devices. This article aims at the scope of interventional radiological procedures, including those performed at SRMC & RI.

MATERIALS AND METHODS:
Invasive angiography and interventions performed by the interventional radiologist has been analyzed. A total of 2732 angiographic / interventional procedures were performed (M = 1737; F = 995; Age range = 1day to 87years, N = 48years). The procedures were performed using Biplane DSA 3D rotational angiography system (LCN + GE Milwaukee). The indications for interventional procedures included acute vascular emergencies, control of acute bleeding, acute stroke, acute limb, several difficult clinical problems such as AVM, aneurysms etc. and for preventive measures, to manage therapeutic complications, as an aid to palliative care, pain management and infertility. This article aims to highlight the variety and the scope of these procedures in patient care.

E) INTERVENTIONS IN CHRONIC PROBLEMS
E.1 Hypertension - Intervention for renal artery stenosis (Fig 15)
There are many causes for obstructive lesions of the renal artery, common ones being atherosclerosis (90%) and fibromuscular dysplasia (FMD). The clinical manifestations of renal artery occlusive disease are hypertension, renal failure or both. Hypertension secondary to renal artery stenosis accounts for 1-5% of patients with hypertension. Angioplasty and stent placement are the percutaneous techniques commonly used in the treatment of obstructive lesion of the renal artery.

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Fig. 15 Renal PTA and Stenting.
Renal artery angioplasty (Fig. 15) and stent placement is a challenging procedure and depends on the types and locations of lesions, the size of the vessels and the angles between the aorta and the renal arteries. Metallic stents improve the technical and clinical outcomes in atherosclerotic disease, particularly for ostial lesions. Unless complicated by a dissection, stents are not necessary for most forms of FMD.

E.2 Claudication / Chronic limb

The prevalence of atherosclerotic peripheral arterial disease increases with age from 3% in individuals aged 40-59 to 20% in older than 70 years. Most common symptom is pain upon ambulation (claudication). A smaller percentage have rest pain, tissue loss or gangrene. A wide variety of technologies have been applied to this vascular bed including angioplasty, stents, stent – grafts, mechanical atherectomy and laser atherectomy. Occlusive disease of the SFA and popliteal artery can be effectively treated with angioplasty when the stenoses or occlusions are focal (Less than 5cm in length). The technical success rate for percutaneous SFA and popliteal interventions is greater than 95% for stenoses and 85-90% for occlusions. Similar interventional techniques are also available for upper limb and visceral arteries. The major advantage of endovascular therapy includes repeatability in primary or secondary failure whereas redo procedures are cumbersome in surgery.

F) INTERVENTIONS FOR THERAPEUTIC COMPLICATIONS

F.1 Intravascular foreign body removal

The most common cause for accidental embolisation is transection of an indwelling polyvinyl or polyethylene catheter when it is withdrawn across a sharp needle bevel. Intravascular foreign bodies can cause serious complications such as death, transient arrhythmia, sepsis, thrombus and pulmonary emboli. These can be successfully removed using retrieval devices such as the loop snare, hooked catheter, helical basket, hook guidewire etc.

G) INTERVENTION IN DIFFICULT CLINICAL SITUATIONS:

G.1 Carotid cavernous fistula (Fig. 16)

These are spontaneous or traumatic connections between the carotid artery and the cavernous sinus and can be classified as direct or indirect. The traumatic carotid cavernous fistula occurs following head injury where there is a tear connecting the carotid artery and cavernous sinus, whereas spontaneous CCF are dural AVM’s at the level of the cavernous sinus. Reversal of direction of the flow through the ophthalmic veins and or sphenoparietal sinus is possible if an arterio-venous connection develops in the cavernous sinus. Elevated venous pressure in the veins draining the orbit may produce orbital venous congestion, transudation of interstitial fluid, proptosis, increased intra ocular tension and secondary glaucoma. Less frequently reversal of flow into the sphenoparietal sinus with resultant cortical venous hypertension poses a risk of intracerebral hemorrhage and warrants emergent therapy.

Endovascular treatment options available include transarterial balloon embolisation for symptomatic direct CCF and trans arterial or transvenous coil embolisation in case of indirect CCF.

Fig. 16: Carotid Cavernous Fistula


G.2 Dural AVM (Fig.17)

Intracranial dural AV Fistulae are acquired arterio venous shunts located inside the duramater. They account for 10-15% of all intracranial arteriovenous lesions. Their presentation and prognosis are variable, symptoms are due to arterialization of the venous system. Endovascular treatment is particularly tricky. The choice of treatment depends on the natural risk of the disease which may be estimated for each patient according to the type of venous drainage. The possible modes of treatment include arterial embolisation with particles or glue, sinus occlusion with coils or even direct puncture and coil embolisation. The major symptoms in patients with dural AV fistulas include tinnitus, proptosis, raised ICP or IOP and mentation changes. The transvenous embolisation or direct puncture embolisation through a burr hole provides a new opportunity to treat these highly complex and challenging problems with good clinical results.
Fig. 17: Dural AVM

**Dural AVM:** 43 year old gentleman presented with proptosis, chemosis of the right eye with right third nerve palsy. **A:** Right carotid angiogram showing dural AVM at the level of the right transverse sinus. **B:** Coil mass in situ in the right transverse sinus. **C:** Post coil embolisation angiogram showing obliteration of the AVM. **D:** Pre procedure clinical photograph showing proptosis and chemosis of the left eye. **E:** Post procedure clinical photograph showing full resolution at three months follow up.

G.3 Pial AVM (Fig.18 & 19)

Arterio-venous malformations (AVMs) are the most common intracranial vascular malformation. AVM’s occur in about 0.02% to 0.05% of the population. In treating AVMs, the nidus of the lesion must be removed or obliterated. The more the nidus of the AVM is occluded, the less collateral supply it will develop. The materials available for embolisation include N-butyl-cyanoacrylate (NBCA) and absolute alcohol or onyx.

**Pial AVM:** 35 year old man presented with sudden onset of hemiplegia and aphasia. **CT scan revealed large right intra cerebral hematoma. A:** Carotid angiogram AP view shows a motor cortex AVM. **B:** Microcatheter placed distally within the nidus. **C:** Following selective catheterization and embolisation with NBCA, total disappearance of the nidus. Patient made a remarkable recovery.

G.4 Spinal AVM (Fig.20)

In patients with spinal AVM or spinal dural fistula embolisation is the choice of treatment as surgical excision carries high morbidity and mortality.

**Spinal AVM:** 64 year old gentleman presented with sudden onset of paraplegia. **A & B:** Spinal angiogram reveals spinal dural AVF in the right side of the thoracic spinal cord. **C:** Post embolisation shows complete occlusion of the AVM. **D:** Embolic cast.
Rapid advances in neuroimaging and improvements in neuro angiography have led to better understanding of spinal cord vascular malformation. The clinical presentation of spinal AVM is directly influenced by the location as well as the angioarchitecture. Significant technical improvement in catheters and delivery systems as well as in various embolic materials have led to marked technical improvements in endovascular therapy.

G.5 Tracheo-bronchial stent

Patients with large airway obstruction arising from either benign or malignant processes and those with tracheo oesophageal fistula have a particularly challenging clinical problem. Common causes of local airway obstruction include overgrowth of granulation tissue, fibrosis, tracheomalacia, endoluminal neoplasm and extrinsic neoplasm. The recent development of an endoluminal stent offers a relatively simple and non invasive method of relieving clinical problems due to obstruction\(^{26,27}\). Being a simple procedure, the placement of endoluminal tracheo-bronchial stents has been advocated for treating mechanical causes of airway compromise that are not amenable to surgery. After the placement of endoluminal tracheobronchial stents, patients experience quick relief of respiratory symptoms and enjoy a relatively comfortable life. Stents also provide relatively long lasting relief against malignant obstruction.

G.6 Pulmonary AV Fistula

Congenital pulmonary arterio-venous fistulae (AVF) and malformations (AVM) are abnormal direct communications between a pulmonary artery and pulmonary vein. Blood is shunted directly from the right heart to the left, without benefiting from two major functions of the pulmonary capillary bed: oxygenation and filtration. Patients with such lesions may present with symptoms of hypoxia due to shunting, resistant polycythemia, effort intolerance, high output cardiac failure or more seriously paradoxical embolisation. The therapy of pulmonary AVFs is embolisation. Occlusion of the feeding artery can be accomplished with coils or detachable balloons. Recurrence after embolisation is unusual (less than 5%).

G.7 Venous malformation / hemangioma (Fig. 21 & 22)

Vascular malformations are composed of dysplastic vessels with a normal endothelial turnover. These can be categorized into slow flow vascular malformation (capillary, venous and lymphatic and high flow vascular malformation (Arterio venous vascular malformations). These venous malformations are usually diagnosed based on clinical examination and demonstrate phleboliths on X-ray. Therapeutic strategies for these malformation is based on multi disciplinary approach involving dermatologists, vascular surgeons, plastic surgeons and hematologists in addition to interventional radiologists.

**Fig. 21 : Superficial Venous Malformation**

**Superficial Venous Malformation:** 35 year old lady presented with multiple tortuous swelling in the right side of neck. A: Common carotid angiogram revealed no arterial feeders. B: Direct puncture embolisation was done using ethanol and lipiodol mixture. C: Pre embolisation clinical photograph with turgid neck swelling. D: Post embolisation clinical photograph reveals almost marked reduction of the swelling.

Percutaneous embolisation help us to achieve significant reduction in size there by helping the surgeon to achieve adequate reduction with limited blood loss. Hemangiomas are pediatric vascular lesions which usually manifest within the first month of life. More than 90% show spontaneous regression at 5-7 years of age. Most hemangiomas do not require any treatment. Embolisation is performed in those patients who presents with high output failure to minimize the shunt and in patients who present with cosmetic disfigurement.

**Fig.22 : Upper Eyelid Hemangioma**

Upper Eye lid haemangioma. 1 year old child presented with swelling of the right upper eye lid of six months duration. A: Right ICA angiogram reveals haemangioma supplied by ophthalmic artery. B: Right ECA angiogram shows feeders from internal maxillary artery. C: Post embolisation angiogram shows significant reduction in vascularity of the haemangioma. D: Pre embolisation clinical picture with mechanical ptosis. E: Post embolisation clinical picture shows remarkable reduction in size of the haemangioma and improvement of ptosis.
H) INTERVENTION FOR PAIN RELIEF

H.1 Vertebroplasty (Fig.23)
Vertebroplasty with acrylic glue (polymethyl methacrylate, PMMA) is a procedure aimed at preventing vertebral body collapse and relieving pain in patients with pathologic vertebral bodies/collapse due to benign or malignant disease.

![Fig. 23: Vertebroplasty](image)

Vertebroplasty. 60 year old female presented with severe back ache. X-ray revealed collapse of the L1 vertebrae. **A:** AP view shows acrylic glue injection with filling of the entire vertebral body. **B:** Lateral view showing transpedicular approach.

The pain reducing effect of cement cannot be explained by the consolidation of the pathologic bone alone. The PMMA is cytotoxic owing to its chemical and thermal effect during polymerization. The temperature during polymerization is high enough to produce coagulation of tumoral cells. The procedure is contraindicated in patients with hemorrhagic diathesis and in the presence of infection. An anterior approach is used in the cervical area, a transpedicular or intercosto vertebral route in the thoracic area and a postero-lateral or transpedicular route in the lumbar area.

H.2 Percutaneous management of Osteoid Osteoma
Osteoid osteoma produces local pain that is worse at night and improves dramatically with aspirin. Effective treatment of this tumor depends on complete removal of the tumor nidus. The conventional treatment is surgical or percutaneous excision. The ability to precisely control the treated area, a high degree of precision, applicability in joints and an excellent dose–response characteristic makes interstitial laser photocoagulation (ILP) a valuable treatment method for Osteoid osteomas. It consists of percutaneous insertion of optical fibers into the tumor. The tumor is coagulated and destroyed by direct heating. The procedure is performed under CT guidance. A single needle and a single laser fibre are sufficient for nidus diameter up to 10mm. Success rates of 60-90% are reported. Other interventions for pain relief include intraarticular injection of corticosteroids in faget syndrome, percutaneous epidural and nerve root block.

H.3 Percutaneous laser disc decompression (PLDD)
Percutaneous removal of the nucleus pulposus has been performed using a variety of chemical and mechanical techniques for the past several years. These techniques consist of percutaneous removal all or part of the nucleus purposes to induce more rapid healing of the abnormal lumbar disc.

H.4 Pelvic congestion syndrome (Fig.24)
Chronic pelvic pain is a perplexing and disturbingly frequent problem. An estimated 10 million women are affected, but a reasonable explanation can be found in fewer than half. A wide variety of gynaecologic conditions can be responsible for chronic pelvic pain. When dilated gonadal and periuterine veins are determined to be the etiology of the pain, the term “pelvic congestion syndrome” is applied. Reflux of blood in gonadal veins is the underlying etiology of pelvic varicosities in the majority of patients. Rarely, pelvic AV malformations or fistulas may be encountered. Gonadal venography remains the definitive diagnostic imaging modality. As most of the patients with pelvic congestion syndrome are relatively young, embolotherapy represents an attractive alternative. Coils are the embolic agents used most often for gonadal vein occlusion. Embolisation reduces or eliminates symptoms in up to 80% of women.

![Fig. 24: Pelvic Congestion Syndrome](image)

Pelvic Congestion Syndrome. 37 year old doctor presented with severe lower abdominal pain. Doppler revealed multiple venous channels in the pelvis. **A:** Ovarian venogram reveals multiple dilated tortuous venous channels. **B:** Post embolisation venogram shows complete occlusion of the dilated venous channels. Patient made a remarkable recovery.

I) INTERVENTION FOR INFERTILITY

I.1 Varicocele embolisation
Dilatation of the pampiniform plexus termed a “varicocele” results from reflux of blood through incompetent gonadal vein valves in males. These are common lesions, found in 5-17% of males. There is no effective medical treatment for varicoceles. The goal of intervention is to interrupt the internal spermatic vein in order to prevent retrograde flow of blood into the scrotum. Surgical ligation can be performed at multiple levels. The recurrence rate is approximately 10-20% due to collateral
flow. Percutaneous embolisation can be performed and embolisation with coils is preferred. Multiple coils are deposited along the entire length of the vein. Sperm counts improve in 80% of patients following successful embolisation.

1.2 Vasculogenic impotence

The indications for penile angiography are the evaluation of impotence and trauma. Approximately 50% of males over the age of 40 experience some degree of erectile dysfunction. The least common cause is vasculogenic, and should be pursued only after other etiologies have been excluded. There are two potential vascular causes of impotence, venous leak (inability to trap blood in the corpus cavernosum) and arterial insufficiency. Vasculogenic impotence has a venous etiology in one third of cases and combined arterial and venous in the rest. Patients with venous leak tend to respond well to pharmacologic therapies. Severe arterial insufficiency is more difficult to treat effectively but in an internal iliac artery, angioplasty or microvascular bypass to the penis may be effective.

1.3 Uterine artery embolisation (Fig. 25)

The most common indication for selective uterine artery angiography is for embolisation of symptomatic fibroids. Fibroids are vascular tumors that grow in size and increase in prevalence with age throughout a woman’s reproductive life. The indications for intervention are fibroids that cause heavy, prolonged periods, pelvic pain, dyspareunia, miscarriages and pressure symptoms on adjacent structures. Pharmacologic treatment with GnRH analogues, results in temporary reduction in size, but fibroids will enlarge once medication is stopped. Conventional surgical procedures include hysterectomy and myomectomy, using open, laparoscopic or hysteroscopic techniques. Uterine artery embolisation is an alternative approach to management of fibroids.

Fig. 25: Uterine Fibroid Embolisation

Uterine Fibroid Embolisation. 35 year old lady presented with abnormal uterine bleeding. Ultrasound revealed 6 x 4 cms fibroid in the right anterior wall of uterus. A: Selective uterine artery angiogram reveals hypertrophied feeders to the fibroid. B: Post embolisation angiogram reveals significant reduction of flow and disappearance of the abnormal vascularity.

The basic principle is selective infarction of the fibroids with particulate embolic materials directly delivered into the uterine arteries. The indications are identical to those for surgery, although the procedure is not recommended when the fibroids are pedunculated or largely submucosal. Permanent particles (300-700mm diameter range) are generally used. Embolisation is continued until there is sluggish flow in the uterine artery with elimination of the fibroid blush. Bilateral embolisation is mandatory. Embolised fibroids shrink on average 50-60% in volume, with relief of symptoms in 85-90% of patients.

1.4 Fallopian Tube Recanalisation (Fig. 26)

Isolated obstruction of the proximal fallopian tube amenable to transvaginal dilatation and recanalisation is thought to be the cause of infertility in about 20% of the patients.

Fig.26: Fallopian Tube Recanalisation

Fallopian Tube Recanalisation. Patient presented with infertility following right salpingectomy for right tubal pregnancy. A: HSG showing left cornual block. B: Balloon dilatation of the left cornu done, contrast injection showing peritoneal spillage

Women with unilateral or bilateral proximal tubal obstruction confirmed by hysterosalpingography or laparoscopy are candidates for transvaginal recanalisation. Transvaginal fallopian tube recanalisation results in lower patient morbidity and is less expensive than tubal microsurgery. This procedure is recommended as the intervention of first choice in patients with proximal tubal obstruction. The more invasive therapy should be reserved for patients with distal tubal disease and for those in whom fluoroscopic catheterization fails.

J) INTERVENTION FOR PALLIATION

J.1 Percutaneous Transhepatic Biliary Drainage (PTBD) (Fig. 27)

The major purpose of PTBD has been and is still used to drain retained bile for decompression in obstructive jaundice. PTBD route is indispensable for carrying out various procedures of biliary interventional radiology. Another use of the route is to obtain an access for intracavitary radiotherapy. Cholestasis induces biliary infection and protracted hyperbilirubinemia impairs renal function. In the presence of hyperbilirubinemia, both surgical mortality and morbidity are high at 15 to 25% and 40 to 60% and...
decompression should be done as early as possible in patients with obstructive jaundice. The success rate of PTBD is 90-100%. The application of indwelling stents (endoprostheses) in the biliary system has been well established because it provides sufficient antegrade biliary drainage without encumbrance of an external tube that needs catheter flushing, dressing at the skin entry site and periodic catheter exchanges.

Fig. 27: Percutaneous Transhepatic Biliary Drainage

Percutaneous Transhepatic Biliary Drainage. 70 year old male presented with obstructive jaundice. MRI revealed Klatskin tumor. A: Cholangiogram shows obstruction at the level of hepatic duct confluence. B: Guide wire placed in the duodenum across the stricture. C: Following successful stent placement, flow of contrast through the obstructed segment visualized. Patient experienced marked relief following biliary stenting.

Invention of metallic stents has become a popular procedure of palliative treatment for patients with obstructive jaundice caused by unresectable malignant tumors. The large caliber is associated with a low frequency of occlusion by bile encrustation.

J.2 Tumor ablation (Fig. 28)

Many solid tissue malignancies are poorly responsive to systemic chemotherapy, surgical resection or local radiation therapy. In situ image guided tumor destruction or ablation has become an attractive option. It offers the possibility of an effective minimally invasive and less costly approach, often achievable in an outpatient sitting. Available ablation techniques can be broadly classified as chemical, embolic or thermal. Chemical ablation is achieved by image – guided instillation of a chemical agent. The most common chemical agent used for tumor ablation is ethanol. Percutaneous ethanol injection (PEI) has been shown to be a safe, inexpensive and effective treatment for small (3-5cms) hepatocellular carcinomas. Tissue functions normally in a narrow range of temperatures. If the local temperature is made sufficiently abnormal, the cells within the environment are permanently damaged. If extremes of temperature are applied, the cells are destroyed and coagulative necrosis ensues. Percutaneous, image – guided therapies using heat have utilized diverse thermal energy sources. Thermal energy sources have included sound (high – intensity focused ultrasound, light (laser photocoagulation), microwaves and radio frequency energy.

Fig. 28: RadioFrequency Ablation

Radiofrequency Ablation. 58 year old male known case of adenocarcinoma lung underwent RFA. A: CT Thorax reveals a large tumor in the posterior segment of the right upper lobe. B: Three months follow up reveals cavitation of the tumor with marked reduction of size. C: Six months follow up reveals almost disappearance of the tumor following RFA. Radio frequency energy has been used for surgical electrocautery since the early 1990s. RFA can be achieved with monopolar or bipolar electrode systems.

COMPLICATIONS

Complication related to interventional radiological procedures include puncture site related, contrast agent related, catheterization related and those related to coagulation. Puncture site related complications include puncture site hematoma, arterial dissection, pseudo aneurysm formation and arterio venous fistula. Contrast agents may lead to nephropathy, allergic reaction, congestive cardiac failure. The use of iso osmolar, non ionic contrast media has reduced the incidence of these complications. Injury to vessels may occur during catheterization and is more common in elderly patients due to pre existent atherosclerosis and in patients with collagen vascular diseases. Thrombo embolic complications may occur due to dislodgement of atheromatous plaques and due to formation of platelet thrombi at the tip of the catheter. The incidence of these complications is variable depending on the procedure performed and the disease for which it is performed.

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CLINICO – MYCOLOGICAL PROFILE OF DERMATOPHYTIC SKIN INFECTIONS IN A TERTIARY CARE CENTER – A CROSS SECTIONAL STUDY.

Kennedy Kumar a, Anupma Jyoti Kindo a, J. Kalyani a, S. Anandan b

ABSTRACT

Introduction: The prevalence of dermatophytosis differs from place to place and is governed by environmental conditions, personal hygiene and individual's susceptibility.

Aim and objective: This study is sought to determine the prevalence of dermatophytic skin infections and their causative agent in the population attending the Dermatology Out patient department.

Material and methods: A total of 117 patients with skin lesions resembling tinea infections attending out patient dept during a six month period (February 2004 to August 2004) were taken for study. Diagnosis was confirmed by microscopy and culture.

Results: Out of the 122 samples collected from 117 patients dermatophytes were isolated from 54.9 %, (67isolates) non-dermatophytes 6.6% (8 isolates) and candida from 4.1% (5 isolates). Tinea corporis accounted for 70.8 % (82 cases) followed by tinea cruris 18.8% (22 cases). Tinea faciei and tinea manuum 3.4% (4cases) each and mixed infection in 5 patients. The male to female ratio of the skin infection was 1.12:1. T. rubrum was the most common etiological agent in 45 cases (67.5%). T. mentagrophytes 18.0% (12 cases), other dermatophytes isolated were T. schoenleinii 4 , two each of E. flocossum and M.audouinii and one of M. nanum and M. canis.

Conclusion: Among the dermatophytic skin infections tinea corporis was the predominant clinical type and T.rubrum was the most common dermatophyte isolated.

Key words: Cutaneous fungus, dermatophytes, Tinea

INTRODUCTION

Mycotic infections are world wide in distribution. However, superficial mycosis is more prevalent in tropical and subtropical countries including India, where heat and moisture play an important role in promoting the growth of these fungi [1, 2].

Fungal infections have attracted the attention of physicians and microbiologists in recent years due to various reasons like indiscriminate use of antibiotics, anticancer therapy and immunodeficient diseases like AIDS.

Sex, race and occupation have little recognized differential influence upon the frequency of dermatophytosis [3], however change in trends are noticed in the studies done by the later researchers.

In the current study, we have undertaken a clinico-myecological approach, correlating various demographic data such as age, and sex with identification of the fungus using standard techniques [3]. As the dermatophytic skin infections are more frequent when compared to those of hair and nails the study was confined to skin infections alone.

Materials and methods

A total of 117 consecutive patients with skin lesions resembling tinea attending the Dermatology Out Patient Department of Sri Ramachandra Medical College and Research Institute, Porur, Chennai, Tamilnadu were taken for the study over a period of six months (February 2004 to August 2004).

A detailed history of selected cases was taken in relation to name, age, sex, address, occupation, duration of illness and involvement of more than one site.

Samples were collected from the site of the lesion. Scrapings were taken with a blunt sterilized scalpel from the active site of the lesion using standard technique described earlier [3].

All the samples collected were subjected to microscopy and culture.

Following direct microscopic examination with 10% KOH, the scrapings were inoculated into slopes of duplicate sets of tubes containing

a) Sabouraud’s dextrose agar with chloramphenicol
b) Sabouraud’s dextrose agar with thiamine
c) Sabouraud’s dextrose agar with chloramphenicol and cycloheximide (To prevent contamination with saprophytic fungi and bacteria.)
d) Dermatophyte test medium.

One set of the tube was incubated at 37°C and the other set at 25°C. The cultures were examined every two days for a period of one month for the presence of growth. The growth was observed starting from sixth day onwards. If no growth was found after 45 days it was considered negative for the growth of fungi.

Growths obtained were identified based on the colony morphology, microscopic appearance and other relevant tests as described by Emmons. The growth on the tubes was examined for gross morphology, pigmentation if any and slide cultures were put to identify up to species level.
Results

A total of 11847 patients attended the OPD section of Dermatology, Venereology and Leprology during the period of our study. Hundred and seventeen of them fulfilled the criteria (not taken any treatment and also had infection for the first time) and hence taken up for the study. There were 62 men (53%) and 55(47%) women. The age of the patients ranged from 4-83 years, the mean age being 35.8 years. There was preponderance of males in the 11-30 years and females in the age group of 31-50 years. Very few cases were encountered in the extremes of age. Males were marginally more affected than females, male to female ratio being 1.12:1. The sex distribution of various clinical types is depicted in Table1.Tinea corporis was the predominant lesion in the present study occurring in 82 (70.08%) patients followed by tinea cruris in 22 (18.8%), tinea faceii and tinea manuum each in 4 (3.41%) and mixed infection in 5 (4.27%) patients.

Table 1

<table>
<thead>
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<th>Clinical types</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<tr>
<td>T.corporis</td>
<td>32</td>
<td>50</td>
<td>82</td>
</tr>
<tr>
<td>T.crusi</td>
<td>20</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>T.manum</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>T.faciei</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>55</td>
<td>117</td>
</tr>
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</table>

Tinea corporis was found to be the most common clinical presentation.

The mixed infection included a combination of tinea corporis and tinea cruris in three patients, tinea corporis and tinea capitis in one and tinea faceii and tinea manuum in the other. The dermatophytic infections were more common in the third decade.

Dermatophytes formed the majority accounting for 67 out of the total 122 samples (54.9%). The principal dermatophyte was T. rubrum 45(67.5%) followed by T. mentagrophytes 12(18.0%), 4 of T.schoenleinii 2 each of E. floccosum and M. audouinii and 1 of M. nanum and M. canis.

A single species of dermatophyte could cause different clinical manifestation as seen with T. rubrum being the major isolate, from all the clinical types of tinea; and a single clinical type like tinea corporis had several etiological agents, from all the three genera of dermatophytes namely Trichophyton, Epidermophyton and Microsporum. (Table 2)

Non-dermatophytic molds were grown from 8 (6.6%) and candida from 5 samples (4.1%). The various non-dermatophytic molds isolated were as follows: Exophiala spp two in number and one each of Exserohilum spp., Fusarium spp.,

Table 2

<table>
<thead>
<tr>
<th>MycoLOGICAL TYPES OF DERMATOPHYTOSIS</th>
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<tr>
<td>Dermatophytes isolated</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>T.rubrum</td>
</tr>
<tr>
<td>T.mentagrophytes</td>
</tr>
<tr>
<td>T.schoenleinii</td>
</tr>
<tr>
<td>E. floccosum</td>
</tr>
<tr>
<td>M. audouinii</td>
</tr>
<tr>
<td>M. nanum</td>
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<tr>
<td>M. canis</td>
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</table>

...Nigrospora spp., Scopulariopsis spp., Cladosporium spp. and Acremonium spp. All these non-dermatophytes were isolated in pure culture and from KOH positive samples and no dermatophytes were isolated along with them.

In our study culture was negative in 42 samples (34.42%).

DISCUSSION:

As stated earlier the present study focused only on skin lesions caused by dermatophytes. Earlier studies confirm that dermatophytic skin infection were more common in males than females as reported by Bhaskaran et al. from Tirupati and Maheshwari Amma et al [4,5], the ratio being 2:1. While most studies in and around Chennai showed a male dominance. In contrast, one study reported a female preponderance (67.26%) (Kamalam.A Thambiah et al) [6]. In our study among the 117 patients who were clinically diagnosed as dermatophytosis the percentage of males (53%) was only marginally higher than the females (47%) with the male female ratio 1.12:1, which can be attributed to the increased health awareness among the women and their positive attitude towards treatment without inhibition and their increased cosmetic consciousness.

The present study has revealed that the majority (46.2%) of the infection by dermatophytes has occurred during the 3rd and 4th decades of their life, an observation which is in par with those of the earlier studies [7,8]. The probable reason for this age predilection is excessive sweating due to excessive physical activity, as a consequence, in addition the tropical climatic conditions.

Tinea corporis was diagnosed in 82 (70.08%) of the 117 patients with dermatophytosis of the skin (fig-1).
Our results are comparable to those from other places like Kashmir, Jabalpur and Manipal [9,10,11]. Tinea corporis had been reported to be the most common clinical type even in few other countries like Spain and Brazil [12,13].

Tinea cruris (18.8%) followed tinea corporis as the next most common clinical variety in our study (fig-2). This report substantiates that published by other authors [7].

Fig-2: Tinea cruris showing hyperpigmented lesion in the genital area

Mixed infection of tinea cruris and tinea corporis were observed only in negligible numbers and so also was tinea manuum, tinea capitis and tinea faceii.

The high incidence of tinea corporis and tinea cruris as concluded from our study is probably due to its symptomatic nature (pruritis) which leads the patient to seek medical advice [7].

Tinea corporis was prevalent more in females (42.7%) than males (30.8%) and tinea cruris affected males (19.6%) more than females (1.7%). The reason for the higher incidence of tinea corporis is probably due to the type of attire, favours dermatophytic infections to thrive [14].

Tinea cruris is much more common in men than in women. The reason for this preference may be because men wear more occlusive clothing and are more physically active. Moreover, they are at a greater risk of acquiring infection at other sites (e.g. tinea pedis) due to their nature of work. This may act as a reservoir for new cases of tinea cruris [15].

Trichophyton species have been isolated with increasing frequency as compared to Microsporum and Epidermophyton species. This has been noticed by many of the Indian studies on dermatophytosis in India as well as the Western countries. T. rubrum was the most common isolate (56.25%) which is comparable with the reports of other authors [7] and was associated more with tinea corporis (46.6%) than with tinea cruris (16.25%). (Fig-3)

It was found from our study that T. mentagrophytes had an occurrence of 17.8% amongst the dermatophytes (n = 67). Therefore it occupied the second place with regard to the frequency with which it was isolated, which is comparable to other studies from India and abroad during the last 50-60 years, only the percentage of isolates differed (ranging from 11-17%). We also found that 50% of T. mentagrophytes was from tinea corporis and 33.3% from tinea cruris. Only two cases (16.7%) were from tinea faciei.

Out of the total dermatophytes isolated it was found that 5.9% of them were T. schoenleinii. This particular species of trichophyton is rarely reported in literature. T. schoenleinii has more often been an isolate from cases of favus. Hence this is a new observation pertaining to the current study and has to be ascertained by further extensive studies covering a larger population in the same area over an extended period of time.

E. floccosum formed only 2.98% of the total dermatophytes (n = 67) in our study. Only few studies from India and abroad have shown E. floccosum as one of the dermatophyte isolated [5,16].

Pure growth of non-dermatophytic molds were isolated on repeated cultures from 8(6.6%) of the total samples. There were five isolates of Candida spp., (4.1%). As with the reports of Wg Cdr Sanjiv Grover, Lt Col P Roy [17] this is a striking finding in our study. Though commonly considered as contaminants, they have been reported to colonize damaged tissues and cause secondary tissue destruction. Their role in causing cutaneous infections is not yet proven and a primary pathogenic role of non-dermatophytes is controversial at best [17]. But the fact that these non-dermatophytic molds were isolated on repeated cultures, without association of dermatophytes and only from KOH positive samples, bears some significance on etiology. The overall findings suggest that our studies are well comparable to the studies conducted by other researchers and the pattern of dermatophytes prevalent in our subjects under study is similar to that in other parts of the country.

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LABELLING TRANSFORMED MITOTIC CELLS WITH FLUORESCENT ANTIBODY CONJUGATE


ABSTRACT

The functions of most living cells are characterized by well-governed events that encompass the cell cycle. Cell division primarily occurs as mitosis, which is regulated and follows a defined series of mechanisms. A key player in regulating mitosis is a group of proteins collectively called the mitotic factors, which are initially localized in the cytoplasm. These appear gradually and peak at the mitotic stage of cell cycle. Protein profile analysis of interphase and mitotic cells show distinct differences and this was utilized for the premature condensation of interphase DNA through techniques involving somatic cell hybridizations and mitotic extracts. Studies of cell cycle kinetics and the underlying mechanisms have become possible with the continuous cultures of cell lines in vitro. We have raised polyclonal antibodies to the mitotic proteins of Chinese Hamster Ovary cells and the predominately IgG antibodies from the whole serum were purified following induction of a secondary immune response. The purified IgG was conjugated to FITC, a fluorescent dye and the conjugate was used to stain interphase and mitotic cells of immortalized cells in culture and also cultured peripheral blood human lymphocytes. It was observed that the conjugate selectively stained immortalized mitotic cells either in suspensions or in preserved tissue sections. While the cross reactivity with immortalized mitotic cells of varied types was evident, the non-reactivity to untransformed cells gives us a means of further utilizing these antibodies to potential applications such as in cancerous conditions where cell transformation and uncontrolled mitotic events are the underlying factors.

Key words: Chinese hamster ovary cell line, polyclonal antibodies, mitotic factors, immunofluorescence.

INTRODUCTION:

Cell growth and division comprises a series of discrete stages, collectively known as the cell cycle (1). The cell cycle begins when the division of a single parental cell forms two new cells and ends when one of these cells divides again into two cells. This division process, called the “M phase”, involves two overlapping events, the karyokinesis followed by cytokinesis (2). Although the cells of a multicellular organism divide at varying rates, most studies of the cell cycle involve cells growing in culture where the length of the cycle tends to be similar for different cell types. Determining the overall length of the cell cycle - the generation time – for cultured cells has been achieved and reported earlier (3). For mammalian cells in culture, S phase is about 6-8 hours in length. Similarly, estimating the length of M phase by multiplying the generation time by the percentage of the cells that are actually in mitosis at any given time is possible. This percentage is called the Mitotic index. The mitotic index for cultured mammalian cells is often about 3-5 %, which means that M phase lasts less than an hour (usually 30 -45 minutes) (4).

The cell cycle control system is based on two key families of proteins. The first is the family of cyclin dependent protein kinases (Cdk), which induce downstream processes by phosphorylating selected proteins on serines and threonines. The second is a family of specialized activating proteins, called cyclins that bind to Cdk molecules and control their ability to phosphorylate appropriate target proteins. The cyclin assembly, activation and disassembly of cyclin-Cdk complexes are the pivotal events driving the cell cycle (3). Cyclins undergo a cycle of synthesis and degradation in each division of the cycle of the cell. There are two main classes of cyclins: mitotic cyclins, which bind to Cdk molecules during G2 and are required for entry into mitosis, and G1 cyclins, which bind to Cdk molecules during G1 and are required for entry into S phase.

Cell fusion experiments suggested that specific molecules present in the cytoplasm are responsible for moving the cells through the G1 and G2 checkpoints (that is, for triggering the onset of DNA replications phase) and mitosis (M phase) (5). Evidence regarding the mitosis-triggering signal has come from experiments involving frog eggs. During development of the frog oocyte (an egg cell precursor) the cell cycle is arrested in G2 until hormones stimulate meiosis. The oocyte then proceeds through most of the phases of meiosis but is arrested during metaphase of the second of two meiotic divisions. If cytoplasm is removed from a mature egg cell and injected into the cytoplasm of an immature oocyte, the oocyte immediately begins meiosis (6). It was hypothesized that a cytoplasmic molecule, which were named maturation promoting factor (MPF), induces this oocyte “maturatin”.

Subsequent experiments revealed that in addition to inducing meiosis, MPF can also trigger mitosis when injected into fertilized frog eggs (6). Similar MPF molecules were later discovered in the cytoplasmas of a broad range of dividing cell types, including yeast, marine invertebrates, and mammals. Because of the general role played by MPF in triggering passage through the G2 checkpoint and into mitosis, MPF, which originally stood for “maturation promoting factor” is now used to mean “mitosis promoting factor”, a term that describes this molecule’s role.

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As distinct protein profiles are evident in the interphase and mitotic phases of the cell cycle, we utilized the antigenic properties of proteins present in the mitotic stage to raise polyclonal antibodies against the same. Antibodies being very specific in their nature of activity, we attempt to demonstrate the specific reactivity of these antibodies to cells at the mitotic stage of transformed cells. Immunofluorescent techniques provide us with the best possible visualization in cytological applications and therefore we used FITC conjugated antibodies to demonstrate the localization and reactivity to varied cell types in both interphase and the mitotic stages.

MATERIALS AND METHODS:

Polyclonal antibodies to CHO mitotic cytosolic proteins:

A continuous culture of CHO cell line was maintained according to standard protocol. Briefly, the cultures were maintained in DMEM with 5% serum supplementation at 37° C under 5% humidified atmosphere (Fig1).

FITC conjugation to Anti-CHO mitotic protein antibodies

Figure 1: CHO cells in culture. Typically, cells show attachment in a few hours after passaging and attain healthy confluence in about 48 hours in a T-25 flask. Culture conditions are in DMEM supplemented with 5% FBS; 37° C with 5% CO₂.

Mitotic CHO cells were harvested and the cytosolic proteins extracted as previously described. Rabbit polyclonal antibodies were raised against the extracted mitotic cytosolic proteins and identified IgG as the predominant isotype further to the induction of secondary immune response (7). IgG was affinity purified from whole serum, concentrated to obtain a final titre of 1:8 and was used for the FITC conjugation. The chromatogram of IgG affinity purification is given in Fig 2.

FITC conjugation to Anti-CHO mitotic protein antibodies

Figure 2: Chromatogram of IgG purification from rabbit anti CHO mitotic cytosolic protein whole serum. Further to the secondary booster, the predominant antibody Isotype was IgG as determined by Ouchterlony Double Diffusion. IgG was purified to avoid the usage of whole serum to minimize nonspecific interactions.

Fluorescein isothiocyanate (1.0 mg) was reconstituted with 200 µL of the solvent (sterile water or 40-50% glycerol). 250 µL of affinity purified IgG fraction (after concentration) was mixed with 1/10th volume (25 µL) of sodium carbonate bi carbonate buffer [7.5 mL of 0.2 mol/L sodium carbonate solution diluted with 42.5 mL of 0.2 mol/L sodium bicarbonate solution to 200 mL with distilled water]. 15 µL of reconstituted FITC solution was added to the above and incubated at room temperature for 2 hours on a shaker in dark. Following incubation, 1/20th volume (30 µL) of 1M ammonium chloride was added and incubated at room temperature for 1 hour on a shaker in dark. Sephadex G-25 column was washed with 20 mL 1X PBS to obtain base line UV and conductivity levels. 300 µL of reaction mixture (IgG labeled with FITC) was loaded to the bed of the column and eluted through the column using 1X PBS as the wash buffer. The elution profile was recorded and the first fraction (containing the IgG-FITC conjugate) was collected. The IgG-FITC conjugate was stored at 4°C with 1%(w/v) BSA and 0.1% sodium azide.

Target cell preparation and immunostaining:

While interphase cells of the cell lines in culture (CHO, Vero and HEK) remain attached to the culture flasks, those in the mitotic phase round off and detach from the culture flasks (Fig 3). Mitotic cells were collected from cell lines by gentle mechanical agitation from confluent flasks and were individually processed by washing (1000 rpm; 10minutes) thrice in 1X PBS.

A 72-hour human lymphocyte (whole blood) culture was set up according to conventional cytogenetic
procedures. The culture was initiated in RPMI 1640 medium with 10%FCS and incubated at 37°C, 5% CO₂. Phytohaemagglutinin was added to stimulate lymphocyte proliferation in vitro. 100µL of 0.01% Colchicine (mitotic blocker) was added at the end of 70 hours and incubated for 90 minutes; following which the cells were harvested by a brief exposure (20 minutes) to hypotonic solution (0.075M pre warmed Potassium Chloride), centrifuged at 1000 rpm for 10 minutes and the cell pellet was collected.

The cell pellets thus collected of the various cell types employed for this study were incubated in 150 µL blocking buffer for 30 minutes at room temperature and following incubation they were washed twice in 1X PBS and the cell pellets were resuspended in minimum quantity of 1X PBS. To each of the cell pellets 200 µl of IgG-FITC conjugate was added and incubated at 4°C overnight. Following overnight incubation the cells were washed (1000 rpm; 10 minutes) in 1X PBS and layered on glass slides and observed under the fluorescence microscope (Flow chart 1).

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**Flowchart 1:** Protocols employed for obtaining target cells and their processing for immunofluorescent staining.

**Figure 3:** CHO Interphase and Mitotic cells; upon trypsinization, cells detach from culture flask enabling passaging and maintenance of the cell line. In a healthy culture, Interphase cells remain attached to the flask with distinct spindle shaped morphology and the Mitotic cells round-off and detach from the flask surface; thus enabling selective harvest of cells at the Mitotic stage.

**Figure 4:** Immunofluorescent staining of epithelial tissue section by the Anti CHO mitotic cytosolic proteins IgG-FITC conjugate revealing selective staining of cells.

Epithelial tissue that was paraffin embedded and preserved was washed in 1X PBS for 5 minutes three times. The section was dehydrated in alcohol series (95%, 90%, 85% ethanol) for 2 minutes in each three times. The slide
was rinsed in 1X PBS for 5 minutes twice. The section was incubated in blocking serum for 15 minutes. Following incubation 250 µL of IgG-FITC conjugate was added to it and the slide was incubated at 4°C overnight in a humid chamber. Following overnight incubation, the slide was rinsed once with 1X PBS and observed under the fluorescence microscope.

RESULTS:

Concentrated IgG fraction (250µL) was conjugated with FITC and purified by desalting to remove unbound dye from the conjugate. A final volume of 2 ml of the purified IgG-FITC conjugate was collected.

A mixture of interphase and mitotic cells from CHO, Vero, HeLa and human peripheral blood lymphocytes harvested and stained with IgG –FITC conjugate demonstrated selective staining of mitotic cells. The same was observed on paraffin embedded epithelial tissue sections; only a few cells in the tissue were preferentially stained by the IgG-FITC conjugate (Fig 4).

DISCUSSION:

Mitotic cells of human origin (HeLa), upon fusion, can induce Premature Chromosome Condensation in cells from a variety of animal species including mammals, birds, amphibians, fishes, and insects, and mitotic cells from these species can induce PCC in HeLa cells indicates that the factors involved in the induction of this phenomenon are common over a wide range of animal species. In fact plant cells have been shown to induce PCC in mammalian cells. The factors involved are most likely proteins since, if the mitotic inducer cells are prelabeled with radioactively tagged aminoacids, labeled protein can be observed to be transferred from the mitotic component to the interphase chromosomes (8, 9).

The mitotic factors being proteins biochemically, their antigenic properties can be fairly well exploited and suitable antibodies produced that can specifically react with the same. Such antibodies, moreover if polyclonal in nature, by virtue of the affinity to antigenic epitopes can be useful tools for evaluating homogeneity of mitotic cytosolic protein antigens. Also, the cross reactivity of the antibodies to structurally similar antigenic epitopes might prove to be valuable in comparing soluble proteins. Immunofluorescent and immunohistochemical techniques employing suitable antibody conjugates become useful detection tools for specific cell populations or those that express unique antigenic epitopes.

The cross reactivity of polyclonal rabbit Anti CHO mitotic extract with mitotic cells of CHO, Vero, HeLa and normal human lymphocytes was earlier demonstrated to evaluate the homogeneity of the cytosolic protein antigens (10). The results demonstrated the reactivity of the whole serum and IgG fraction only to the mitotic extracts from immortalized cell lines (CHO, Vero and HeLa) and failed to react with the protein extracts from normal human lymphocytes indicating the presence of unique protein or antigenic epitope in mitotic extracts of cell lines with altered cell cycle kinetics which is not present in normal cells (human lymphocytes). It was also inferred that the reactivity of the antiserum is strongly influenced by the presence of this particular protein or the epitope.

CONCLUSION:

The fluorescent antibody conjugate employed in this study was effective in detection and selective labeling of immortalized mitotic cells from a mixed population either in suspension or in preserved tissue section. While demonstrating the presence of shared epitopes in altered cell cycle conditions, also gives us the possibility of exploitation of these antibodies for a variety of applications especially in cancerous situations where, the breakdown of the mitotic machinery is the hall mark that results in unregulated mitotic proliferation of cells.

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INTRODUCTION:

Fluorescence in situ hybridization (FISH) refers to the use of labeled nucleic acid sequence probes for the visualization of specific DNA or RNA sequences on mitotic chromosome preparations or in interphase cells (Plate 1). Exciting advances in FISH are changing the nature of cytogenetics, in both basic research and molecular diagnostics. Cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in tumorigenesis and the three-dimensional organization of the human genome. The high resolution that is achieved by these techniques, particularly by microarray technologies such as array comparative genomic hybridization, is blurring the traditional distinction between cytogenetics and molecular biology. The current review focuses on the advances that FISH has undergone to suit the requirements of present day diagnostics.

CHROMOSOME PAINTING AND ITS VERSATILITY IN MODERN DIAGNOSTICS

Harpreet Kaur a, Teena Koshy a, Venkateswaran N a, Venkatachalam P a and Solomon F.D. Paul a

ABSTRACT

The last two decades have seen the advent of a novel and versatile technique, well known as Chromosome Painting or Fluorescence In Situ Hybridization (FISH). During its maturation, various methodologies and modifications have been introduced to optimize the detection of DNA and RNA. The pervasiveness of this technique is largely because of its wide variety of applications and the relative ease of implementation and performance of in situ studies. Chromosome painting allows precise visualization of unique sequences, chromosomal sub-regions, or entire genome (DNA on metaphase chromosomes and interphase nuclei). It plays an increasingly important role in a variety of research areas, including cytogenetics, prenatal diagnosis, tumor biology, gene amplification and gene mapping. This review describes the applications of some FISH based techniques in human disease diagnosis.

Key words: Chromosome painting, nucleic acid hybridization

INTRODUCTION:

Fluorescence in situ hybridization (FISH) refers to the use of labeled nucleic acid sequence probes for the visualization of specific DNA or RNA sequences on mitotic chromosome preparations or in interphase cells (Plate 1). Exciting advances in FISH are changing the nature of cytogenetics, in both basic research and molecular diagnostics. Cytogenetic analysis now extends beyond the simple description of the chromosomal status of a genome and allows the study of fundamental biological questions, such as the nature of inherited syndromes, the genomic changes that are involved in tumorigenesis and the three-dimensional organization of the human genome. The high resolution that is achieved by these techniques, particularly by microarray technologies such as array comparative genomic hybridization, is blurring the traditional distinction between cytogenetics and molecular biology. The current review focuses on the advances that FISH has undergone to suit the requirements of present day diagnostics.

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Probes and samples used for FISH:

As mentioned earlier, FISH uses fluorescently labelled probes for the visualisation of DNA sequences on metaphase spreads or interphase nuclei. Both numerical and structural aberrations can be determined. Probes can be for the whole chromosome, centromere, or locus specific. FISH probes for the entire genome are also often used. Interphase nuclei can be obtained from a range of clinical specimens including touch preparations, fine needle aspirates, bone marrow smears, and archival material (1).

Variations of FISH:

Interphase FISH

One application of FISH involves the hybridization of probes to interphase cells. This is extremely beneficial when it is not possible to prepare metaphase spreads as in the case of primary tumors. In addition, interphase FISH can be performed on paraffin-embedded, formalin-fixed tissue sections thereby allowing researchers to retrospectively analyze samples and correlate chromosome aberrations with biological and clinical endpoints. Interphase cytogenetics also allows one to precisely define the cell pool carrying chromosomal abnormalities, to identify whether aberrant
cells exist in clonal patches or as isolated events and to observe aberrations on a cell-to-cell basis rather than as a population (2).

**Telomeric FISH (Q-FISH)**

Subtelomeric probes are a relatively new addition to the arsenal of cytogenetic tests. This test is a collection of 41 different FISH probes that are used to identify rearrangements that cannot be seen by conventional cytogenetic methods (3). The subtelomeric probes target the regions right behind the ends of the chromosomes that enables to visualise if they are involved in rearrangements. Each of the probes is a different color so that the specific chromosomal segment can be identified. This is a very expensive and labor-intensive process. However, it can be used when a geneticist suspects a chromosomal abnormality and routine chromosomes are normal (4) or when there is chromosomal material of unknown origin.

**RNA In Situ Hybridization (RISH)**

In many situations, transcription of genes at the cellular level needs to be studied. Several groups have developed methods for FISH of RNA. This is a potentially important application of the FISH technique because it provides direct visual evidence of gene expression from a particular chromosome. House keeping genes, which are abundantly expressed, can be detected reliably. Further optimization and amplification of the signal can even allow detection of genes expressed at baseline levels (5).

**Primed In Situ Labelling (PRINS)**

The efficacy of FISH may be limited in specific applications by low-resolution sensitivity. The primed in situ labelling (PRINS) method is an alternative to in situ hybridization for chromosomal detection based on the use of chromosome-specific oligonucleotide primers. In this procedure, chromosomal identification is done by the in situ annealing of specific oligonucleotide primers, followed by primer extension by Taq DNA polymerase in the presence of labelled nucleotides. It has been demonstrated that the PRINS technique is more specific and considerably faster than classical FISH for chromosomal identification (6).

**Fiber FISH (Dynamic Molecular Combing)**

The term Fiber FISH refers to the common practice of fluorescence in situ hybridization (FISH) conducted on preparations of extended chromatin fibers. FISH on DNA fibers is useful in assessing the length of DNA probes, and to map probes relative to one another, as it can reveal even their degree of overlap. Thus, Fiber FISH has superior mapping resolution compared to interphase FISH. It can resolve DNA loci separated by a few kilobases and study loci as large as two megabases in a single experiment (7; 8).

**Comparative Genomic Hybridization (CGH)**

Comparative Genomic Hybridization serves as an important global screening test for chromosomal aberrations present within a tumor genome. This technique requires only genomic tumor DNA and metaphase preps from a normal donor, thus circumventing the preparation of high-quality tumor metaphase spreads. Tumor DNA extracted from archived, formalin-fixed paraffin-embedded tissue can also be used. This allows identification of chromosomal aberrations and facilitates the correlation of cytogenetic findings with histologic / histochemical information, clinical course, and prognosis. Analysis of small subregions of a histologically defined lesion is also possible (9). Once regions of gain or loss have been identified, these regions can be defined further using FISH or molecular genetic techniques (10). CGH coupled with microarray also known as array CGH has proved to be very informative in many clinical settings (11).

**Combinatorial Binary Ratio Labeling (COBRA) FISH**

Combinatorial fluorescence in situ hybridization (COBRA FISH) of the DNA of the 24 different human chromosomes with 5 fluorophores in conjunction with spectral or filter-based microscopic imaging has greatly advanced molecular cytogenetic analysis of chromosomes. Use of 5 fluorophores allows the identification of up to 31 different chromosomal targets on the basis of color combinations. (12) COBRA–FISH allows color discrimination of all of the p and q arms of each chromosome and permits detection and elucidation of intra and interchromosomal rearrangements (13).

**Spectral karyotyping (SKY) FISH**

Spectral karyotyping (SKY) is a molecular cytogenetic technique that allows differential visualization of all human chromosomes in distinct colors with a single hybridization and image exposure (14). After the chromosomes are classified and aligned in a karyotype table, interpretation and comparison of all aberrations is summarized in the karyogram.

**Multiplex-FISH (M-FISH)**

The M-FISH technology has the ability to identify the twenty-four different human chromosomes in a metaphase spread by the simultaneous hybridization of chromosome-specific DNA probes, each labeled with a different combination of fluorescent dyes. M-FISH differs from SKY only in that it is a filter-based system where separate images are acquired sequentially for each fluorochrome used. The individual fluorochrome files are then combined to generate the final image (15).

These techniques – M-FISH and SKY FISH, have combined the advantages of FISH with traditional chromosome banding techniques and spawned many variations resulting in diverse applications. They permit the detection of interchromosomal structural aberrations, such as translocations and insertions resulting in balanced as well as unbalanced rearrangements. SKY and M-FISH have the potential to identify cryptic translocations and clarify complex aberrations (marker and ring chromosomes), which are typically unidentifiable by conventional banding techniques. In addition, other aberrations such as double minutes can be better resolved, leading to the identification of critical oncogenes (16, 17).
Applications of FISH

FISH is generally used either to complement classic staining methods or as a substitute for chromosome identification at metaphase or interphase. FISH has proved useful in several clinical settings to determine prognosis. Discrete information is obtained for each cell, which is an important advantage of the technique. In particular, FISH demonstrates the qualities listed below for various diagnostic and/or prognostic applications:

Sensitivity: FISH can detect cryptic chromosomal deletions and rearrangements, not detectable by conventional means: submicroscopic microdeletions at the chromosomal level (18) and DNA level (19). FISH also helps in detection of single gene disorders - Duchenne’s muscular dystrophy (20) and microduplications - mental retardation (21).

Specificity: By using a particular probe or probes, chromosomal material of unknown or uncertain origin can be identified - marker chromosomes (22) and chromosomal variants or polymorphisms (23).

Efficiency: FISH allows rapid screening of a large number of metaphases or interphases for a particular chromosome or other target sequence. Mosaicism (24) and chromosomal aneuploidies in prenatal samples (25) can be studied. FISH has also proved invaluable in monitoring residual disease status in patients with cancer (26).

Applicability: FISH allows interphase cells to be screened from a wide variety of tissues not directly accessible with conventional cytogenetics. Some of the studied samples are: human lung carcinoma tissue (27), endometrial tissue (28) and uncultured chorionic villus samples (29). FISH may also be applied to buccal smear samples (30) where venous blood is unavailable for cytogenetic analysis, or to blood smears, where an extremely rapid result is required (31). The FISH technique has also provided a great deal of information about chromosome behaviour at meiosis. FISH allows normal and abnormal chromosomes to be tracked through all stages of meiosis (32) Rapid, direct analysis of large numbers of the chromosomal complements of sperm, has been successfully performed using FISH by Chantot-Bastaraud et al (33).

Thus, FISH related applications allow information to be mined irrespective of whether a single locus needs to be studied or the entire genome needs to be scanned. The following section gives details on the impact of FISH on certain disciplines.

Cytogenetics

Classic cytogenetics has evolved from black and white to technicolor images of chromosomes as a result of advances in fluorescence in situ hybridization (FISH) techniques, and is now called molecular cytogenetics. Improvements in the quality and diversity of probes suitable for FISH, coupled with advances in computerized image analysis, now permit the genome or tissue of interest to be analyzed in detail on a glass slide. It is evident that the growing list of options for cytogenetic analysis has improved the understanding of chromosomal changes in disease initiation, progression, and response to treatment. The contributions of classic and molecular cytogenetics provided scientists and clinicians alike with new avenues for investigation.

Small, submicroscopic, genomic deletions and duplications (1 kb to 10 Mb) constitute up to 15% of all mutations underlying human monogenic diseases. Novel genomic technologies such as microarray-based comparative genomic hybridization (array CGH) allow the mapping of genomic copy number alterations at this submicroscopic level, thereby directly linking disease phenotypes to gene dosage alterations. At present, the entire human genome can be scanned for deletions and duplications at over 30,000 loci simultaneously by array CGH (approximately 100 kb resolution), thus entailing an attractive gene discovery approach for monogenic conditions, in particular those that are associated with reproductive lethality. (34)

Fluorescence In Situ Hybridization (FISH) showed three signals for chromosome X (green) and two signals for chromosome 18 (blue) confirming the karyotype results of a structural anomaly - dicentric X.

Plate – 2

Plate 2 shows the identification of a chromosome anomaly by FISH in a case of primary amenorrhoea referred to the Department of Human Genetics for cytogenetic investigation. Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed three signals for chromosome 21 (orange) and two signals for chromosome 13 (green) indicating Trisomy 21 – Down’s Syndrome.

Plate – 3

Plate 3 shows the confirmation of Down’s Syndrome – Trisomy 21 by FISH, in a paediatric patient referred to the Department of Human Genetics for cytogenetic investigation.
Prenatal Diagnosis

Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus. Numerical chromosome abnormalities are the major cause of inherited diseases with an incidence of 21% in spontaneous abortions. Of these, trisomies for sex chromosomes and chromosomes 13, 16, 18 and 21 account for 50% of chromosomally abnormal abortions. (35).

Prenatal diagnosis needs a rapid, accurate and overall genome analysis. FISH is a powerful tool for detecting some genetic diseases as well as microscopic or submicroscopic chromosome rearrangements in metaphases cells or interphase nuclei involving chromosomes commonly implicated in aneuploidies - 13, 18, 21, X and Y (36). Interphase FISH is very useful in urgent high-risk cases. The use of FISH overcomes the difficulties of conventional banding on metaphase spreads. The ability to generate accurate results in a few hours with FISH as compared to the two weeks typically needed for standard karyotype analysis has been instrumental in relieving the anxiety of many women, or in allowing them, their families and their physicians to make difficult decisions more swiftly. It is usually employed as an adjunctive tool to conventional cytogenetics (37).

Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed two signals for chromosome 21 (orange) and two signals for chromosome 13 (green) indicating no numerical anomalies associated with chromosomes 13 and 21.

Preimplantation Genetics

Preimplantation genetic diagnosis (PGD) identifies genetic abnormalities in preimplantation embryos prior to embryo transfer. As mentioned earlier, the correlation between aneuploidy and declining implantation rates with maternal age demands screening of chromosome aneuploidies in human embryos by FISH using 13, 18, 21, X and Y probes should significantly reduce the risk of older patients undergoing in vitro fertilization (IVF) delivering trisomic offspring (35). PGD is being explored through polar body biopsy, biopsy of the single cell from the eight-cell embryo, and trophoderm biopsy of the blastocyst (38). Interphase FISH using multi color, subtelomeric and centromeric probes is used to test single cells for structural or numerical chromosome abnormalities. It helps in identifying embryos free of specific genetic abnormalities (39). Results with PGD indicate a significant decrease in spontaneous abortions, from 81% before PGD to 13% after PGD (40).

Fluorescence In Situ Hybridization (FISH) for chromosomes 13 and 21 showed two signals for chromosome 21 (orange) and one signal for chromosome 13 (green) indicating that the blastomere biopsied from the embryo was abnormal.

Plate 5 shows FISH done on a blastomere obtained by embryo biopsy prior to IVF performed for research purpose.

Cancer Genetics

Chromosomal aberrations are found in cancer and tumor cell lines. Some of them are already characterized and correlated with specific syndromes and some of them have yet to be associated with a clinical outcome. Cytogenetic analysis is now a routine part of the diagnosis and management of a significant number of malignancies. Whilst conventional cytogenetics remains the most comprehensive method for assessing chromosome abnormalities, the technical difficulties associated with conventional cytogenetics in most cancers has resulted in increased use of FISH to identify specific abnormalities that are useful in either the diagnosis or management of these disorders. Aberrations such as aneuploidies, translocations, deletions, and gene amplifications are investigated in samples. This is accomplished using probes for centromeres, whole chromosome probes, and/or probes for specific aberrant sequences (41).

In chronic lymphocytic leukemia (CLL), genetic analyses by FISH and DNA sequencing have greatly improved the understanding of pathogenic events and prognostic markers (42). The combination of metaphase and interphase analyses and the investigation of specific structural aberrations by FISH have definitely made tumor diagnostics much rapid and accurate.

Plate 6 shows the identification of the bcr-abl gene fusion as identified by FISH and typical of Chronic Myeloid Leukemia (CML), in a patient referred to the Department of Human Genetics for cytogenetic investigation.
Fluorescence In Situ Hybridization (FISH) of interphase cells showed a fusion signal (yellow) confirming the presence of Philadelphia chromosome in a patient referred for chronic myeloid leukemia (CML) with green / red signals representing normal chromosomes – 22 / 9.

Plate - 6

Radiation biodosimetry

In almost every instance involving accidental, unexpected or suspected radiation exposure, biodosimetry comes to the picture. Biodosimetry involves the identification and scoring of certain biomarkers specific to and induced by radiation. To be useful, a biomarker for exposure and risk assessment should employ an end point that is highly quantitative, stable over time, and relevant to human risk (43). Biodosimetry is usually performed by enumerating the number of unstable chromosome aberrations – Dicentric Chromosomes and Centric Rings in peripheral blood lymphocytes of exposed individuals (44) Radiation induced unstable chromosomal exchanges are eliminated from the body within 1-3 years depending on the exposure condition. As a result, there is a considerable uncertainty in this dosimetry for past exposures (45, 46).

This is circumvented by scoring stable chromosomal exchanges such as translocations. Studies of the Japanese A-bomb survivors and patients receiving radiotherapy have shown translocations to persist in peripheral blood lymphocytes many years after exposure and repeated cytogenetic analyses have also indicated that the frequencies of cells with translocations remain unchanged (47). Thus, they are potentially a better indicator of cumulative dose.

The advent of Chromosome Painting (FISH) has revolutionized biodosimetry in simplifying the analysis of translocations. FISH assay not only makes the identification of translocations very easy, but also increases the sensitivity by its ability to score events, which conventional banding may fail to detect. The usefulness of this technique is demonstrated by its ability to resolve Complex chromosomal aberrations associated with high radiation doses, which are quite cumbersome and time-consuming to deduce by routine conventional cytogenetic techniques such as Banding by Trypsin and Giemsa (GTG Banding) (48).

Dual color FISH with whole chromosome and pan-centromere probes facilitates rapid detection of translocations. This approach allows analysis of translocations for assessment of genetic damage at long times after exposure or as a result of chronic exposure during a long period of time. Multi-color FISH with locus specific probes allows assessment of the frequency of cells carrying specific aberrations known to be associated with tumorigenesis, analysis of the series of genetic changes that occur during tumor evolution and correlation between genotype and phenotype (49).

The metaphase shown below shows complex chromosomal aberrations involving chromosome 1 (green) and chromosome 3 (orange), an indication of high doses of radiation exposure.

Plate – 7

Plate 7 shows some of the complex aberrations that have been identified by FISH (Whole Chromosome Painting) in peripheral blood lymphocytes exposed to gamma radiation.

FISH - past, present and future

Although the basic principles of FISH have remained unchanged, high sensitivity detection, simultaneous assay of multiple species, and automated data collection and analysis have advanced the field significantly. Efficiency and sensitivity have been improved by combining methodologies. In the future, this technique is likely to have significant further impact on live-cell imaging and on medical diagnostics.

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LAPAROSCOPIC BILE DUCT INJURIES-CONTROVERSIES AND CONSENSUS

S. Sankar a, M. Subramaniam a

ABSTRACT
Among the various advancements witnessed in the field of surgery towards the end of the last millennium, none has been so dramatic as Laparoscopy Cholecystectomy. Laparoscopic cholecystectomy is the prototype amongst the minimally invasive surgeries; however this unprecedented rapid advancement was plagued by the increased incidence of bile duct injuries. Frequency of bile duct injuries is almost double in Laparoscopy. The mechanism of injury is different. Laparoscopic anatomy is different. Though the recognition of injury is relatively early, the magnitude of injury can be devastating. The implications of post-laparoscopic cholecystectomy biliary injuries are serious in terms of the physical and fiscal inconvenience for the patients and the legal problems for the surgeon. For obvious reasons, accrual of data on this entity is extremely difficult. Despite the completion of learning curve, bile duct injuries continue to occur. This article reviews the controversies and consensus on the subject of post-laparoscopic cholecystectomy biliary injuries.

Key words: Laparoscopic, Cholecystectomy, Bile duct diseases

INTRODUCTION
Born in secrecy and viewed with skepticism, Laparoscopic cholecystectomy (LC) stormed into the surgical arena, and in an unimaginable span of time has become the gold standard, for the treatment of Gall stone disease. The benefits of LC were so obvious that, till now there are no Randomized Controlled Trial comparing LC and Open Cholecystectomy (OC). In a lighter vein, the highest level of evidence (class A) is lacking, to support the advantages of LC. The incidence of bile duct injury in LC is 0.4% - 1.3% (1,2,3,4,5,6) compared to 0.2%-0.3% (6,7) for OC. Despite the inherent difficulty in collecting data on laparoscopic bile duct injuries, these injuries occur twice more frequently in Laparoscopic cholecystectomy. This statistical data may look innocuous, but nevertheless there are major differences between the bile duct injuries associated with LC and OC.

Are Post laparoscopic Bile duct injuries anyway different
1. The so-called “classical Laparoscopic injury” (8) is characterized by significant segmental loss of bile duct, and often the proximal level of injury may reach the biliary confluence. This is because, the surgeon divides the CBD mistaking it for the Cystic duct, and further in the process of removing the GB, he divides the CHD proximally and finally ending up in a significant loss of length. Basically the cranial traction exerted on the GB fundus to lift the Liver makes the Cystic duct to align with the CBD and hence the mistake. This complex kind of injury is unique for Laparoscopy and unlikely to happen in OC.

2. Many a time bile duct injuries are associated with Right Hepatic Artery injury (RHAI) because of its close proximity to the CHD, which is invariably damaged in the classical LC bile duct injury. Normally it is thought that RHAI leads to increased incidence of complications like liver abscess, necrosis, bile leaks, unsuccessful repairs etc. In a retrospective study of 261 LC bile duct injuries, Stewart and Way analyzed the mechanism and consequences of RHAI (9). The incidence of RHAI was an amazing 32%, but ironically this was not associated with increased mortality, and did not alter the success of repair in the hands of experienced biliary surgeons (9). Belghiti’s group has also reported the incidence of RHAI to be 36% (40). Most of the times the RHAI goes unrecognized. If the injury is recognized on the table, whether one should try to reconstruct is a matter of debate. But in the presence of pre-existing Liver dysfunction, Right Hepatic artery injury may have serious consequences.

3. Many LC biliary injuries are due to surgeons’ misperception of the anatomy rather than an inadequacy of skill or judgment (10). Without realizing this optical illusion, the surgeon subconsciously feels that, what he was doing was correct. In cognitive psychology this is technically called the “Heuristic process”. This means, biliary injuries will continue to occur, despite the completion of the often-repeated cliché, “learning curve”. But this should not be misinterpreted as a justification for the errors, that carries tremendous physical, emotional, legal and financial implications. There have been suggestions to inculcate “methods of error reduction” in the laparoscopic training of a surgeon, something similar to that followed in sectors like aviation, nuclear technology, as errors in these fields can have serious consequences.

Laparoscopic Anatomy – is it different?
• The commonly described Calot’s triangle is actually different from what it was originally described. In the original Calot’s triangle, Cystic artery forms the boundary and not the content. The Hepato-Cystic triangle (also called as Mooseman’s area or triangle of Buddie), is the one where Cystic artery forms the content and Liver forms a boundary.
• The cranial traction applied on the fundus of GB to retract the Liver, actually narrows the Calot’s Triangle and hence an outward traction on the Hartman’s pouch is needed and causes the Cystic duct to align with the CBD
• The “reverse” or “posterior” dissection of the Calot’s triangle gives a different view of the anatomy
• “Rouvier Sulcus” can be demonstrated in a majority of patients, this sulcus separates the right lobe and caudate process and corresponds to the porta hepatis harboring right hepatic pedicle. Hence the dissection should start anterior to the sulcus. Thus it provides an extra biliary reference point, which does not get altered due to the pathology of Gall bladder (41).
• In LC, the junction of cystic duct and Hartman’s pouch is demonstrated. Whereas in OC the junction of Cystic duct and CBD is demonstrated.

Factors predisposing for biliary injuries include

A. Difficult anatomy
   i. Anomalies of cystic duct (low insertion, high insertion, short duct, parallel duct)
   ii. Right sectoral duct anomalies (in 20% of the cases one of the sectoral duct join the CHD)
   iii. Intrahepatic GB
   iv. Arterial anomalies (accessory Right hepatic artery, arterial humps)

B. Difficult pathology
   i. Acute cholecystitis
   ii. Scleroatrophic GB (contracted GB)
   iii. Mirrizi syndrome
   iv. Chronic cholecystitis
   v. Frozen calots’s triangle

C. Difficult in terms of technique
   i. Casual attitude towards a “simple Gall bladder”
   ii. Improper placement of trocars
   iii. Bulky and hanging falciform ligament
   iv. Bulky Quadrate lobe
   v. Undue haste exercised in clamping or clipping in the event of bleeding
   vi. Injudicious use of electro cautery in Calot’s triangle
   vii. Too much skeletonization of the Bile duct leading to ischemic strictures
   viii. Surgeons’ experience- the “learning curve effect”, which implies that the bile duct injuries and experience of the surgeon are inversely proportional. There is considerable evidence to support this view (2, 5, 11, 12, 13). However there are also reports of major injuries, inflicted by surgeons of considerable experience (14)

Classification

Bismuth classification (15) is shown in figure 1. This is used for strictures but the main drawback is, it does not include all possible biliary injuries during Laparoscopic Cholecystectomy.

![Fig. 1](image)

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Stricture &gt; 2cm from the confluence. More than 2 cm of CHD is available.</td>
</tr>
<tr>
<td>Type II</td>
<td>Stricture &lt; 2 cm from the confluence. Less than 2 cm of CHD is present.</td>
</tr>
<tr>
<td>Type III</td>
<td>No CHD, but the confluence is patent and the right and left systems are communicating. Sikora et al have proposed a sub classification of type III strictures. III A, where both the floor and roof of the confluence are healthy. III B, where the roof of the confluence is healthy, but the floor is scarred (24). Their contention is, this sub classification has therapeutic and prognostic significance. Type III B strictures are difficult to treat and resembles that of type IV strictures.</td>
</tr>
<tr>
<td>Type IV</td>
<td>Confluence is stricturous. The two systems are isolated.</td>
</tr>
<tr>
<td>Type V</td>
<td>Stricture at the junction of the aberrant sectoral duct.</td>
</tr>
</tbody>
</table>
**Strasburg classification** (6) is represented in figure 2. This classification includes all possible types of injuries, both leaks and strictures.

**Stewart & Way classification** (16) (figure 3)

This is more practical based on the different operative scenario, where injuries occur.

**Clinical presentation**

Depending on the temporal relation with the injury, three situations are possible, namely injuries recognized on the table, immediate postoperative period and delayed presentations.

1. **At surgery:**

The surgeon recognizes this by finding welling of bile in the operative field (excluding the bile spillage from the GB). Ideally at this point surgeon should convert and may request for expert help. In some cases bile duct injuries may become evident after an intra-operative cholangiography. Non-visualization of proximal Ductal system indicates major injury. Uncontrollable bleeding also mandates conversion instead of blindly clipping and cauterying. If any one of the major ducts is clipped and if it is recognized immediately, the clip should be removed and usually nothing more is needed.

The role of intra-operative cholangiogram, whether routine or selective has been a matter of debate. Intra-operative cholangiography may only help to identify injuries and correct it, but it is unlikely to prevent occurrence of injuries (36). There are reports of low yield of intra-operative cholangiography and its routine use is discouraged (37, 38, 39). Selective use of intra-operative cholangiography is preferred. When the anatomy is distorted, intra-operative cholangiography is indicated and it is recommended to convert to OC, since Laparotomy is less dangerous than undetected biliary injury.

2. **Injuries detected in the immediate post operative period**

Bile duct injuries sustained in Laparoscopic cholecystectomy are likely to be recognized relatively earlier than its counterpart, namely open cholecystectomy (8, 17), which probably reflects the differences in the pattern of injuries and the surgeon’s awareness of the “potential of laparoscopy” in inflicting injuries (6)

On the first post-operative day, the patient should be “one hundred percent fine” in an otherwise smooth LC, and anything short of this recovery should alert the surgeon. The clinical features depend on various factors. Whether it is a bile leak or an occluded duct, occlusion may be partial or complete. Leaks may be “minor or major” and “contained or uncontained”. Strasburg A, C, D and E are likely to present with leaks. Clinical features also depend on whether a drain is placed or not and whether the drain is draining or not. Most of the times drains help in detecting bile leaks. But, “a drain is useful only if it drains” and should not be an alternative to clinical monitoring. Drains can get blocked and can provide a false sense of security. Significant bile in the drain necessitates the surgeon to order for ultrasonogram. The use of drains in laparoscopic cholecystectomy should be selective. Sometimes patients are discharged early and get readmitted with features of sepsis.

Tachycardia, fever and general weakness may be the subtle signs of bile leak. In grossly obese individuals often
the signs of peritonitis are absent and one has to rely on indirect evidences (in these patients, a sense of "impending doom" may be an indicator of a serious problem). Bile leaks may range from being trivial to massive. Large subhepatic collections may compress the vena cava; reduce the preload to the heart and a fall in the systolic pressure (Waltman Walter syndrome). Undetected or neglected massive bile leaks may be fatal especially when they are uncontaminated and if the patient is allowed to slip into sepsis and its sequelae.

When the extra hepatic bile duct is occluded, patient present with varying degree of icterus and rising enzymes and vague abdominal pain. Usually it takes more than two weeks for the IHBR dilatation to occur. Isolated sectoral duct occlusion may not manifest with jaundice, but only enzymes are elevated.

Late presentation

Occurs typically in biliary stricture patients. Recurrent fever with mild icterus characterizes this group of patients. Recurrent cholangitis leads to general ill health and malaise. Often the bilirubin is elevated to moderate levels. Once again clinical features depend on the magnitude of occlusion and the location of stricture. Strictures of the right sectoral ducts may lead to recurrent infections, abscesses or atrophy of liver sector. Long standing biliary obstructions may predispose to secondary biliary cirrhosis and portal hypertension. Presence of portal hypertension may also be due to associated portal venous injury. Total occlusion of the duct may cause deep jaundice. Sometimes an elevated alkaline phosphatase may be the only abnormality. Even asymptomatic or minimally symptomatic patients may need therapeutic intervention, as secondary biliary cirrhosis is likely to occur.

Pathological consequences of biliary stricture

1. Fibrosis:

High local concentration of bile salt at the level of canalicular membrane sets a cascade of molecular and cellular changes, leading to fibrosis and scarring of bile ducts and ductules, which may sustain the cholestasis. However the fibrosis is not equivalent to cirrhosis as the liver architecture is maintained. Early drainage of the liver may produce reversal of changes and in this context a concept of "latent portal hypertension" has been proposed by some (18). In one study, duration of biliary obstruction and the trend of ALT levels were independent predictors of Hepatic fibrosis (32)

2. Atrophy

The liver mass is regulated by mechanisms that are poorly defined, but biliary drainage and portal venous flow has a bearing on it. Sectoral or segmental ductal obstruction may lead to atrophy of the corresponding segments. This is associated with contralateral lobe hypertrophy. Atrophy-hypertrophy complex is clinically significant as it has implications in diagnosis and surgical treatment. Atrophy-

3. Portal hypertension

The presence of portal hypertension in biliary stricture may be due to one of the following three reasons.

1. Secondary biliary cirrhosis
2. Portal vein injury
3. Pre-existing liver disease.

It takes nearly 2 years for the secondary biliary cirrhosis to contribute to portal hypertension. In alcoholics this period may be shortened. The concept of “Reversible” or “Latent portal hypertension” is interesting (18). Whatever may be the etiology of portal hypertension, it carries a poor prognosis, and for the surgeon, enough of technical difficulties. In the presence of portal hypertension some prefer a two-stage approach i.e.: a porto systemic shunt followed by hepaticojejunostomy or a single stage with venovenous bypass (19). Some prefer single stage procedure with acceptable morbidity, i.e: Hepaticojejunostomy without a preliminary shunt surgery (20)

Management

Management depends on the scenario of injury. Three situations are possible.

1. On the table
2. Immediate postop period
3. Late (Biliary stricture)

Intra operatively detected injuries

Usually bile is found welling in the operative field or the anatomy is distorted and uncomfortable. An operative cholangiogram is indicated at this point to find out the cause of leak. Non-filling of proximal ducts on cholangiogram may also indicate CBD injury. Mostly this situation warrants conversion and the need for help. It is best to accept that any potential morbidity from a laparotomy is minor compared to a bile duct injury.

1. CBD transected, but no segmental loss.

If the CBD has been transected and there is no segmental loss, the best option is to do a Choledochojjunostomy. But fashioning a bili enteric anastomosis in an undilated duct may be technically demanding. The other option is to do a primary repair with a T tube brought out separately. Though primary repair of CBD is associated with high rate of stricture formation (50% of cases), these strictures are the ones, which can best be managed with endoscopic methods. The high rate of stricture after primary repair is due to the axial blood supply, 60% coming from distal side.

2. CBD transected with segmental loss.

If CBD transected with segmental loss then it warrants a hepaticojejunostomy. If the surgeon is not confident or
no expertise is available, a good drainage is accomplished and patient is referred to a higher center where services of a Hepatobiliary surgeon is available.

3. Lateral injuries.

Lateral injuries of the CBD once again need biliary enteric anastomosis, as suturing over a “T-tube” may compromise the lumen and end up in a sticture. Inadvertent application of clips on the bile duct if recognized, immediate removal will suffice.

Immediate postoperative period

Reports of successful early repair of biliary injuries continue to appear (21). But the key issue is “a stable patient and absence of sepsis”. The first priority in biliary injuries detected in the immediate postoperative period is to stabilize the patient hemodynamically. An USG abdomen is done to know whether the leak is contained or not. A CT is better than USG, because ultrasound is less sensitive in the evaluation of post operative fluid collections. If the leak is not contained, it has to be contained by proper drainage, either by a Radiological procedure or may be a Relaparoscopy. Once a leak is contained and the patient is hemodynamically stable, the next question to be answered is whether the biliary continuity is preserved or not. Non-invasive methods of knowing this is by MRCP or Isotope scan. MRCP and contrast (Mangafodipir) enhanced MRCP are increasingly being used in the evaluation of biliary injuries and are very reliable (22, 23). Isotope scan suffers from the drawback of not being an anatomical investigation, but when the duodenum shows activity, it is indirectly inferred that biliary continuity is maintained. Having established the biliary continuity, the best option is to do an ERCP. If the leak is due to cystic duct blow out a stent or a nasobiliary drain with or without a sphincterotomy will settle the issue. With this modality the leak settles very quickly. Since majority of the cystic duct leaks tend to heal, some surgeons tend to treat conservatively without an ERCP, as long as the patient is stable without signs of sepsis. But the inherent problem with this approach is that the surgeon presumes that bile leak is from the cystic duct and not from major Ductal injury. Conservative treatment without an ERCP may also prolong the hospital stay.

When the biliary continuity is lost (classical LC bile duct injury), and the surgeon is experienced, early hepaticejejunostomy is accepted. “How early is early” may depend on the surgeon’s philosophy and experience, apart from the logistics. In delaying, Hepaticejejunostomy may be postponed to a later date after containing the leak, as the first repair should be the best repair, and with every further attempt, surgery becomes less successful and more difficult.

Late presentation

This is essentially the typical biliary stricture. A good cholangiogram is an essential mandatory preop investigation. MRCP is an ideal modality and has almost replaced PTC (31) as it gives all the information that the surgeon wants, besides being non invasive. Of course the gold standard is PTC, which provides a good quality when compared to MRCP. If the patient is having cholangitis that is not responding, one may have to embark on a PTBD. Routine PTBD is not needed. When one decides to do a PTC, it is timed just before the surgery for fear of cholangitis.

The basic principle in the treatment of biliary stricture is to relieve the symptoms and prevent the development of secondary biliary cirrhosis. Surgery has been the standard treatment and the gold standard against which other treatments are compared. Any treatment modality should be durable and long lasting with less morbidity and no mortality. Surgery always carries morbidity and the remote possibility of mortality. The results of the surgery are variable. In the hands of experienced biliary surgeons results have been excellent. Long-term success rate of 80-90% has been reported from high volume centers (33, 34, 35). Biliary sepsis and portal hypertension are two factors hindering surgical success. The recurrence of stricture is reported in 10% of the patients. Treatment of choice for recurrent strictures after surgery is balloon dilatation. Some cases of recurrent stricture may warrant redo Hepaticojejunostomy.

A good Hepaticojejunostomy is the treatment of choice for biliary stricture. The classical Hepp Couinaud approach wherein a wide opening is made in the dilated CHD and extending onto the left duct and a side-to-side anastomosis is fashioned to a Roux Loop of Jejunum. In a nutshell the following are the principles of surgery.

- Careful meticulous dissection
- Duodenum, Colon and Stomach will be adherent to the GB fossa, which are taken down.
- Identification of dilated bile duct
- Lowering of hilar plate
- Bile duct opened and the incision is extended on to the left duct for a wide anastomosis
- Roux loop of jejunum is fashioned
- Single layer interrupted, mucosa to mucosa anastomosis with Vicryl or PDS
- Access loop in selected cases
- Appropriate Biliary stent and drains
- Long follow up

Surgery vs. Endoscopy

Traditionally management of biliary stricture has been a Surgeon’s domain. An Endoscopist has a definite role to play in the rescue of the surgeon in the immediate postoperative period. With the advancements of endoscopic technique and accessories, endoscopic management is emerging as an alternative modality to surgery in the management of established biliary strictures. There are few studies directly comparing the results of surgery and endoscopic treatment. The available data is from retrospective
CONCLUSIONS:

Biliary injury is the Achilles’ heel of Laparoscopic Cholecystectomy. William Halsted, the great Surgeon sustained biliary injury during Cholecystectomy and succumbed to it after resurgery. Anthony Eden one of the youngest prime minister of Great Britain had his political career curtailed, as he became a biliary cripple following Cholecystectomy and subsequent repeated operations. However these were in the prelaparoscopic era. Despite all the precautions and learning curves, bile duct injuries will continue to occur, and be the nemesis for the Laparoscopic Surgeon. Errors happen at so many levels, but it is up to the Surgeon who is the last level in the Swiss cheese model, not to allow it to pass through him. Management of such complex problems should integrate the services of Hepato-biliary Surgeon, Interventional Endoscopist, Interventional radiologist and the Intensivist, to optimize the results.

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ABSTRACT

The post-genome era has heralded the beginning of incorporating genome-wide approaches in processes beginning from drug discovery to clinical prescription with the ultimate aim of viewing modern medicine as personalized molecular medicine. A pioneering step in this direction is the development of Pharmacogenomics – the study of the interplay of genotype and drug efficacy that seeks to identify the variant genes affecting the response to drugs in individual patients. These variations can be used to predict whether a patient will have a good response to a drug, a bad response to a drug, or no response at all. Pharmacogenomic studies encompass the sum of all genes, i.e., the genome with respect to its impact on pharmacokinetics and pharmacodynamics. Initially termed as Pharmacogenetics, it is now synonymous with Pharmacogenomics.

A classical example cited in literature is the existence of a polymorphism in the metabolism of the antituberculous drug – Isoniazid (INH) that affects the catalytic activity of the acetylating enzyme, thus leading to slow acetylation of INH. Certain populations of the world are associated with this polymorphism making them slow acetylators. Similar polymorphisms have also now been studied in many other genes of pharmacogenomic significance.

The use of non-automated and automated methods for profiling genotypes has tremendously helped to reveal the association of single nucleotide polymorphisms, with their corresponding phenotype.

Apart from the pharmaceutical impact, another significant role of pharmacogenomics has been found in preventive medicine. It also suggests restructuring the current design of clinical trials by considering the relevant genetic variations that may considerably affect the trial outcome. Critical clinical decisions will also be benefitted by the understanding of the genotype-phenotype associations. Thus it has a promising role to play in diverse areas of clinical healthcare including diagnostics, therapeutics, disease prevention and target-based drug discovery.

Pharmacogenomics - the rational genomics-based therapeutic approach requires several important issues to be addressed such as the quantum of information that can be and needs to be divulged to the patient, clinician acceptance and critical ethical and legal questions.

This review gives a brief insight into this developing field that has a vast potential in clinical research and healthcare. A pharmacogenomic approach is a vision for the future where personalized medicine will be focused on the patient who has a disease and not just looking at the disease per se.

Key words: Single nucleotide polymorphism, pharmacogenomics, pharmacokinetics

INTRODUCTION

The completion of the Human Genome Project (1,2) in 2003, signified a turning point in the field of biosciences and development of high-throughput technologies. It paved the way for numerous innovations and many scientific advancements of the Post Genome Era in biological sciences. During the course of the project, the presence of sequence variations led to the inclusion in the last part of their last five-year plan to identify and map these variations. With their occurrence roughly every 1000 base pairs, single nucleotide polymorphisms (SNPs) are among the most common genetic variations (3).

The National Centre for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH) in U.S. has been actively involved in the genome sequencing efforts and is now a popular resource for molecular biology information (1). It played a key role in launching an initiative towards identifying and mapping the SNPs which culminated in the NCBI establishing the Single Nucleotide Polymorphism database (4) (http://www.ncbi.nlm.nih.gov/SNP) in collaboration with the National Human Genome Research Institute (NHGRI). This has now become an international effort and many research institutions in various countries are involved in this task. Currently, many SNP databases exist that can be accessed on the internet. E.g. A similar Japanese population based SNP database has also been created (http://snp.ims.u-tokyo.ac.jp/) (5).

Role of Polymorphisms in Pharmacogenomics

Genetic polymorphisms contribute greatly to the inter-individual variations observed in complex human phenotypes, such as disease susceptibility and our responses to drugs or environmental chemicals (6). Hence, understanding these variations will assist in unraveling the relationship of individual/population genotype(s) with the response to therapeutics. The study of association between genetics and drug response is called pharmacogenomics. The potential role of genomics and pharmacogenomics in clinical research and medical healthcare is in modifying the existing disease management strategies that will
additionally incorporate the knowledge of genetic polymorphisms in drug disposition and effects. This will make a significant contribution to the pursuit of developing better, safer drugs as a part of personalized drug therapies in an era of personalized medicine where the right drug will be given to the right patient (7,8). Pharmacogenomics is thus, a science that examines the inherited variations in genes that dictate drug response and explores the ways these variations can help predict the outcome of administering therapeutic medications as good, bad or no response to the drug(s).

This is closely related to Pharmacogenetics which has been mainly concerned with the study of the genetics of drug metabolism and genetic factors affecting drug targets such as receptors. Presently, these terms are used synonymously.

**Trends: Past and Present**

Many phenotypic differences in drug absorption, distribution, metabolism and elimination (ADME) are partly a reflection of polymorphisms in genes encoding drug metabolizing enzymes, drug transporters, and/or drug targets (e.g., receptors, enzymes) (9). Initial pharmacogenetic studies focused on monogenic traits where these variations affected drug metabolism. Current studies look at high-throughput techniques that reveal the significance of many candidate proteins, influencing pharmacokinetics and pharmacodynamics of a drug – the former involving mechanisms that influence the concentration of a drug reaching its target(s) and the latter, the drug target itself (10).

For many drugs, the difference in patterns of patient response has been attributed to variations in pharmacokinetic rather than pharmacodynamic. But recently, it seems that pharmacodynamic variability is usually more pronounced than in pharmacokinetics. Some examples of the role of pharmacogenomics on pharmacokinetics are cytochrome P-450 isoenzymes, dihydropyrimidine dehydrogenase, and thiopurine methyltransferase; some examples of pharmacogenomics revealing variations in pharmacodynamics involve cholesteryl ester transfer protein, angiotensin-converting enzyme, and serotonin transporter (11). Genes of pharmacogenomic significance can be classified as 1) polymorphisms affecting metabolism of drugs, 2) affecting drug transport and disposition and 3) polymorphisms affecting drug targets. The first category includes cytochrome P450s which is involved metabolizing drugs such as Nicotine, Warfarin, Omeprazole, Cyclosporine etc., N-acetyltransferase -2 ( associated drug – INH) and Thiopurine S-methyltransferase ( drug metabolized – 6 Mercaptopurin, 6-Thioguanine and Azathioprine). Polymorphisms affecting drug transporters such as MDR-1 ( Multidrug Resistance Protein ) affect digoxin transport. Individuals homozygous for a particular variant with a high MDR-1 gene expression had lower levels of plasma digoxin levels following oral administration of the drug. Polymorphisms affecting drug targets such as dopamineD3 receptor affects response to some antipsychotic drugs, those in angiotensin converting enzyme influence the response to ACE inhibitors and polymorphisms in cholesteryl ester transfer protein modify response to cholesterol lowering statin – Pravastatin. (12)

The majority of drug responses involve many proteins and their corresponding genes could have several polymorphisms making it unlikely that a single polymorphism in a single gene could result in a high degree of drug response variability in a consistent fashion. Thus, genomic approaches seem more appropriate to study the contribution of polymorphisms of several genes involved in a particular drug response. Such approaches to pharmacogenomics have shown promise in relating drug target polymorphisms to response or toxicity, and incorporating this strategy in drug discovery and development is a new approach that is being used by many pharmaceutical companies (13). Two such FDA approved pharmacogenomics-based drugs available currently in the market are Herceptin® (Genentech) and Velcade® (Milleneum). Herceptin® (Trastuzumab) is indicated for treatment of HER2 protein overexpressing, metastatic breast cancer. Velcade® (Bortezomib), a proteasome inhibitor is indicated for treatment of Multiple Myeloma.

A combination of pharmacogenomics and personalized medicine will eventually change the method of current drug prescription to one where drug selection and dosage process for any given patient will be advanced by genomic knowledge and information gained by these strategies (14).

Metabolic phenotyping studies can be done by administering a probe drug or substrate and measuring the metabolites with the clinical outcomes if any. The disadvantage of this approach is that it tends to be labor intensive and requires repeated sample collection from the individual or patient, being tested. On the other hand, genotyping allows determination of individual DNA sequence variants associated with particular traits. DNA sample is commonly obtained from whole blood cells and buccal cells. Genomic DNA is isolated from other cellular material. After a specific region of interest is identified and amplified, using polymerase chain reaction (PCR). Subsequently, gel electrophoresis is often performed to verify that PCR was successful and that the amplified target sequence is the correct size. Some of the commonly used genotyping methods include gel electrophoresis-based techniques, such as polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism analysis (RFLP) does not rely on automated technology and is practical for laboratories genotyping a limited number of samples. Other gel electrophoresis-based techniques include multiplex PCR, and allele-specific amplification. Automated genotyping methods include Pyrosequencing, in which the principal allele discrimination method is a primer extension reaction coupled with a luciferase-based enzyme reaction. TaqMan uses fluorescence-labeled probes,
in addition to PCR primers, in the reaction mixture, enabling PCR amplification and allele discrimination in the same step. Mass spectrometry differentiates DNA molecules based on their mass. Denaturing high-performance liquid chromatography (DHPLC) using a reverse-phase ion-pair column, helps discriminate between variant and nonvariant. Some other fluorescent dye-based high-throughput genotyping procedures include including oligonucleotide ligation assay and direct heterozygote sequencing. The latest advances in genotyping include Microarrays. (15,16)

The development of a new related field that serves to complement pharmacogenomics is that of pharmacoproteomics. The combined effort will endeavour to enhance the development of personalized medicines. The high-throughput techniques in pharmacoproteomics have a potential contribution in the area of molecular diagnostics that forms an essential component of personalized medicine. As the functional impact of genetic variations is seen in the functioning of the encoded protein, pharmacoproteomics will explain the functional phenotypic consequence of the patient-to-patient variation, to a greater extent than that offered by the genotype alone. Another role foreseen is in characterization of multifactorial diseases where it can help match a particular target-based therapy to a particular marker in a subgroup of patients (17).

An innovative and exciting prospect, this specialization of genomics has an immense potential in successful development of better, effective therapeutics as it will play a role in overcoming important setbacks in the traditional process of drug discovery. Genomics and its derivative fields along with their high throughput technologies promise to influence different stages of drug discovery process such as target selection, lead identification, lead optimization, thus yielding safer and effective drug candidates (18).

**Role of Pharmacogenomics**

- **In Disease Management & Therapeutics – Some recent examples:**

Many biological systems and their diseases have been the focus of pharmacogenomic studies with a potential impact in areas ranging from screening, diagnosis, monitoring therapy response, choice of drugs etc. Some highlighting examples in this regard include diseases such as Acute Lymphoblastic Lymphoma, Asthma, Gastric Cancer, Multiple Sclerosis and Osteoporosis.

**Acute Lymphoblastic Leukemia**

The childhood-onset disease Acute Lymphoblastic Leukemia is a notable case where pharmacogenomics models have been suggested which could aim at providing maximum efficacy with minimum adverse effects of existing therapeutic medications when prescribed using the patient genotype with favourable response and low toxicity, or to identify novel therapeutic targets in lymphoblasts that are resistant to conventional antileukemic drugs (19). These drug targets possessing polymorphisms that favour optimum drug response with low toxicity and lesser chances of relapse will be ideal for developing newer, safer and more effective chemotherapeutic agents for cancer therapy. ALL is also perceived to serve as a model for similar pharmacogenomic application in other human cancers. Early treatment response involving measurement of minimal residual disease reflects, both drug responsiveness of leukemic cells and host pharmacodynamics/pharmacogenomics, has been considered the most reliable prognostic indicator for gauging the intensity of treatment. (20).

**Asthma**

Beta2-agonists are the most effective bronchodilators available providing rapid relief of asthma symptoms and the degree of the drug response varies greatly between patients and genetic factors have a major role in it (21). Pharmacogenomic analysis of the commonly used anti-asthmatic agents such as β-agonists, leukotriene modifiers, and corticosteroids showed that relative risk–benefit ratio of a particular therapeutic course for an individual patient could be identified by using “panels” of polymorphisms. Targeted therapy will thus help provide optimum benefit for asthma patients who respond preferentially to certain medications and avoid toxicity by prescribing drugs after assessing the likelihood of adverse drug reactions (22).

**Gastric Cancer**

Therapeutics based on the genotyping of each patient is also predicted as the future goal of personalized medicine (or tailor-made medicine) for chemotherapeutic management of advanced gastric cancer with drugs such as 5-fluorouracil, Paclitaxel, Docetaxel etc that are associated with side effects and drug resistance. These drugs play a role in management of advanced gastric cancer and complements surgical management of one of the common malignancies in the world (23).

**Multiple Sclerosis & Osteoporosis**

Another area of potential impact is foreseen in diseases that presently, have no definitive treatment eg. the neurodegenerative disease - Multiple Sclerosis (24). and diseases where therapy is typically required for several years before outcomes can be evaluated for an individual eg. the systemic skeletal disease – Osteoporosis (25). These are being investigated currently.

- **Understanding Disease Mechanisms of Biological Systems**

There has been a significant increase in understanding of biological systems and their disease mechanisms eg. the cardiovascular system. Pharmacogenomic approaches are emerging for many medications used in different cardiovascular conditions that would assist clinicians in decision-making being more precise to optimize the benefit-to-risk ratio. When combined with molecular imaging, molecular pathway mechanisms can be studied that will allow us to monitor health and disease and enable the enormous shift from genetic to genomic medicine (26).
• **Critical Clinical Decisions**

The use of the anticoagulant Warfarin, is difficult due to the variations in the dose required to have a therapeutic effect, and the adverse effect of serious bleeding. The drug interferes with the recycling of vitamin K in the liver which reduces the activation of several clotting factors. Many genes seem to be involved in the biotransformation and mode of action of this drug and the outcome of large ongoing studies of these along with assessment of environmental factors, will suggest a method to predict the required warfarin dose with minimized risk of haemorrhage (27).

Administering anti-fungal drugs in the immunocompromised state or for refractory/ persistent mycoses is another daunting scenario. Genes with potential pharmacogenomic significance include a) drug transporters involved in absorption and excretion, b) phase I enzymes (e.g., cytochrome P450-dependent mixed-function oxidases) and phase II enzymes (e.g., glucuronosyltransferases) contributing to metabolism, and c) molecules involved in drug distribution (e.g., albumin, A1-acid glycoprotein, and lipoproteins). Tools of population genetics can be used to define inter-individual variation in drug ADME and yield pharmacogenomic models for the observed genetic variations in antifungal pharmacokinetics (28).

Monitoring and management of adverse drug reactions of chemotherapy in cancer patients is another application of Pharmacogenomics. The impact of polymorphism in cytidine deaminase that metabolizes the anticancer drug - Gemcitabine was studied in Japanese cancer patients. A particular genotype harboring a nonsynonymous SNP, (Ala70Thr) was associated with decreased clearance of gemcitabine, and increased incidences of neutropenia when patients were coadministered platinum-containing drugs or fluorouracil.(29)

**Enhanced Diagnostic Tests**

Several technologies used in personalized medicine include SNP genotyping, haplotyping, gene expression studies by biochip/microarrays and proteomics. Molecular diagnostics will play a key role in personalized medicine where therapy and diagnosis will be integrated (30). In January 2005, the US Food and Drug Administration (FDA) granted market approval for the first pharmacogenetic test using a DNA microarray, the AmpliChip CYP450 GeneChip(R) (AmpliChip), which genotypes cytochrome P450 CYP2D6 and CYP2C19 (31). It classifies individuals into two CYP2C19 phenotypes (extensive metabolizers [EMs] and poor metabolizers [PMs]) by testing three alleles and into four CYP2D6 phenotypes (ultrarapid metabolizers [UMs], EMs, intermediate metabolizers [IMs], and PMs (32). Cytochrome P450 is one of the best studied drug-metabolism associated protein complex in the light of pharmacogenomics.

The application of this knowledge is relevant to the population type being considered for therapy. Poor metabolizers (PMs), lacking the enzyme, account for up to 7% of Caucasians for CYP2D6 and up to 25% of East Asians for CYP2C19. Patients having three or more active CYP2D6 alleles; the Ultra Rapid Metabolizers account for up to 29% in North Africa and the Middle East. As CYP2D6 metabolizes many of the psychiatric drugs, psychiatry has become the pioneer for the practical clinical use of pharmacogenetic testing that has relevance in the usage of drugs such as tricyclic antidepressants and antipsychotics (33). Evaluation of pharmacokinetics of citalopram in relation to genetic polymorphism of CYP2C19 was done in Chinese volunteers whose genotypes and phenotypes were known before administering the drug and studying its varied effects. Citalopram, a potent and selective serotonin reuptake inhibitor is used as an antidepressant. It is mainly metabolized to N-desmethylcitalopram and further demethylated to didesmethylcitalopram, the latter being catalyzed by CYP2C19, CYP2D6 and CYP3A4. This study supported the involvement of CYP2D19 in catalyzing this reaction and showed the varied genotype of this gene leads to differences in drug pharmacokinetics when comparing poor metabolizers (PM) and extensive metabolizers (EM). Some of the findings included moderately higher t_{1/2} values of citalopram in PMs than the EMs. The CL oral (oral clearance ) of citalopram in poor metabolizers (17.1 ± 2.0 l/h) was significantly lower than that of extensive metabolizers (24.1 ± 1.6 l/h). PMs showed significantly longer t_{max} values of desmethylcitalopram than in extensive metabolizers (34). CYP testing is also applicable in toxicology and ADME profiling to guide drug development. Combined with pharmacotherapy, it can aid in effective decision-making regarding choice of drugs and dosage (35).

Another landmark test to identify metastatic colorectal cancer patients with indications for use of topoisomerase I interactive drug – irinotecan was approved by FDA in 2005. The genetic test (Invader UGT1A1 Molecular Assay), conducted on genomic DNA from peripheral blood, identifies homozygosity for the UGT1A1*28 allele. Such patients clear irinotecan and its metabolites more slowly than the rest of the population, and so have greater exposure to active drug after a standard dose. The FDA-approved test label states that “a reduced initial dose should be considered for patients known to be homozygous for the UGT1A1*28 allele”(36,37).

**Role in Clinical Trials**

The post-genome era has led to advances such as high-resolution SNP maps and technical approaches like microarray have enabled incorporating genome-wide association studies in clinical trials and can contribute by identifying disease-susceptibility genes useful as diagnostic indicators, process of drug discovery, and selection of therapy. An SNP map containing genetic variants relevant to drug transport, metabolism, and receptor interaction can be significant in drug selection and point to conditions where careful drug dosage monitoring is required (38).

At the 42nd American Society of Clinical Oncology (ASCO) annual meeting in Atlanta, Eli Lilly and Company,
a leader in thoracic cancer, unveiled two breast cancer studies involving pharmacogenomics and its chemotherapies GEMZAR® (Gemcitabine HCl) and ALIMTA® (Pemetrexed). They are among the early trials involving pharmacogenomics in breast cancer treatment and have so far shown signs of being able to predict response related to both general and drug-specific chemotherapy and biomarker analysis.

Clinical Data Inc. has launched the Phase III clinical trial for the depression drug Vilazodone and concurrently looking forward to develop a diagnostic test based on genetic biomarkers that can predict drug efficacy and response.

Many pharmacogenomics based clinical trials are currently underway in different phases of study. Eg. Some at the US National Institute of Health are mentioned at URL: http://www.clinicaltrials.gov/.

Improved Prospects in Preventive Care

If risk for a given disease is predicted to be high, as judged by the SNP pattern of a patient, preventive therapy and lifestyle adjustments (diet, exercise, etc) may be implemented (38). This could help prevent the onslaught of diseases that can be managed by preventive measures before the onset of the actual disease.

Current Databases

**PharmGKB** - The Pharmacogenetics and Pharmacogenomics Knowledge Base, is a publicly available Internet research tool developed by Stanford University with funding from the National Institutes of Health (NIH) and is part of the NIH Pharmacogenetics Research Network (PGRN), a nationwide collaborative research consortium. Its aim is to aid researchers in understanding how genetic variation among individuals contributes to differences in reactions to drugs. The database is a central repository for genetic, genomic, molecular and cellular phenotype data and clinical information about people who have participated in pharmacogenomics research studies. The data includes, but is not limited to, clinical and basic pharmacokinetic and pharmacogenomic research in the cardiovascular, pulmonary, cancer, pathways, metabolic and transporter domains (39) available at URL: http://www.pharmgkb.org/

**Human Membrane Transporter Database** - for Drug Transport Studies and Pharmacogenomics is another related database which currently contains data on more than 250 human membrane transporters and related proteins, their structure, function, sequence variants, and substrates, especially drugs. As this database is intended to support pharmacogenomic studies, it also provides information on sequence variants, altered functions caused by polymorphisms/mutations, and the (patho) physiological role and associated disease (40) available at URL: http://lab.digibench.net/transporter/.

Limitations and Obstacles

Though found to influence many clinical situations, there are others where it may not be very useful. An example for this is the assessment of variations in metabolism of and clinical response to many commonly prescribed non-steroidal anti-inflammatory drugs (NSAIDs), though cytochrome activity plays an essential role in the drug metabolism (CYP2CB9/9) (41). The market withdrawals of rofecoxib (Vioxx) and valdecoxib (Bextra) led to great interest in assessing the side effect profiles of cyclooxygenase (COX) inhibitors. CYP2C9 genotype is considered a risk factor as many COX inhibitors are CYP2C9 substrates in vitro. But the study showed that apart from the effect of CYP2C9 genotype, a major determinant of clearance of this category of drugs, it was also felt necessary to consider CYP2C8 genotype for some of them and, possibly, CYP3A4 activity for some others (42). Hence prescribing these over-the-counter drugs with the relevant genotypic information may not be easily accepted by the clinicians and patients alike as some of them are prescribed for simple symptomatic relief.

There are inherent obstacles that have to be surmounted for the widespread use of pharmacogenetics and include difficulties such as economic feasibility and awareness among medical practitioners regarding the application and interpretation of results (31, 43).

Apart from educating the healthcare personnel and assessment of questions in ethical and legal concepts, public awareness about consent and implications of genetic testing in drug therapy and disease management is also a pressing need (44). This would aid interpretation of risk assessment tests where a predisposition to a pathological condition may not mean actual suffering from the disease. Adequate preventive measures can be instituted where tests indicate a higher probability of disease in later years, ultimately preventing many illnesses. But the burden of the information and fear may be unintended outcomes that also have to be weighed carefully.

**CONCLUSION**

The traditional approach of one-drug-fits-all does not cater to the need of smaller populations/subpopulations that show a similar phenotypic profile with the others but have an underlying distinct genetic signature that consequently leads to variation in drug response.

Pharmacogenomics combining the potential of genomics with pharmacology has tremendous practical potential to significantly enhance the ability of clinicians to use medications in a safe and effective manner (13). The future is not far away when these will be a part of the personalized drug prescription and estimates foresee this by the year 2015, according to the lay journal Time(45) and 2020, by JAMA(46).

An overall decrease in the number of adverse drug reactions, the number of failed drug trials, the time taken for approval of a drug, the duration of medication administered, the number of medications taken by patients before eventually finding the effective therapy, the pathological effects of a disease (through early detection), and a simultaneous increase in the range of possible drug targets will promote a net decrease in the cost and improve the quality of health care.
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PSYCHOLOGICAL AUTOPSY: the Psychological Assessment of an equivocal Death

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ABSTRACT

There are times when physical evidence and evidence found at autopsy does not reveal the cause and mode of death. This is known as equivocal death. The psychological autopsy is a retrospective construction of a decedent’s life initiated to get a better understanding of his death. It is used to determine the victim’s psychological intent, using interviews and examination of documents to reconstruct the behaviour, personality, lifestyle, habits and history of the victim prior to death. Psychological autopsy aids as an investigative tool which is at the outer edge of professional knowledge and practice in that it requires an application of skills, experience, and training to assess a variety of factors including the behaviour, thoughts, feelings, and relationships of an individual who is deceased. There may be issues that the deceased or his or her family does not want facts to be revealed that require special handling. Hence the interviews of family members, friends, and relatives may be adequate. So the interviewer has to be flexible. The interviewer should establish mutual respect and confidence, with the informant, and ensure confidentiality and anonymity, and also obtain an informed consent before the interview. Hence one should be qualified and skilled to conduct the interview. False information also can be given due to lack of memory or it may be intentional. Suicide note, Personal documents, Medical records, School records, Military records, Employment records, should be carefully analysed. With the above mentioned information, a psychological autopsy report is produced, the final conclusion depends on the accuracy of the data collected from the interviews, examination of relevant documents and other materials. Therefore the interviewee’s probabilities and limitation to science should be noted. Thus the final judgement as to the mode of death is based upon a review of all the known facts and circumstances; including the coroner’s report, forensic medical report, police reports, crime scene analyst reports, and the psychological reconstruction so that people may learn from the tragedy and, hopefully, be cautious and reduce the chances of similar occurrence in future.

Psychological autopsy is most often used in cases of suspected suicide or homicide in an attempt to reconstruct the personal life and character of the deceased, to uncover hidden secrets that may help to give family members peace of mind and also plays a role in many legal suits, malpractice suits, insurance claims, and criminal investigations.

Mesh words: Cause of death, autopsy, coroners and medical examiners.

“Human consciousness is an unusual template of experience and emotions”

Introduction

In every instance of death, a physician must distinguish both the cause and mode of death. The cause of death is defined as “the original underlying medical condition which initiates the lethal chain of events culminating in death”. It is the duty of the medical examiner, coroner, and law enforcing authorities, to determine the mode of death in all violent and suspicious death investigations. There are times when physical evidence and evidence found at autopsy does not reveal the mode of death. This is known as equivocal death. Equivocal deaths often involve questions that surface around suicide, homicide, accidental or some natural deaths. Those deaths that fall under the category of “undetermined” are based on what is found at the scene of crime and autopsy, but which frequently require a closer “psychological” investigation and examination. The psychological autopsy was developed as a further post mortem investigative tool that aids in the determination of the person’s death. The psychological autopsy is a retrospective construction of a decedent’s life initiated to get a better understanding of his death. It is used to determine the victim’s psychological intent, using interviews and examination of documents to reconstruct the behaviour, personality, lifestyle, habits and history of the victim prior to death. The concept and technique of the psychological autopsy was developed by Dr. Edwin S. Shneidman who defined the psychological autopsy as:

“A behavioural scientific impartial investigation of the psychological (motivational, intentional) aspects of a particular death. It legitimately conducts interviews (with a variety of people who knew the decedent) and examines personal documents (suicidal notes, diaries, and letters) and other materials (including autopsy and police reports) that are relevant to the role in the individual’s death”.

Behind the scene of an equivocal death investigation

Role of the medical examiner in the investigation:

The medical examiner, who is the forensic pathologist defines the cause of death, provides details about the manner and circumstances of death and the results of a medicolegal autopsy. The prime function of the medical examiner is medicolegal certification of the manner of death, which has important legal, social, medical and research implications.
Role of the investigators:

An investigator in the death team investigation includes the magistrate / coroner, medical examiner, psychologist, psychiatrist, a psychiatric social worker, or a police investigating officer and other law enforcement authorities, as psychological autopsy aids as an investigative tool which is at the outer edge of professional knowledge and practice in that it requires an application of skills, experience, and training to assess a variety of factors including the behaviour, thoughts, feelings, and relationships of an individual who is deceased. Therefore, the interview with the family, friends, co-worker, relatives, neighbours, physicians and other acquaintances to prepare a psychological autopsy report are carried out mainly by the mental health professionals and behavioural science investigators.

Primary goals of these mental health professionals and behavioural scientists are:

a) To determine the mode of death.
b) Reasons for death at that particular period of time.
c) Assessment of lethality (suicide).
d) Psychotherapeutic value to the survivors.

Protocol to conduct an equivocal death interview

a) Ethical considerations concerning the interview, is of prior importance, the integrity of the deceased must be respected (Young, 1992). There may be issues that the deceased or his or her family does not want revealed or facts that requires special handling.
b) History of the actual events must be reviewed, later the interviews of family members, friends, relatives, neighbours, physicians and other acquaintances should be conducted.
c) Approaching the informant to conduct an interview is an important consideration, the informants are contacted by mail and later a phone call, to avoid contact refusal rate. So the interviewer has to be flexible and ready to reschedule the interview.
d) The interviewer should establish mutual respect and confidence, with the informant, and ensure confidentiality and anonymity, and also obtain an informed consent before the investigation.
e) The skills of interviewing includes, proper language, clarity, listening more than speaking, no threatening questions, no repetitions, avoid loaded questioning and more than one informant in a single interview.
f) The interviewee may have motives of giving exaggerated information or concealing facts, or give pertinent information to protect the image of the victim and family. False information also can be given due to lack of memory or it may be intentional. Therefore it is important to explain to the interviewee the significance of the information he / she are providing. It is the role of the interviewer to be able to assess distorted or irrelevant information.

Salient features in an equivocal death interview:

1) Description of the deceased: the personal views about the deceased.
2) Period of association with the deceased: how long they know the deceased, how often they see each other, type of relationship between them.
3) Any changes noticed in behaviour or emotional distress associated with the deceased.
4) Any problems noticed by the interviewee, or discussed with deceased.
5) Observed or expressed mental status of the deceased to situation of depression and stress.
6) Recent changes physically observed: pain, signs of illness, fatigue, tension, or loss of appetite, changes in sleep pattern, insomnia, wakes up throughout the night.
7) The interviewee’s reasons behind the death: what would have probably happened and why.

Other sources:

- **Suicide note:** This play an important role to solve the whole issue if it is proved that the deceased had written the suicide note (verified by a forensic document examiner), the contents, language (specific references to suicide or morbid content). Suicide note also plays as an experimental control for the mental health professionals in interviewing process, when they are not disclosed about the evidence, and to derive their opinion to provide an estimate of validity.
- **Personal documents:** Letters (family, friend, relatives, or acquaintances), dairies, videos, and literature read recently and in past (morbid content), e-mail, threats notes or messages received recently, bills, tickets, and pornographic collections if any.
- **Medical records:** Visits to physicians, medical illness, family history of illness, whether under medications.
- **School records:** Information such as change in academic performance or absenteeism, conduct and character in general.
- **Military records:** Reveal education and training background, areas of deployment, promotions, efficiency and obsession for weapons.
- **Employment records:** Performance, conduct, alibi of work and absenteeism.

With the above mentioned information, a psychological autopsy report is produced, and later reviewed by the death investigation team to determine the mode of death. The psychological autopsy is considered ultimately to be an expert opinion; therefore it depends on the accuracy of the data collected from the interviews, examination of relevant documents and other materials. Therefore the interviewee’s probabilities and limitation to science should be noted.
Evaluation of psychological autopsy report

The psychological autopsy report provides detailed information about the death using various sources including the autopsy report, medical records, relevant documents and information gathered from interviews with key informants. These sources and information provided is to be clearly documented for evaluation and its potential validity. The behavioural scientist and other mental health professionals would be expected to provide more systematic details about the important psychological stages in the person’s thought processes e.g. motivation and personality, to deliver a formal evidence for the conclusion. Actually there is no well developed conceptual or theoretical basis for deriving conclusions from various sources of information, due to lack of standardised technique or specific procedure in conducting the psychological autopsy there is an area of potential weakness of this procedure. Here, the investigator is merely interpreting a prior set of facts rather than predicting future behaviour based upon the limited facts available before assessment. The final judgement as to the mode of death is based upon a review of all the know facts and circumstances; including the coroner’s report, forensic medical report, police reports, crime scene analyst reports, and the psychological reconstruction1. Finally, to analyse and conclude suspicious deaths or ambiguous fatalities, and to facilitate the expansion of knowledge so that people may learn from the tragedy and, hopefully, be cautious and reduce the chances of similar occurrence in future.

Our Experiences an example:-

A 23 year male was rushed to the causality with bleeding injury abdomen, with strong smell of alcohol, with alleged history of tripping and falling over an sharp vegetable cutting weapon in an inebriated condition. He maintained the same history up to his death. By practising our guidelines of psychological autopsy the investigation revealed that there was a scuffle between two brothers with a double edged weapon, which lead to his death. Hence the opinion as to accidental injury was changed as homicidal.

![Surgically sutured wound on the lateral side of the abdomen showing the homicidal injury](image)

Conclusion

Psychological autopsy is most often used in cases of suspected suicide or homicide in an attempt to reconstruct the personal life and character of the deceased, to reveal that may help to give family members peace of mind and also plays a role in many legal suits, malpractice suits, and insurance claims, including criminal investigations. Psychological autopsy has become a valuable tool in the investigation and, at times, resolution of questionable and equivocal death cases. Despite the weakness of the evidence and procedures used in this technique, mental health professionals face problems while reconstructing a psychological autopsy in both civil and criminal matters, when questions can be raised regarding the mental state of the deceased prior to death. Therefore, it is important to establish the value that the report may add to the proceedings, and that includes the admissibility of psychological autopsies as evidence in court hearing which will be in the near future.

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PULSE THERAPY IN DERMATOLOGY

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ABSTRACT

Pulse therapy means the administration of large (suprapharmacologic) doses of drugs in an intermittent manner to enhance therapeutic effects and reduce adverse effects. Dexamethasone and cyclophosphamide in bolus doses have been widely used in pulse form to treat various dermatological disorders. Pulse corticosteroids have also been tried in other conditions like renal graft rejection, lupus nephritis and other autoimmune disorders. This form of therapy has given excellent treatment response with very few side effects. Corticosteroids, immunosuppressives, antifungals, antivirals are the commonly used drugs administered in pulse doses.

Key words: Dexamethasone, pulse drug therapy, cyclophosphamide, autoimmune diseases.

INTRODUCTION

Pulse therapy means the administration of large (suprapharmacologic) doses of drugs in an intermittent manner to enhance the therapeutic effect and reduce the side effects 1.

PULSE CORTICOSTEROID THERAPY

The first reported use of pulse administration of corticosteroids is attributed to Kountz and Cohn who used it to successfully prevent renal graft rejection 2. Thereafter, pulse doses of corticosteroids were used for other diseases such as lupus nephritis, rheumatoid arthritis and pyoderma gangrenosum 3, 4.

The introduction of dexamethasone cyclophosphamide pulse (DCP) therapy for a blistering disorder, pemphigus vulgaris, by Pasricha et al has revolutionized the therapy for pemphigus 5. The therapy has since been used to treat a large number of patients at several centers in India 6 and abroad 7. The dermatological disorders in which corticosteroid pulse therapy has been advocated are pemphigus vulgaris, an autoimmune blistering disorder, systemic sclerosis, systemic lupus erythematosus, dermatomyositis, pyoderma gangrenosum, toxic epidermal necrolysis, Steven Johnson’s syndrome, lichen planus, alopecia areata, sarcoidosis, and systemic vasculitis. Pulse corticosteroid therapy has been proposed as a means of rapidly controlling life-threatening or serious conditions with minimal toxicity, allowing for less aggressive long term maintenance therapy. The selection of drugs and their dosages has been completely arbitrary and based upon intuition and convenience. Conventionally, methyl prednisolone was the agent most commonly used. The choice of dexamethasone made the treatment considerably more affordable and accessible to patients.

There was concern among some workers about the equivalence of 1000mg of methylprednisolone and 100mg of dexamethasone and some groups have administered pulses of 136mg of dexamethasone. However, a dose of 1000mg of methylprednisolone is as arbitrary as a dose of 100mg of dexamethasone and in the absence of evidence that 136mg pulses of dexamethasone are more effective nearly all centres continue to use 100mg boluses 8. Moreover, the cost of dexamethasone cyclophosphamide pulse therapy works out to be 1/3rd of the total cost of methylprednisolone pulse.

Dexamethasone Cyclophosphamide Pulse (DCP) therapy - divided into 4 phases.

Phase I consists of Dexamethasone 100 mg in 5% Dextrose as a slow IV infusion over 2 hours for three consecutive days along with cyclophosphamide 500 mg infusion on one of the days. This constitutes one DCP. Such DCPs are repeated every 28 days till no new lesions appear between pulses. Cyclophosphamide 50 mg / day is given orally. During this phase the patient may continue to develop recurrences of clinical lesions in between the DCPs and can therefore be given conventional doses of oral corticosteroids to achieve quicker clinical recovery. After the skin and mucous membrane lesions have subsided completely and the additional medication withdrawn, the patient is considered to have entered phase II 9.

Phase II consists of the DCP schedule given for a fixed duration of 9 months.

Phase III, only oral Cyclophosphamide 50 mg / day is given for 1 year

Phase IV, all the drugs are withdrawn and the patient is followed up.

There was initial alarm and anxiety about the large doses of corticosteroids and cyclophosphamide, but today this therapy is given as a routine infusion, often in a day care or OPD setting, with the patient going home a few hours after completion of the infusion.

There have been modifications of DCP therapy 10. Cyclophosphamide is known to cause oligo / azosperma and amenorrhoea. For unmarried patients who have not
completed their family, cyclophosphamide is replaced with azathioprine 50 mg (DAP therapy). Methotrexate, 7.5 mg (DMP) was instituted in patients who were not completing Phase I even after 12 pulses (1 year) of DCP or DAP therapy.

Contraindications of DCP therapy

DCP therapy can be given to patients of all ages but the doses have to be reduced to half for children below the age of 12 years. It can also be administered to patients having other medical problems like diabetes mellitus, hypertension, osteoporosis, tuberculosis. Diabetic patients need to be given 10 units of soluble insulin for every 500 ml bottle of 5% dextrose dissolved in the same drip. In addition they must be given the routine treatment for diabetes mellitus. Similarly patients having concomitant diseases like hypertension and tuberculosis must receive the respective medication. If there is serious infection elsewhere or if the patient has severe bacterial, viral or fungal infections, the pulse may be delayed for a week or two till the infection has been brought under control. Pulse therapy is absolutely contraindicated in pregnant, lactating and unmarried patients.

Laboratory monitoring

As a routine, it is mandatory to admit every patient enrolled for pulse therapy and undertake a complete baseline clinical and laboratory evaluation before starting the first pulse. Haemogram, serum electrolytes, renal and liver function tests, blood sugar (including HbA1C), urine microscopic examination, chest X-ray, electrocardiogram and pregnancy test are some of the preliminary laboratory tests to be done at the first visit of the patient. Blood sugar, serum electrolytes, urine microscopic examination, body weight and blood pressure should be monitored at base line and at each visit of the patient.

Adverse effects of DCP therapy

The adverse effects of pulse therapy are those of its constituent drugs. Corticosteroids precipitate infections, diabetes mellitus, hypertension, hyperacidity and osteonecrosis. Side effects attributed to cyclophosphamide are leucopenia, hematuria, gonadal failure, hyper pigmentation and hair loss. These side effects are infrequent compared to daily corticosteroid therapy. Side effects peculiar to pulse therapy include hiccups, facial flushing, diarrhea, weakness, generalized swelling and weight gain, joint and muscle pains, arrhythmias and shock. Arrhythmia is attributable to rapid efflux of potassium and influx of sodium in the myocardium.

MINI PULSE CORTICOSTEROID THERAPY

Oral betamethasone has been given at a dose of 10 mg once weekly in dermatoses like vitiligo, lichen planus, alopecia areata with variable success. 10 mg of betamethasone is split in 2 equal doses on 2 consecutive days a week.

OTHER FORMS OF PULSE THERAPY

1. Cyclophosphamide pulse therapy

Gokhale et al evaluated the response to pulse intravenous cyclophosphamide therapy in patients of pemphigus vulgaris. Cyclophosphamide 500 mg pulses are given in 500 ml of 5% dextrose solution slowly intravenously. These pulses are given monthly for 12 months and 2 monthly for further 6 pulses. Close monitoring of kidney and bladder function was done. The response to therapy was good to excellent in more than 60% of patients. This form of pulse therapy has been tried in other conditions like lupus nephritis, serious central nervous involvement in lupus erythematosus and Wegener’s granulomatosis.

2. Pulse therapy with antifungals

Itraconazole, fluconazole are used to treat superficial and deep fungal infections. These drugs are administered in weekly doses every month.

Itraconazole - 400 mg / day for 1 week every month for 3 months, for treatment of Tinea unguium.
Fluconazole - 400 mg once a week for 1 month, for treatment of Pityriasis versicolor

Each pulse is given for 7 days every month.

3. Isotretinoin therapy for acne vulgaris

1 mg / kg / day of isotretinoin is administered for 7-10 days every month.

4. Pulse therapy in Psoriasis

Weinstein Frost regimen - Methotrexate 7.5 mg – 25mg , administered every week. It is used for the treatment of psoriasis

CONCLUSION:

Pulse therapy appears to be novel path breaking therapy for pemphigus vulgaris, a host of autoimmune dermatoses and systemic diseases. In India, maximum work has been done on pulse therapy in pemphigus vulgaris. However, the steroid molecule used is dexamethasone, not methyl predinolone. Pharmacokinetic and immunologic studies are grossly lacking in Indian perspectives which would help us to understand the mechanism of action and refine this path breaking therapy further. For practical purposes, every evaluation of pulse therapy should address few clinical outcomes: the time to clinical remission, the duration of remission while on treatment and duration of remission after withdrawal of treatment.

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TOOTH WITHIN A TOOTH
Balagopal Sundaresan*, Arathi Ganesh*, Nivedha Rajendran*

ABSTRACT

Endodontic treatment for teeth that exhibit the dental anomaly called “Dens Invaginatus” is very difficult due to the operator’s inaccessibility to the diseased pulp in the complex root anatomy. Surgical intervention and extraction are the common approaches to deal with this condition.

INTRODUCTION

‘Dens in dente’ meaning ‘tooth within a tooth’ is a rare developmental anomaly of the tooth affecting mostly the permanent maxillary lateral incisors and less commonly the other permanent teeth and the primary dentition. It is also called as ‘dens invaginatus’ or ‘dilated composite odontome’. This condition is characterized by the infolding or inversion of the enamel into the tooth structure, during the early stages of tooth bud formation. This gives an appearance of an in – growing tooth.[1] Dens invaginatus is commonly diagnosed as an incidental radiographic finding unless the patient presents with pain or swelling associated with the involved tooth. Severe form of dens in dente affects the entire morphologic structure of the tooth. The incidence of dens invaginatus is reported to range between 0.04% and 10% [2] with a higher male predilection than females.

Several causes of this condition have been proposed. These include localized external pressure, focal growth retardation and focal growth stimulation in certain areas of the tooth bud. Roentgenographically it is recognized as a pear – shaped invagination of the enamel and dentin with a narrow constriction at the opening on the surface of the tooth. The significance of dens in dente is its predisposition to early decay, pulp necrosis and periapical cyst. [3] This is believed to be due to the patients’ inability to keep the defect free of cariogenic plaque. [4]

Oehlers FAC[5] in 1957 classified this defect into three possible variations:
Type I: The enamel invagination is limited to the dental crown short of the cementoenamel junction(CEJ).
Type II: The defect penetrates beyond the CEJ into the dental root; the pulp chamber may or may not be involved.
Type III: The invagination extends beyond CEJ reaches the periodontal tissues and has a separate apical foramen communicating with the periapical tissues.

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This case report describes a dens invaginatus with bizarre root canal anatomy which was successfully treated by non-surgical endodontics.

Key words: Dens in dente, Dens Invaginatus, case reports.

CASE REPORT

A 22-year old female presented with swelling and pain in the gums in the anterior region of the upper jaw (Patient reported on 7th April 2005). The patient gave a history of recurrent swelling in the same region for the past six months which subsided on its own. On examination a mild firm swelling was present in the gingiva in relation to the upper left lateral incisor. A sinus tract opened in the facial gingiva in relation to this tooth. There was no evidence of pus discharge from this sinus tract. The tooth exhibited a different external morphology resembling a conical tooth [Fig 1] unlike the trapezoidal morphology of a normal lateral incisor [Fig 2]. It was tender on percussion and showed delayed response to tooth vitality tests which indicated that the tooth was turning non-vital.

Management of malformation aims to prevent degeneration of the invaginated enamel. Variations such as type II and type III require complicated endodontic or surgical intervention. This case report describes the non-surgical management of a complicated type III variant of dens in dente.

Fig 1: Conical appearance of the Dens in Dent lateral incisor
Fig 2: Trapezoidal appearance of a normal lateral incisor
The radiographic examination revealed that the tooth was invaginated till the apical third of the root with the presence of periapical radiolucency. The root canal morphology appeared more complex radiographically for the invaginated tooth [Fig 3] unlike a normal lateral incisor [Fig 4]. This led us to diagnose the tooth as type III dens invaginatus with periapical abscess.

![Fig 3: Radiographic appearance of dens in dente](image)

![Fig 4: Radiographic appearance of normal lateral incisor](image)

Root canal treatment was initiated for the lateral incisor. On opening the access to the root canal, two canals mesial to and separate from the main root canal system were present [Fig 5]. This finding is in contrast to the normal morphology of a maxillary lateral incisor which has only one root canal [Fig 6]. All the three root canals in this tooth were separate from each other throughout its length. Mild pus discharge was evident from all the canals suggesting the presence of an abscess in the periapical tissues.

![Fig 5: Access opening of dens in dente showing three root canals](image)

![Fig 6: Access opening of normal lateral incisor showing one large root canal](image)

Root canal treatment was completed in two visits with an inter-appointment dressing of calcium hydroxide. Radiographs were taken post operatively to confirm the integrity of the root canal filling. Figure 7 shows the post operative sixth month follow-up radiographic appearance of the filled root canals of the invaginated tooth showing satisfactory bone healing periapically. This can be compared with the radiographic appearance of a root canal treated normal lateral incisor with single canal in figure 8. At the second appointment two weeks later, the sinus tract was found to be completely healed and there was no evidence of swelling in the gingiva. The final restoration of the tooth was done and then patient was reviewed for clinical and radiological signs of healing after 1, 3 and 6 months (October 2005). The patient was asymptomatic and did not have any recurrence of symptoms in the affected tooth.

![Fig 7: Radiographic appearance of obturated (filled) dens in dente showing three canals (sixth month follow-up)](image)

![Fig 8: Radiographic appearance of obturated (filled) normal lateral incisor showing one root canal](image)
DISCUSSION

Bacterial contamination of the invagination resulted in development of infection and periapical inflammation. Treatment of dens invaginatus is a complex procedure because of its atypical root canal anatomy. The success of the root canal treatment in these teeth depends on identifying all the additional canals and restoring the same. This case showed the presence of three root canals instead of one. Identification of the complex root canal anatomy of this case and management of all the canals nonsurgically, has improved the prognosis of the tooth. Calcium hydroxide, used as an inter - appointment dressing in the root canals, helped in healing the periapical infection and disinfecting the root canals owing to its anti – microbial and tissue dissolving properties. This resulted in early elimination of the root canal infection thereby avoiding surgical intervention. The success of this non surgical endodontic treatment of the dens invagination was demonstrated by the resolution of the patient’s signs and symptoms with radiographs showing periapical bone healing in the subsequent follow – up examinations.

CONCLUSION

Dens invaginatus can be recognized before the eruption of the tooth from periapical radiographs. So these teeth should be treated prophylactically as soon as possible after tooth eruption. Early diagnosis and intervention can definitely prevent pulpal necrosis and the potential loss of tooth. The nonsurgical endodontic management of the complex root canal morphology of these teeth is a successful alternative to the more invasive surgical intervention. Operator skill in locating these abnormal courses of the root canals will eliminate the procedural errors which could occur while searching for the canal in its normal position. Knowledge about such unexpected variations in root canal anatomy and its immediate conservative treatment is the key to the successful management of the anomalies of tooth.

REFERENCES:

CORTICAL VENOUS THROMBOSIS IN A CASE OF PREECLAMPSIA

Usha Vishwanatha *, Sonali Sood *, Shalu Gupta *

ABSTRACT

A 23 year old primigravida presented to the Obgyn outpatient department with severe early onset fetal growth restriction and oligohydramnios with persistent diastolic notch in the uterine artery. Patient subsequently went on to develop full blown early onset severe preeclampsia which further manifested itself in the post partum period as generalized clonic tonic convulsions due to cerebral hemorrhage & infarction with cortical venous thrombosis. This is a rare but dreaded complication of preeclampsia. This late onset eclampsia was then managed conservatively with anticonvulsants and thrombolytic agents.

Key words: pre-eclampsia, postpartum period, venous thrombosis, intrauterine growth retardation, oligohydramnios, case reports.

INTRODUCTION

Preeclampsia a pregnancy specific syndrome clinically manifesting as elevated blood pressure and proteinuria is one of the major contributors of maternal morbidity and mortality of pregnant mothers in the developing countries. (1,2)

World wide, 50,000 women die each year from preeclampsia and it still remains the most significant and intriguing unsolved problems in obstetrics. (3) Cerebral lesions have rarely been demonstrated with preeclampsia but more commonly associated with eclampsia, range from cerebral edema, hyperemia, ischemia, thrombosis to hemorrhage (ranging from petechiae to gross bleed). These lesions may be wide spread, focal and seldom fatal. Cortical cerebral venous thrombosis is a rare but dreaded complication of preeclampsia and this case is reported for its rarity and dramatic response to therapy.

CASE REPORT

A 23 years old primigravida presented to the Department of OBGYN in October 2006 with the anomaly scan at 23 weeks showing features suggestive of intrauterine growth restriction (symmetrical) with all fetal parameters below 5th percentile with severe oligohydroamnios. The Doppler study showed features suggestive of utero placental dysfunction and the uterine artery had persistent diastolic notch and the umbilical artery had absent diastolic flow. The Doppler study showed features suggestive of utero placental dysfunction and the uterine artery had persistent diastolic notch and the umbilical artery had absent diastolic flow. These lesions may be wide spread, focal and seldom fatal. Cortical cerebral venous thrombosis is a rare but dreaded complication of preeclampsia and this case is reported for its rarity and dramatic response to therapy.

On clinical examination, the patient was moderately built with normal blood pressure 120/80mmHg. Systemic examination was within normal limits. On abdominal examination there was a lag of > 4 wks with grossly decreased liquor with fetal heart rate of 156/min by Doppler.

In view of severe early onset IUGR with oligohydramnios and uterine artery showing persistent diastolic notch (which is a very sensitive marker for prediction of preeclampsia -78% sensitivity), the patient was explained regarding the poor fetal prognosis and possible maternal complications of continuing the pregnancy and counselled for termination. However the patient decided to continue the pregnancy and was lost in follow up. Subsequently, in the next 3 weeks she developed severe preeclampsia with uncontrolled blood pressure, for which termination of pregnancy was done. She expelled a dead fetus weighing 300 gm with no obvious external congenital anomalies in the foetus and placenta showed no obvious external areas of infarction or calcification. After delivery and discharge patient did not go for postnatal check up and presented to the Emergency department of SRMC & RI with an episode of generalized tonic clonic seizures on the 10th post natal day. The seizures were preceded by a headache (temporoparietal) for 3 days. The patient was immediately treated with parenteral anticonvulsants and antiedema measures. Investigations for preeclampsia were within normal limits. The Fundus showed Grade I hypertensive changes.

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MRI brain was done which showed haemorrhagic infarcts in the left temporoparietal region (fig 1 and fig 2) and in view of patient's history MR venogram was performed in the same sitting which revealed left transverse
and sigmoid sinus thrombosis (fig 3). Therefore patient started on therapeutic low molecular weight heparin. Meanwhile patient was evaluated for thrombophilic disorder, all of which showed negative results. Patient showed drastic improvement symptomatically. After 5 days of overlap, anticoagulation was changed to Warfarin and patient was discharged and is presently doing fine. Repeat MRI has been planned after 6 months for evidence of complete cure and to do antiphospholipid workup after anticoagulant is stopped.

DISCUSSION

Previously the controversial existence of delayed postpartum eclampsia is now acknowledged by most experts. Evidence suggests the increasing incidence of late onset eclampsia (6). By definition, convulsions with initial presentation more than 48 hours but less than 4 weeks after delivery are commonly referred to as late postpartum eclampsia. The presentation of late postpartum preeclampsia or eclampsia may differ from that occurring during pregnancy. This contributes to the difficulty in the diagnosis (4).

Lubrasky and Charms reported 44% and 79% patients respectively with late onset postpartum eclampsia who had not been identified as having preeclampsia before the onset of seizures (5, 6). Late postpartum eclampsia is most commonly preceded by a prodrome of visual and cerebral symptoms (headache/diplopia) which should not be ignored in any postnatal patient.

Our patient had convulsions on the 10th postnatal day. The response to anticonvulsants with MRI showing haemorrhagic infarct with venous thrombosis with Fundus showing grade I hypertensive changes, with exclusion of metabolic, thrombophilic and infectious causes, strongly support the diagnosis of ECLAMPSIA. (Spontaneous cortical venous thrombosis which is a rare occurrence in pregnancy is a possible differential diagnosis for this case)

Lesions in MRI cannot predict whether damage is permanent or likely to be reversible. To reduce the rate of postpartum eclampsia, efforts should be directed to educate the patient and her health care providers regarding prompt reporting and evaluation of symptoms of preeclampsia during the postpartum period.

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INTRODUCTION
Complications arising from infection of the paranasal sinuses still occur in paediatric sinusitis, despite the use of broad spectrum antibiotics. Inflammatory orbital complications can cause loss of vision. Orbital complications can be divided into several overlapping stages: inflammatory edema, orbital cellulitis, orbital periostitis, subperiosteal abscess, orbital abscess and cavernous sinus thrombosis.

Functional endoscopic sinus surgery is an established standard for surgical treatment of infections of the paranasal sinuses.

Case report:
A 4 year old boy was referred from the Paediatric department with complaints of swelling in left eye for 2 weeks. The swelling was initially in the medial aspect of the left eye and then progressively increased. The swelling was associated with fever and pain at the onset. Patient was being treated with antibiotics and steroids as prescribed by the Ophthalmologist and pain and fever subsided but the swelling persisted. There was no visual disturbance. There was no history of trauma or nasal surgery. Examination revealed a small swelling 1 X 1 cm just above the left medial canthus with associated edema of the upper eyelid. Reddish discoloration of the whole upper eyelid was noted. The swelling was tense and tender on palpation. The left eyeball was displaced forwards, downwards and outwards (fig. 1). All the extraocular movements were normal except for restricted upward gaze. Ophthalmologic examination revealed normal fundus with no visual impairment. Diagnostic nasal endoscopy showed enlarged middle turbinate on left side with crowded ostiomeatal complex. Mucoid discharge was seen in choana with adenoid hypertrophy. CT scan of Paranasal Sinuses & Orbit with contrast showed homogenous opacity involving the whole of the left maxillary sinus and ethmoidal sinus with extension into the extraconal compartment of left orbit through a defect in the lamina papyracea (fig. 2). Peripheral enhancement of the lesion was noted. With the provisional diagnosis of left orbital subperiosteal abscess with orbital cellulitis, the patient was taken up for endoscopic drainage of the abscess. Under general anaesthesia, using a 0° degree Hopkin’s telescope uncinectomy and middle meatal antrostomy was performed on the left side. Polypoidal tissue was removed from the maxillary antrum and the maxillary ostium was made patent. The ethmoidal air cells were cleared till the lamina papyracea could be visualized. The papery thin bone of the lamina was removed almost completely and 10 ml of frank pus was drained out and swab was sent for culture and sensitivity. Patient was continued on broad spectrum intravenous antibiotics. In

ABSTRACT
Intraorbital abscess is a serious complication of sinusitis. The recommended surgical procedure at present for drainage of abscess is endoscopic approach. Here we report a case of complicated paediatric sinusitis which was treated successfully.

Key words: Sinusitis, Subperiosteal abscess, Orbital complications, case reports.
in the immediate post operative period, the proptosis subsided and the eye movements were back to normal. Nasal packs were removed the subsequent day and patient was discharged after 5 days of antibiotics and steroids. However the pus did not grow any organism. Patient has been followed up for three months with no evidence of recurrence (fig. 3).

Discussion:
Complications of sinusitis can be life threatening and often require surgical therapy. Complications originate from progressive spread of inflammation along the bony dehiscences in the lamina papyracea, directly by infectious breakdown of the lamina along the vessels, and in children along the open suture lines. The commonest sinus to be involved in paediatric age group is the ethmoid sinus. Compression of small nutrient vessels of the optic nerve might be responsible for loss of vision. The infectious agents are often Haemophilus Influenza (especially in small children), Streptococcus pneumoniae and Staphylococcus aureus. It is difficult to estimate the incidence of orbital complications because many patients are treated effectively by paediatricians or general practitioners. In the initial stage i.e. orbital cellulitis, genuine proptosis of the globe occurs and this can be difficult to distinguish from the oedema of the lids. The proptosis will be exaggerated by a specific collection of pus which is most often sub or extraperiosteal. Pus readily collects between the sinus and orbit, stripping the periosteum from the adjacent lamina papyracea. The position of the abscess will determine the angle of displacement, usually combining a degree of axial proptosis with lateral and inferior displacement, as was seen in our patient. The movements of the globe may be restricted by the presence of the mass and/or oedema of the ocular structures. Visual loss will depend upon the extent and rapidly with which the displacement develops. Colour vision is often impaired first. Once vision is lost it is exceptional for it to return even after surgical and medical decompression. Radiological imaging is mandatory to confirm the diagnosis and for exact planning of the surgery.

Computed Tomographic scans are the imaging modality of choice for chronic sinusitis and orbital as well as further complications such as septic cavernous sinus thrombosis.

An ophthalmologist consultation to document visual acuity should be performed in every case. Endoscopic sinus surgery is a functional and minimally invasive technique and is the treatment of choice at present. The advantages of endoscopic surgery over external approach are minimal surgical trauma, no drains, no visible scars and the healing can be followed by the use of an endoscope. Early diagnosis and intervention is mandatory to prevent life threatening complications of sinusitis.

Reference:
LAPAROSCOPIC NEPHRECTOMY IN CHILDREN: A CASE REPORT AND REVIEW OF OUR EXPERIENCE

Ramesh Babu a, Vikram a, Shakir a

ABSTRACT

Laparoscopic approaches are fast becoming popular for pediatric urology problems. Here we report a case of ectopic ureter managed laparoscopically. Also our experience with laparoscopic nephrectomy in children is reviewed. A 11 month old boy was referred with UTI and recurrent left epidydymo orchitis. Antenatally he was diagnosed to have left multicystic kidney. Ultrasound postnatally confirmed dysplastic left kidney with poor function on DTPA scan. An MCU revealed reflux into the ectopic ureter. Laparoscopic transperitoneal left nephroureterectomy was performed. At the same sitting right orchidopexy was also performed. The child recovered well and is devoid of UTIs. Laparoscopy can be a very useful tool to remove distal ureter as low as possible without another incision. It reduces hospital stay and hastens patient recovery. All the six patients who underwent laparoscopic nephrectomy last year have performed well. Our results are comparable to those published in the literature.

Key words: Urinary tract infection, child, laparoscopy, nephrectomy, case reports.

INTRODUCTION

Laparoscopy has become the mainstay of treatment for many conditions in adult surgery and urology. However it was a bit slow to develop in pediatric surgery and pediatric urology. With the availability of expertise and equipments, more centres worldwide have started using the laparoscopic approach for the management of pediatric urological problems [1].

The advantages of laparoscopy in children include reduced pain, reduced hospital stay and quick recovery which in turn reduce complications and help them return to school soon. However pediatric laparoscopy is not without its limitations. Limited space available inside the pediatric abdominal cavity can result in difficulty with manipulation of instruments and dissection. Laparoscopic renal surgery was initially performed via retroperitoneal route. However the lack of landmarks and limitation in space made training in this approach [2,3] difficult. Transperitoneal approach has now become more popular for laparoscopic renal surgery. In this article we present a challenging case managed in our unit and discuss our experience of laparoscopic nephrectomy in children, at SRMC.

CASE REPORT

A eleven month old baby boy was referred with recurrent urinary infections, recurrent epidydymo orchitis and failure to thrive. He was the second child of a consanguineous marriage, diagnosed antenatally to have left multicystic dysplastic kidney. The other gross abnormality noted at the time of birth was absence of nipples (athelia) and right undescended testis (Figure 1a and 1b). In view of this a karyotyping was done which was found to be normal.

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There were no other dysmorphic features or other systemic abnormality. Examination of the scrotum revealed absence of right testis, the left testis was enlarged and tender due to epididymsgo orchitis. Hematology and Biochemistry examination were within normal limits. Ultrasound abdomen revealed a normal right kidney and dysplastic left kidney with its dilated ureter running ectopically up to the bladder neck. Renal nuclear scan revealed very poor function in the dysplastic left kidney (Figure 2). Voiding cystourethrogram revealed a bladder diverticulum and reflux into the dilated ectopic left ureter. The contrast from the dilated ureter was also seen refluxing into the vas deference.

Figure 1. a) Eleven month old boy with dysplastic left kidney, ectopic ureter. Clinical examination revealed absent nipples (athelia) b) Same patient having right undescended testis

Figure 2. DTPA renal scan showing poor function in the left kidney
making it more prone for epidydymo orchitis (Figure 3). In view of these findings it was decided to remove the dysplastic left kidney, ectopic left ureter and also perform a right orchidopexy. It would have normally required three different incisions for all the three steps. With the availability of pediatric laparoscopy, it was decided to combine all three via the laparoscopic approach.

Pneumoperitoneum was established via 10mm umbilical port, inserted by open Hassan’s technique. Two further 5 mm ports were placed above and below the umbilical port (Figure 4). After mobilising the left colon, the left kidney was dissected and vessels were clipped. The ureter was traced as low as possible and resected at the level of the bladder neck. The right testis which was located at the level of deep ring was mobilised and brought down with the assistance of laparoscopy. The resected kidney specimen along with the ectopic ureter was removed via the umbilical port. Histology confirmed dysplasia. The patient was advised to continue prophylactic antibiotics. At one year follow up he was free of urinary infections or epidydymo orchitis. The testis brought down laparoscopically was felt in the right place in the scrotum.

A retrospective analysis was performed to identify the children who underwent laparoscopic nephrectomy in our unit over the last one year. Table 1 summarizes the different age groups, indications, and the outcome. The operating time for laparoscopic nephrectomy has declined dramatically with the learning curve and is at the moment comparable to that of open nephrectomy (2 hours). No complications were encountered in any of these patients.

<table>
<thead>
<tr>
<th>No</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 yr; boy</td>
<td>PUV, Valve bladder + Non functioning kidney</td>
<td>Lap nephrectomy + Ureterocystoplasty</td>
</tr>
<tr>
<td>2</td>
<td>14 yr; girl</td>
<td>PUJ obstruction + Non functioning kidney</td>
<td>Lap nephrectomy</td>
</tr>
<tr>
<td>3</td>
<td>5 yr; boy</td>
<td>PUJ obstruction + Non functioning kidney</td>
<td>Lap nephrectomy</td>
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<td>4</td>
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<td>Lap nephrectomy</td>
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<tr>
<td>5</td>
<td>11 month; boy</td>
<td>Ectopic Ureter + Multicystic kidney + undescended testis</td>
<td>Lap nephrectomy + Lap Orchidopexy</td>
</tr>
<tr>
<td>6</td>
<td>10 yr; girl</td>
<td>Multicystic Dysplastic Kidney</td>
<td>Lap nephrectomy</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Children with urinary infection have to be carefully evaluated as up to 50% of these children can have underlying renal abnormality. Vesicoureteric reflux is the commonest cause of childhood urinary infection [1]. Abnormalities like ectopic ureter should be suspected in the presence of recurrent epidydymo orchitis in a boy or constant wetting in a girl. Often multicystic dysplastic kidney is associated with ectopic ureter.

Our case depicts a text book description of ureteric bud developmental anomaly where, the abnormally developed ureteric bud, leads to ectopic insertion of ureter and recurrent epidydymo orchitis [2-4]. Due to abnormal induction of nephrogenesis, ectopic ureter is often associated with dysplastic or non functional kidney on that side. Laparoscopic nephroureterectomy results in cure of the symptoms in such patients.

In our institute apart from diagnostic laparoscopy, laparoscopic orchidopexy, laparoscopic varicocele ligation, laparoscopic pyeloplasty and laparoscopic nephrectomy are being performed. Open Hassan’s technique is always used to avoid any possible damage to viscera by blind insertion of the Veres needle. Transperitoneal approach is preferred as the landmarks are better within the peritoneum and space constraints seen with retroperitoneal approach is not there.

Although the operative time was slightly higher at the beginning (3.5 hours), with the learning curve, the current
operative time for laparoscopic nephrectomy has approached that of open nephrectomy (2 hours). Post operative recovery is excellent and the cost is not higher as the hospital stay is reduced significantly compared to open surgery.

A review of literature showed that our results are comparable to those reported in the literature [2-6]. With the increasing awareness of patients and physicians about ‘key hole surgery’ it is essential that we are able to offer the best available treatment for our patients. The reported case and the other cases of laparoscopic nephrectomy show that pediatric laparoscopy can be successfully performed despite the young age and SRMC has taken a lead in this direction at the right time.

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Dr. Gayathri, PICU consultant,

REFERENCES:
INTRODUCTION

Acrodermatitis enteropathica was recognized in 1936, by a Swedish dermatologist Thore Brandt. Zinc absorption in young patients with this entity is low, about 2-3% compared with 27 – 65% in normal adults. The cause of this specific zinc malabsorption is not known. The decreased absorption can be overcome by an oral zinc load but also improves with age.

We report here a case of acrodermatitis enteropathica who showed a dramatic clinical improvement to oral zinc therapy.

CASE REPORT

A 3 year old, male, child, born normally at full term to 2nd degree consanguinous parents presented with a generalized, pruritic, eczematous eruption. Birth history was normal. Skin was apparently normal till 3 months of age.

At the time of presentation, cutaneous examination revealed an eczematous eruption on the extremities, front of the trunk and periorificial areas (Fig 1). The back of the trunk was relatively spared. There were erythematous, scaly, crusted plaques on the central part of the face (Fig 2). The palms and soles showed exfoliation (Fig 3). A few white plaques were seen on the tongue which were suggestive of candidiasis. There was redness and fissuring at the angles of the mouth. All the finger and toe nails showed dystrophy, Beau’s lines and were associated with various degrees of paronychial inflammation (Fig 4). Scalp hair was sparse, thin and light brown in colour.

ABSTRACT

Acrodermatitis enteropathica is a rare, inherited disorder, transmitted as an autosomal recessive trait. The clinical syndrome is characterized by a phenotypic triad of acral dermatitis, alopecia and diarrhoea. The acral and periorificial distribution of the rash is recognized as a virtually pathognomonic cutaneous marker for zinc deficiency. It may be hereditary or acquired in gastrointestinal disorders or in patients on prolonged total parenteral nutrition. Therapy with zinc gives an excellent clinical response and reduces mortality.

Key words: acrodermatitis enteropathica, zinc, treatment, case reports.
Milestones were normal. He was fully immunized for his age. The child had been exclusively breast fed since birth. He developed intolerance (diarrhoea) to cow’s milk when weaned at 3 months of age and was started on infant formula feeds. The first sibling, a male child had died of similar complaints at 6 months of age.

Examination revealed an afebrile, alert but irritable child weighing 12kg. He had a height of 82cm, weight of 12kg, chest, arm and head circumference of 52cm, 15cm and 49cm respectively. Systemic examination was unremarkable. He had average intelligence and linguistic skills. A complete haemogram, liver and renal function tests were normal. Serum albumin was 3.9gm % and serum globulin 4.2gm % with a reversal of the albumin: globulin ratio. Chest X-ray was normal. Ultrasonography of abdomen revealed a polycystic left kidney. Histopathological examination revealed mild intracellular oedema in the upper part of epidermis, parakeratosis, diminution of granular layer and mild psoriasiform hyperplasia(Fig 7). Urine and stool examination was normal. Alkaline phosphatase enzyme was 76 IU/L Plasma zinc level was low 52ug/dL (60 – 120ug/dL).

On the basis of clinical and biochemical findings, we arrived at a diagnosis of acrodermatitis enteropathica. The patient was treated with oral zinc sulphate supplements 2mg/kg/day, topical corticosteroids, warm saline compresses. Oral fluconazole 50mg daily was given for 1 week. There was an overall dramatic response to treatment in 7 days. The child became active and playful by the second day. The skin lesions started healing by the 5th day and completely healed in one month (Fig 5,6).

Discussion:

Acrodermatitis enteropathica is a rare, inherited disorder, transmitted as an autosomal recessive trait. The clinical syndrome is characterized by a phenotypic triad of acral dermatitis, alopecia and diarrhoea. The acral and periorificial distribution of the rash is recognized as a virtually pathognomonic cutaneous marker for zinc deficiency. The cutaneous lesions are psoriasiform or annular, erythematos, scaly, crusted plaques. As the disease progresses these plaques become vesicobullous, pustular and erosive. Other features include stomatitis, apathy, irritability, growth retardation, failure to thrive and delayed wound healing. Delayed puberty and hypogonadism in developing males are some of the long term effects. Classic hereditary acrodermatitis enteropathica begins within days to weeks after birth in infants bottle fed with bovine milk or soon after weaning from the breast in older infants. Acquired zinc deficiency usually occurs in patients receiving prolonged total parenteral nutrition for inflammatory bowel disease and chronic diarrhoea. It may occur in alcoholic liver cirrhosis, pancreatitis, cancer chemotherapy. Acrodermatitis enteropathica needs to be differentiated from other dermatological causes of anogenital rash such as diaper dermatitis, candidiasis, seborrheic dermatitis, psoriasis. Certain other disorders such as essential fatty acid, carboxylase and amino acid deficiencies may have similar cutaneous features and need to be excluded especially if the lesions persist despite zinc supplementation or relapse frequently. Riboflavin deficiency closely mimics this condition but in addition presents with a seborrheic dermatitis like picture, corneal vascularisation and interstitial keratitis. Histopathology of acrodermatitis enteropathica is very non specific and not an absolute criterion to diagnose this condition.

Zinc has been recognised as a constituent of the human body. As it forms an integral part of carbonic anhydrase and many other highly purified enzymes, its importance in protein and carbohydrate metabolism is understandable. Zinc is present in unmilled cereals, beans, cheese, whole wheat bread, animal protein especially lean red meat, pork. Most
of the diets provide 10 – 15 ug of zinc per day (150 – 250μmol). High content of phytate and fibre in unleavened bread (chapatti), cereals and vegetables binds the zinc and produces a highly insoluble Zn – calcium – phytate complex that prevents absorption. Leavening of bread with a phytate – splitting enzyme of yeast decreases its phytate content by 15 – 25% and increases zinc absorption. Fats tend to dilute zinc from the total diet. A preadolescent child should receive about 10mg of zinc in the diet per day. The requirement for adolescent male and female are 15mg and 12mg/day respectively.

Zinc sulphate for acrodermatitis enteropathica was first introduced in 1973. Oral zinc given in a dose of about 2mg/kg/day was found to cure all clinical manifestations related to zinc deficiency within 1 or 2 weeks. In most instances dietary supplementation with two to three times the RDA in doses of 30 to 55mg of elemental zinc daily is adequate to restore normal zinc status. Dosage is based on the amount of elemental zinc present in the preparation. As observed in a study treatment with zinc sulphate 1mg/kg/day helped clear the symptoms in 5 days. The serum zinc levels which were as low as 6ug% increased to 75ug% after 3 days of zinc therapy. In yet another case report, zinc sulphate was used in a dosage of 5mg/kg/day. There was rapid improvement of diarrhoea within 24 hours and the skin lesions within 1 to 2 weeks.

This case emphasizes the need for early diagnosis and prompt treatment required to reverse the condition, reduce mortality and prevent the long term consequences of zinc deficiency.

REFERENCES:
UNUSUAL PRESENTATION OF RHINOSPORIDIOSIS - A CASE REPORT
Anupma Jyoti Kindo a, J. Kalyani a, Sandhya Sundaram b, Senthil Kannan M c, A. Ravi Kumar c

ABSTRACT
Rhinosporidiosis is a chronic infestation by the fungus Rhinosporidium seeberi, which predominantly affects the mucus membranes of the nose and nasopharynx. We report a case of rhinosporidiosis with presentation as a mass extending up to the nasopharynx.

Key words: Rhinosporidiosis, nasopharynx, case reports.

INTRODUCTION
Rhinosporidium is a cosmopolitan disease of man and domestic animals endemic in India and Srilanka and is hyperendemic in southern districts of Tamil Nadu.

It usually presents as a polypoidal growth in nasal cavity that involves anterior part of nasal septum and nasal vestibule[1].

The aim of this paper is to report an interesting unusual case of rhinosporidiosis.

Case report: A 38 year old male Assistant Engineer by profession hailing from Ennore, Northern part of Chennai came to the OPD of the ENT Department with complaints of foreign body sensation in the throat for past 8 years. Patient was able to feel the sensation whenever he had an episode of upper respiratory tract infection associated with unproductive cough. He complained of recurrent episodes of nasal obstruction. He would develop throat irritation and cough whenever he tried to feel the mass.

There was no complaint of otalgia, no history of epistaxis and trauma, No medical procedure done in the past.

Local examination showed a pinkish pedunculated mass measuring 3x3 cm hanging from the nasopharynx extending upto the level of the posterior 1/3rd of the tongue along the posterior pharyngeal wall. It was non pulsating or expansile, did not bleed on touch.

Posterior nasal examination showed bilaterally patent eustachian tube, the right choana was filled with a pinkish material, left choana was free.

An endoscopic excision of the nasopharyngeal mass was done and the tissue was sent to the Microbiology and Pathology Laboratory.

Macroscopic and Microscopic findings
The specimen on gross examination showed a soft pink mass with white spots on the surface.

Fig 1. The KOH mount showing numerous sporangiospores with occasional Sporangium (Magnification 400X)

Haematoxylin and eosin staining of tissue sections showed polypoidal fragments lined by stratified squamous epithelium. The subepithelium showed many globular cysts. Each of these cysts represented a thick walled sporangium containing numerous “daughter spores” in different stages of development (fig2). The fibroconnective stroma showed fibroblasts and myofibroblasts and an inflammatory infiltrate consisting of polymorphs and eosinophils. (fig 3). These changes were characteristic of rhinosporidiosis.

Fig 2. Tissue sections stained with Haematoxylin and eosin stain showing “daughter spores” in different stages of development (Magnification 400X)
Fig 3. Tissue staining with haematoxylin and eosin showing characteristic features of rhinosporidiosis: the polypoid fibroconnective stroma with sporangiospores. Surrounded by dense inflammatory infiltrate consisting of polymorphs and eosinophils (Magnification 200X)

Functional endoscopic sinus surgery was performed and the patient was discharged on the second day post operatively.

Discussion

Other than India and Sri Lanka where this disease is endemic, it has been recognized in many other parts of the world, like South America, United states, England, Egypt and South Africa [2].

Mode of transmission is through water or dust, from which the endospores penetrate the nasal cavity mucosa, mature into sporangium within the submucosal compartment and after maturation burst with release of sporangia into surrounding tissue [2]. Clinically, the lesion presents as a polypoid, soft mass, sometimes pedunculated, of the nose (primary site of infection), the eye and its adnexa, above all conjunctiva, or the urethra. Larynx, trachea, skin and lung are less frequently involved. Osteolytic bone infiltration is another clinical presentation [3]. Histologically the infected tissue reveals granulomatous reaction (mixed cell granuloma), pseudocystic abscesses and fibrosis around the causative organism [3].

Surgical excision is the mainstay of treatment. It has been advocated that a wide surgical margin is necessary to reduce risk of recurrence [4].

Rhinosporidiosis is a condition which both clinicians and laboratory personnel should keep in mind when managing patients from endemic places. Moreover, it is very interesting in such cases to follow the clinical course: An eventual recurrence of the lesion in our patient would mean a true relapse excluding the possibility of a reinfection, more probable in the endemic areas.

REFERENCE:
A CASE OF REACTIVE THROMBOCYTOSIS

Sudhakar M K a, Senthil N a, Mohini Singh a, Hissham a

ABSTRACT

Thrombocytosis is a laboratory abnormality which may be encountered on routine evaluation of an unrelated medical problem. The degree of elevation of platelet count was once considered to be an indicator whether the thrombocytosis was reactive or due to a primary hematological disorder. But, current evidence seems to indicate that the degree of elevation cannot be used as a criteria to differentiate between the two. We report a case of reactive thrombocytosis with unusually high platelet counts, who did not have evidence of a primary hematological problem and was identified to have two co-existing pathologies which led to the elevated platelet count.

Key words: thrombocytosis, empyema, anemia, case reports.

INTRODUCTION

Thrombocytosis is quite often discovered as an incidental finding during the laboratory evaluation of an unrelated medical problem. However, when it is detected, it constitutes an important diagnostic challenge. Thrombocytosis can either be due to a reactive process (secondary thrombocytosis) or it can be due to a clonal bone marrow disorder (primary thrombocytosis or clonal thrombocytosis). It is often exceedingly difficult to differentiate between the reactive and clonal types of thrombocytosis on the basis of clinical findings or laboratory test results. Yet there are fundamental differences between them in terms of cause, pathophysiological features, and clinical implications. Here we report a case of reactive thrombocytosis with an unusually high platelet count.

CASE REPORT:

A 26 yr old lady presented to us with history of fever, cough, chest pain, breathlessness of one week duration along with anorexia and weight loss of one month. Patient did not have any other symptoms. General survey revealed that she was conscious, oriented and had a temperature of 102 degree Fahrenheit. Respiratory rate was 33/min, BP was 130/80 mmHg, PR was 116/min she was pale and had no significant lymphadenopathy. Respiratory system revealed diminished breath sound in the right hemithorax with severe intercostal space tenderness. Cardiovascular, Abdomen and neurological system examination were normal.

Chest X ray taken revealed a moderate right sided pleural effusion (Fig 1).

USG guided aspiration revealed the fluid to be frank pus and an ICD was inserted subsequently. Investigations showed TLC of 18,600 (P-88, L-10, M-2), haemoglobin 5.4 gms%, platelets 22.9 lakhs/cu mm, ESR >150mm/hr.RFT, electrolytes, LFT and urine examination were normal. USG abdomen – normal study. Pleural fluid showed WBC-11000, RBC-6000 and cultures grew Staphylococcus aureus. Manual platelet counting done revealed it to be 21.6 lakhs/cu mm. Peripheral smear showed evidence of microcytic, hypochromic anemia along with an MCV of 58. Bone marrow aspiration and biopsy done (in view of very high platelet counts) showed a reactive marrow and no evidence of a primary hematological problem.

A diagnosis of empyema with iron deficiency anemia was made along with reactive thrombocytosis. She was treated with IV cefaperazone and sulbactum, iron supplements, blood transfusions and other supportive measures. Serial monitoring of blood indices revealed a steady fall in the platelet count as the infection and anemia improved, thereby underlining the cause of the severe thrombocytosis as a combination of severe iron deficiency anemia along with a severe purulent infection in the form of an empyema. Serial blood indices are given in Fig 2 A and Fig 2 B.
DISCUSSION:

The most striking feature of this case is the gross elevation in the platelet count. The degree of elevation of platelet count in this patient was such that further work up for a primary hematological problem was warranted. However, further investigations in that direction did not reveal any evidence of a primary hematological disorder. This patient had a severe infection (empyema) and severe iron deficiency anemia which are well established causes of reactive thrombocytosis. The combination of these two illnesses may well have caused a dramatic increase in platelet count.

The cause of reactive thrombocytosis has been ascribed to cytokine mediated increased synthesis of thrombopoietin, which in turn leads to increased synthesis of thrombocytes. The various causes of reactive thrombocytosis are given in Table 1.

We report this case to highlight the following current concepts in reactive thrombocytosis: Firstly, the degree of elevation of platelet count is not an indicator whether the thrombocytosis is primary or secondary. Secondly, the management of secondary thrombocytosis is purely directed towards the inciting cause which in our patient was due to iron deficiency anemia and empyema.

ACKNOWLEDGEMENT:

1. Department of Pathology, SRMC, Chennai.
2. Department of Cardiothoracic Surgery, SRMC, Chennai.

REFERENCES:

ERRATUM

The following corrections are made for the article “Prevalence of extended spectrum β-lactam resistance among bacteria causing hospital infections - detection using PCR”

TYPOGRAPHICAL ERRORS:
1. “bla” genes to read as “blaTEM” and “blashv”
2. All names of microorganisms to have appeared in italics. The typographical errors in this article are regretted.
3. The pictures with the following legends are published in this issue
4. Page 14, Para 3, Line 16 references to read as “8, 9 and 11” and line 20, references to read as “6, 12”.

The above corrections are incorporated in the article and posted in website www.srmc.edu

FIG. 1: Presence of a plasmid(s) seen in drug resistant clinical isolates of E. coli (EC) seen in panel (a)- lanes 2, 6, 10: SRMC 26; lanes 4, 8- SRMC 3; and in panel (b)- lanes 4, 5, 6 – R1759, U8688 & E4159. Plasmid(s) of Klebsiella (Kl) are shown in panel (a)- lanes 3, 7 - SRMC 20; lane 5, 9 - ATCC 70603 (with plasmid); and in panel (b)- lane 2, 3 & 7 - R1738; R1756 & E4146. The lane 1 in both panels contained DNA/HindIII digest. Note the presence of more than one plasmid in some isolates. EC

FIG. 2: A comparison of the sizes of the PCR products on a 1.3% gel from both genes, blashv and blatem indicating that they are of expected lengths. The lanes were loaded with lane 1- 100bp ladder; lane 2- (TEM +ve; 1.2 kb); lane 3- (SHV +ve; 900bp); lane 4 - negative control. EC- E. coli; Kl- Klebsiella.
FIG. 3: Detection and identification of bla$_{SHV}$ genes by PCR was seen in isolates of E. coli (EC)-lane 8 (panel a) and lane 4 (panel b). Isolates of Klebsiella (Kl) that were positive can be seen in lane 9 (panel a); lane 3, 6, 7 (panel b). Lane 1- Lambda DNA/Hind III digest.

FIG. 4: Detection and identification of bla$_{TEM}$ genes by PCR seen in panel (a) of isolates of E. coli (EC)- lane 3, 4 and in isolates of Klebsiella (Kl)- lane 5, 6. A positive E. coli sample (SRMC - 26) showed a ~1.2 Kb product (panel b). Lane 1: Lambda DNA/Hind III digest (panel a) or a 1.0 Kb ladder (panel b).
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