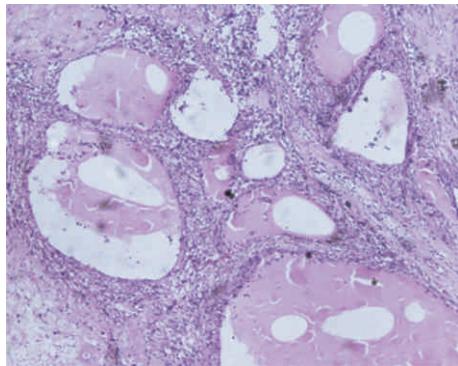


# SRI RAMACHANDRA JOURNAL OF MEDICINE

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## **From the Editor's Desk**

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Dear Readers,

I am happy to bring out this issue of the Sri Ramachandra Journal of Medicine. I would like to place on record our appreciation to the Contributing Authors, Peer Reviewers and the Editorial Board Members.

This issue contains 4 original articles from different specialities, one review article from the Faculty of Dental Sciences and 3 case reports.

I make an earnest appeal to contribute more towards our University Journal.

**P.V. VIJAYARAGHAVAN**

EDITOR

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## CONJUNCTIVAL FLORA IN DIABETICS & NORMAL POPULATION - A COMPARATIVE STUDY

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### ABSTRACT

**Background and objectives:** To compare the normal conjunctival flora of non diabetics individuals with that of diabetics; to isolate and identify the organisms from the conjunctival flora; to study the antibiotic sensitivity pattern of the organisms.

**Methods:** The study group consisted of 50 patients each with diabetes and non diabetics attending the ophthalmology Out Patient Department of Sri Ramachandra University. The conjunctival swabs were collected from both the eyes and sent to microbiology lab for smear and culture. Antibiotic sensitivity test was done using Kirby Baeur's method.

**Results:** The age varied from 17-85 years 49 were males and 51 were females. The total positive cultures were 148 from 100 patients (diabetic-72 and non diabetics-74) and 52 were negative for culture. The most common organism isolated was ***Staphylococcus aureus*** in both

### INTRODUCTION

Normal microbial flora of eye<sup>[1]</sup> constitutes organisms which are present on eyelids and conjunctiva without causing any disease. These organisms are generally considered to be saprophytic but have the potential to become pathogenic when the normal defense mechanisms falter.<sup>[2]</sup>

At birth the eyes are sterile. Soon, the conjunctival sac is invaded by various organisms which then persist throughout life. The bacterial flora of the two eyes are strikingly similar. The source of conjunctival sac flora is usually the eyelid. Lactoferrin, lysozyme and secretory IgA which are present in the tears play an important role in maintaining the normal flora.<sup>[3]</sup>

There are two types of ocular flora - Resident and Transient. Resident ocular flora are permanent flora which represent the colonization and repeated cultures usually reveal the same organisms in large numbers. This includes *Staphylococcus epidermidis*, *Diphtheroids*, *Staphylococcus aureus*, *Lactobacillus*

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groups followed by *Micrococci*. *Diphtheroids* were more commonly isolated in the diabetic patients (18%). The gram negative bacilli ***Enterobacter Species*** (69.9%) being the most common in the diabetic group. We did not come across any fungal isolates.

**Conclusions:** The most common isolate was *Staphylococcus aureus* in both the groups. *Diphtheroids* and ***Enterobacter*** species were more common among diabetics. ***Staphylococcus aureus*** showed maximum resistance to Erythromycin and Ampicillin. Conjunctival flora can become a pathogen in diabetic patients if they develop any injury to the eye. Over usage of antibiotics should be restricted to prevent resistance among the bacteria.

**Key words:** Antibiotic sensitivity, bacterial flora, diabetics, conjunctival flora.

SRJM 2014;7:1-4

species and *Propionibacterium* species (anaerobic diphtheroid).

Transient ocular flora inhabit the eye for short periods and cannot be consistently recovered in consecutive cultures. They are influenced by a number of factors, examples of which are as follows:

Increased recovery of fungi from the conjunctival sac of people living in rural areas.

Increased recovery of *Pseudomonas* from the hospitalized patients.

Increased recovery of *Streptococcus*, *Pneumococcus* and *Haemophilus* in children.

Also in patients with certain ocular disease, there may be ocular flora different from that normally seen. Patients with keratoconjunctivitis sicca tend to harbour *Proteus* and *Staphylococcus aureus*. Topical and systemic immunosuppressive therapy and immunosuppressive diseases like diabetes mellitus also affects the normal flora. Studies have revealed an increase in fungi recovered from eyes on topical steroid therapy and also an increase in the incidence of bacterial lid and conjunctival infections.<sup>[4]</sup> The transient ocular flora include *Staphylococcus* species other than *Staphylococcus aureus*, *Bacillus* species, *Haemophilus* species, *E.coli*, *Klebsiella* species, *Enterobacter* species, Anaerobes: *Peptococcus*, *Bacteroides*, *Clostridium*, *Actinobacterium*,

*Eubacterium* and fungi which includes *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Candida*, *Helminthosporium*, *Rohodotorula* and *Fusarium* species.

### AIM OF THE STUDY

- To isolate and identify the organisms from the conjunctival flora of normal and diabetic population.
- To compare the conjunctival flora of normal and diabetic population.
- To study the antibiotic sensitivity pattern of the organisms.

### INCLUSION CRITERIA

- Diabetic patients without any pre-existing ocular diseases.

### EXCLUSION CRITERIA

- Patients with pre-existing ocular diseases
- Patients with any ocular surface disorders, intensive care unit patients, immunocompromised.

### MATERIALS AND METHODS

The study group consisted of 50 patients each of with diabetes and non diabetics attending the ophthalmology outpatient department of Sri Ramachandra University. After obtaining the informed consent the conjunctival swabs with two moistened sterile cotton tipped applicators without touching eyelid margins or lashes were collected from both eyes of patients separately and to sent for microbiology, one for culture and other for microscopy.<sup>[5]</sup> The swabs were inoculated in thioglycollate broth over night and gram staining was done from the broth and also cultured in blood agar and MacConkey's agar.

The organisms were identified by standard microbiological procedures

- Antibiotic sensitivity test was done using Kirby Bauer's method

### Antibiotic susceptibility tests:

Antibiotic susceptibility testing was done on Mueller-Hinton agar by Kirby Bauer diffusion method using Clinical Laboratory Standards Institute (CLSI) guidelines. It allows the antibiotic to diffuse into the medium. If the organism is susceptible to particular antibiotic, growth of the organism is inhibited around the disc. This is called zone of inhibition. When there is no inhibition, the organism is said to be resistant. The zone diameter was interpreted according to the CLSI chart. The zone diameter was measured in millimeter (mm). The results were interpreted as sensitive, resistant and intermediate sensitive according to CLSI chart.

### RESULTS

A total of 100 patients were screened to study the conjunctival flora in diabetics and non diabetic individuals. Study group consisted of 50 diabetic patients and 50 non diabetics. The age varied from 17-85 years 49 were males and 51 were females.

Culture positives isolated from both eyes in the same individual were not necessarily the same. The total positive cultures were 148 from 100 patients (diabetic-72 and non diabetics-74) and 20 were negative for culture. The distribution of the bacterial isolates has been shown below (Chart 1 & 2). No influence of sex was noted in the colonization of bacteria. The study group did not show any significant difference with

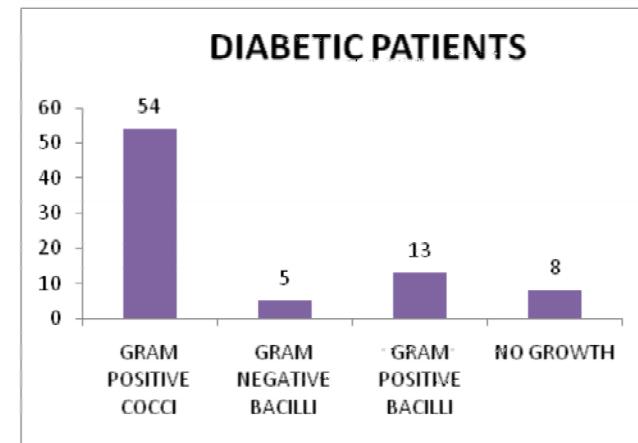


Chart-1 : Distribution of Bacterial Isolated in Diabetic patients.

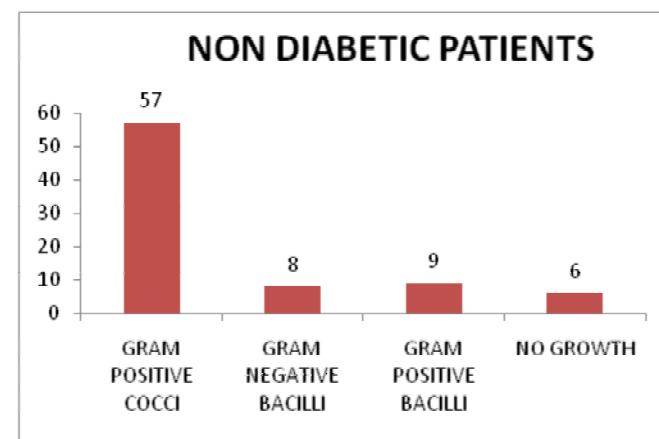
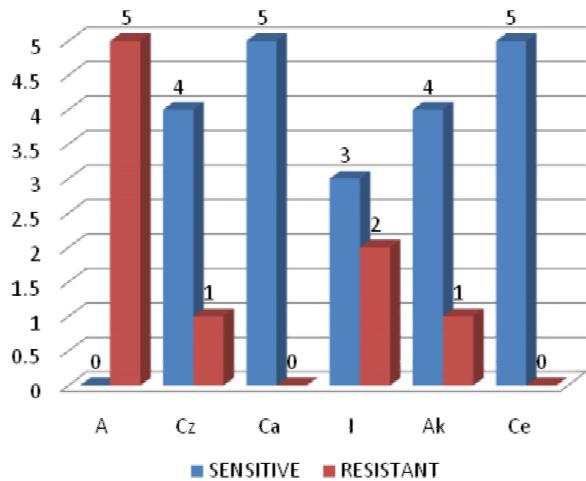


Chart-2 : Distribution of Bacterial Isolated in non-diabetic patients

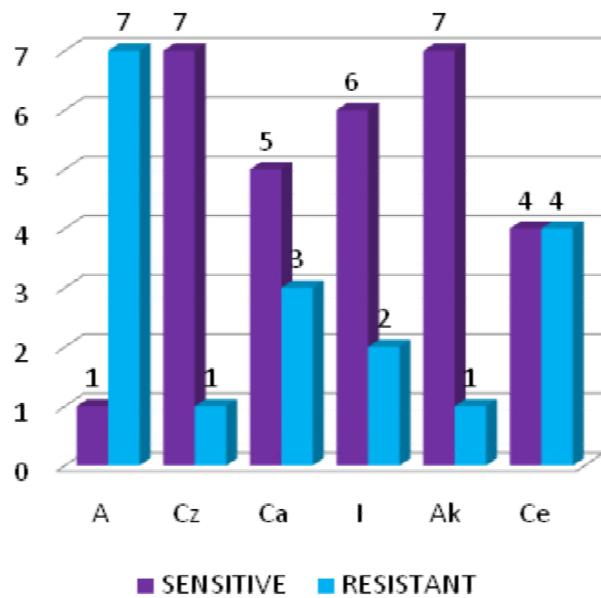
regard to the conjunctival flora. The most common organism isolated was *Staphylococcus aureus* in the diabetic(40.2%) as well as in the non diabetic group(58.1%). Micrococci was the second most frequent organism isolated in both the Groups (diabetic-27.7% and non diabetic-16.2%). Diphtheroids were more commonly isolated in the diabetic patients (18%). The gram negative bacilli were very few in number,

*Enterobacter* Sp (69.9%) being the most common in the diabetic group. We did not come across any fungal isolates.

In both the groups, sensitivity pattern to the various antibiotics were almost similar (Chart 3,4).



**Chart-3 :** Antibiotic sensitivity testing in diabetic patients



**Chart-4** Antibiotic sensitivity testing in non-diabetic patients

Among the gram positive cocci such as *Staphylococci* maximum resistance was seen with Erythromycin (38%) among the diabetic group. Maximum resistance was seen with Ampicillin in the non diabetic group (30%). Among the gram negative bacilli such as *pseudomonas*, maximum resistance was seen with Ampicillin in both the groups (56%). Whereas *Klebsiella* is intrinsically resistant to Ampicillin.

## DISCUSSION

A total of 100 patients were screened to study the conjunctival flora in the diabetics and non diabetics

group in Sri Ramachandra Hospital Ophthalmology OPD.

Our study showed *Staphylococcus aureu*<sup>[6]</sup> to be the most common isolate in both the diabetic and non diabetic group. J.A Capriotti, et al (2009) and Martins, et al (2004) found CONS to be more common among the diabetics.<sup>[7,8,9,10,11]</sup> Similarly Davood Aghadoost, et al (2007) also found CONS to be the most common organism constituting the normal flora of the conjunctiva.<sup>[8,9,10,11,12]</sup>

The second most common organism isolated in the diabetic group was diphtheroids. Habib Bilen, et al (2007) also showed diphtheroids to be the next most predominant organism after *Staphylococcus* Sp in their study. Gram negative formed a very small group (6.9%) among the diabetic and (12.1%) in the non diabetics. Our sample size of the patient is very small. Studies with greater numbers have to be done to get the actual picture of gram negative organism and its relation to normal flora.

Study by J.A. Capriotti, et al (2009) showed 9.8% of gram negative bacteria among the healthy individuals in the rural population<sup>[7]</sup> whereas our study showed 11% among the non diabetics.

The sensitivity pattern of *Staphylococci* is being reported here not only because *Staphylococci* constitute the bulk of the organisms isolated in this study but also because these organisms develop resistance to various antibiotics rapidly. *Staphylococcus aureus* showed maximum resistance to Erythromycin (27%) in the non diabetics group, whereas in the diabetic group it was 38%.

Resistance of 26% to Ampicillin by *Staphylococcus aureus* in the diabetic group and 30% in the non diabetic group was seen in our study. Whereas the study done by Tiago Eugenio, et al (2006) showed no resistance to Penicillin group of drugs in Brazil.<sup>[4]</sup> This could be because of the over use of antibiotic.

There are few studies in India that document the need for many such surveys. The present study was planned to determine the normal bacterial and fungal flora of the eye in both sexes at all ages. However, during the study period no fungi were isolated.

There was not much difference in the frequency of isolation of organisms in the diabetic and non diabetic group. The most common isolate was *Staphylococcus aureus* in both the groups. Diphtheroids were more common among the diabetics as compared to non diabetics. *Enterobacter* Sp was the most common isolate among the gram negative organism among the diabetics. There was no significantly predominant gram negative organism isolated from the non diabetic group.

*Staphylococcus aureus* showed maximum resistance to Erythromycin followed by Ampicillin, whereas gram negative organisms such as pseudomonas was resistant to Ampicillin. Conjunctival flora can become a pathogen in diabetic patients if they develop any injury to the eye. Over usage of antibiotics should be restricted to prevent resistance among the bacteria.

## CONCLUSION

In our study there was not much of difference in the frequency of isolation of organisms in the diabetic and non diabetic group.

The most common isolate in our study was *Staphylococcus aureus* in both the groups.

Diphtheroids were more common among the diabetics as compared to non diabetics.

*Enterobacter Sp* was the most common isolate among the gram negative organisms among the diabetics.

There was no significantly predominant gram negative organism isolated from the non diabetic group. No fungal isolate were recovered from the specimens.

*Staphylococcus aureus* showed maximum resistance to Erythromycin followed by Ampicillin, gram negatives such as pseudomonas showed maximum resistance to Ampicillin.

Conjunctival flora can become a pathogen in diabetic patients following any injury to the eye. Over usage of antibiotics should be restricted to prevent resistance among the bacteria.

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# COMPREHENSIVE SRMC SCORING SYSTEM FOR SURGICALLY TREATED TENDO ACHILLES RUPTURE

**Jambu Nageswaran<sup>a\*</sup>, Ganesan G Ram<sup>a</sup>, S.Ramesh Babu<sup>a</sup>, B.Samuel Chittaranjan<sup>a</sup>**

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## ABSTRACT

**Background:** Achilles tendon rupture is a common injury and accounts 18 per 1,00,000 people. The management of Achilles tendon repair is debatable with literature supporting both nonsurgical and surgical management. Functional outcome of surgically treated tendo achilles were assessed by various scoring systems like Boyden clinical outcome score, Leppilhati score, Ankle hind foot scale developed by American foot society and others. All these scoring systems have merits and demerits. After evaluating all the demerits of the available scoring system a new scoring system Comprehensive SRMC scoring system was evolved.

**Materials and Method:** Study was done in Sri Ramachandra Medical College and Research Institute,

Chennai, from June 2010 to Jan 2014. Thirty patients who were surgically managed for Tendo Achilles rupture were included in the study. A comprehensive scoring system has been developed, Comprehensive SRMC scoring system and all patients were followed using the scoring system.

**Results:** As per our scoring system eighty four percent of patient surgically treated had good results.

**Conclusion:** Surgical management of tendo Achilles gives good and reliable results. As comprehensive SRMC scoring is specific for Tendo Achilles it can be widely used.

**Keywords:** Achilles tendon, Equinus, Gait, Tendon Strength

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## INTRODUCTION

Achilles Tendon is the strongest and thickest tendon in the body. It is almost 15 cm long. Surgical repair and reconstruction are the common ways of treating ruptured Achilles Tendon. In order to assess the surgical treatment, we have evolved our own new scoring system "Comprehensive SRMC Scoring System".

## MATERIALS AND METHOD

Study was done in Sri Ramachandra Medical College and Research Institute, Chennai, from June 2010 to Jan 2014. Thirty patients who were surgically managed for Tendo Achilles rupture were included in the study. The study group had twenty-eight male patients and two female patients. Ten patients had closed injury while others had open injury. Closet injuries were found in eleven patients and rests of the open injuries were due to road traffic accident. There were five patients who had spontaneous rupture. Mean age of open injury patients was twenty-seven while closed was forty-seven years.

Two of the spontaneous rupture patient were having uncontrolled diabetes while one had rheumatoid arthritis. Two patients had recent steroid injection to

back of heel for chronic pain. As far as mechanism of injury was concerned majority of the patients had forcible or sudden dorsiflexion of the foot. One of the patients in the open tendo Achilles rupture had assault using knife.

Emergency wound debridement and primary suturing was done in all open Tendo Achilles rupture. All the open ruptures underwent surgery within eight hours of reaching the hospital. Regional block was given in the causality itself and wound wash was given with atleast 3 liters of saline and sterile dressing done. The time elapsed after injury was noted. Patients were started with third generation cephalosporin's, intravenous Garamycin and Metrogyl after taking cultures in causality. Fourteen cases of open injuries were taken within 6 hours to the OT and after thorough debridement; modified Kessler's method<sup>[1]</sup> end-to-end suturing was done.

We had twenty patients of complete rupture and five case of partial rupture. After intra operative wound debridement and assessment, depending upon surgeons choice various suturing technique of tendons like Modified Kessler's stitch, bunell's Stitch and Krakow stitch were used to approximate the ruptured tendons. Bosworth's technique<sup>[2]</sup> was used to approximate five cases of spontaneous rupture and Lindholm procedure<sup>[3]</sup> was done in two patients for whom end to end approximation was not possible.

All the patients had above knee Plaster of Paris (POP) cast post-operatively atleast for two weeks. POP was given with foot in full plantar flexion and knee in

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30 degree of flexion. A window was created as per the sutured wound size for facilitating regular wound dressing. Patients were mobilized from second postoperative day non-weight bearing with walker support. During third postoperative week the above knee pop cast was converted into below knee POP with foot in maximum equinus for two more weeks. At the end of fifth post operative week walking POP was given

and patients were made to walk full weight bearing. The period of postoperative regimen varies between each individual and none of the patients had POP more than three months. The rehabilitation progresses slowly into strengthening, gait and balancing activities.

All the patients had regular periodic follow up. The minimum follow up in our study was one year and most of the patients were followed upto three years.

**Table-1: Comprehensive SRMC scoring system, which includes the following parameters**

QUALITY OF LIFE	Patient back to his/her occupation as preinjury status	10
	Patient was back to his occupation but not able to perform normally	5
	Change of occupation	0
WOUND HEALING	Healed in time without complication	10
	Wound dehiscence	8
	Superficial infection	6
	Deep infection	2
	Required skin/flap cover to heal	0
TENDON GEOMETRY	Normal	10
	Tenderness/Adherence to skin	5
	Rerupture	0
PAIN	No pain	10
	Pain on jogging or running	8
	Pain on walking and squatting	4
	Rest pain	0
TENDON STRENGTH BY APPARATUS	10 or more kg	10
	2.5 to 10 kg	5
	less than 5kg	0
STRENGTH	Hopping and jumping on single leg (operated)	10
	Ability to stand with single heel raise(operated)	8
	Ability to stand with both heel raise	6
	Ability to squat without support	4
	Ability to squat with support	2
GAIT	Walking without limp	10
	Walking with limp	0
RANGE OF MOVEMENTS	Normal range of movements	10
	Abnormal range of movements	8
	ROM restricted < 50%	6
	ROM restricted > 50%	2
ANKLE	Calf atrophy without influencing the result	10
	Calf atrophy with negative influence in result	5
PATIENT SATISFACTION	Satisfied	10
	Partially satisfied	5
	Unsatisfied	0
<b>TOTAL SCORE</b>		<b>100</b>

Scores between 75 to 100 = Good; Scores between 50 to 74 = Fair; Less than 50 = Poor

We have evolved our own scoring system and named it as Comprehensive SRMC scoring system and all patients were followed using the scoring system.

## ANALYSIS OF RESULTS

Comprehensive SRMC scoring system,<sup>[4]</sup> which includes the following parameters (Table-1)

- ◆ Quality of life
- ◆ Tendon strength by apparatus
- ◆ Wound healing
- ◆ Tendon geometry
- ◆ Pain
- ◆ Strength
- ◆ Gait
- ◆ Rom of ankle
- ◆ Calf atrophy
- ◆ Patient satisfaction

## RESULTS

As per Comprehensive SRMC scoring system, scoring between 75 to 100 were defined as good. We had nearly eighty-four percent good results. The results were tabulated in Table-2. We had complication like calf atrophy in five patients, sural nerve hypesthesia in two patients. We had no case of infection and deep vein thrombosis.

## DISCUSSION

Achilles tendon rupture is a common injury and accounts 18 per 1,00,000 people.<sup>[5]</sup> The management of Achilles tendon repair is debatable with literature supporting both nonsurgical and surgical management. Functional outcome of surgically treated

**Table-2:** Comprehensive SRMC scoring system

SRMC SCORING	Good	Fair	Poor
	25 (83.3%)	3 (10%)	2 (6.7%)

tendo achilles were assessed by various scoring systems like Boyden clinical outcome score,<sup>[6]</sup> Leppilhati Score,<sup>[7]</sup> Ankle hind foot scale developed by American foot society<sup>[8,9]</sup> and Tegner activity score.<sup>[10,11]</sup> All these scoring systems have merits and demerits. After evaluating all the demerits of the available scoring system we have evolved a new scoring system Comprehensive SRMC scoring system.

Ankle hind foot scale is not specific for Achilles tendon. It has components for assessment of pain, function and alignment. Forty points were given for assessment of pain, fifty points for function and ten points for alignment. The advantage with this scoring system is that it can be used for any ankle and foot condition. Disadvantages were lack of assessment for

quality of life and patients satisfaction. Leppilhati et al<sup>[7]</sup> scoring scale has components like pain, stiffness, calf muscle weakness, footwear modification, active range of movements difference between ankles, subjective results and isokinetic muscle strength. The disadvantage with Leppilhati scoring system is that it mainly depends on an expert opinion only.

Tegner<sup>[10,11]</sup> activity scoring system is a graduated list of activities of daily living, recreation, and competitive sports. The patient is asked to select the level of participation that best describes the current level of activity and that before injury. A score of 0 represents sick leave or disability pension, whereas a score of 10 corresponds to participation in national and international elite competitive sports and >6 score can only be achieved if the person participates in recreational or competitive sport. The Tegner activity-scoring disadvantage is that it is not specific for tendo Achilles or foot as it was originally intended and developed for patients with ACL (Anterior Cruciate Ligament) injury.

It is important for the development of clinical practice and research that practical and appropriate patient-reported outcome measures are universally accepted.<sup>[12]</sup> This will allow comparisons and meta-analysis of high-quality randomized controlled trials possible into this ever-increasing injury. So it is mandatory to develop a new scoring system without any flaws. Hence we have evolved our own Comprehensive SRMC scoring system, which includes all necessary parameters. The male: female ratio in our study group was 9:1. Almost all the literature for Achilles tendon rupture had a male predominance. Our study can be comparable to the study of Nicola maffulli<sup>[13]</sup> who had a similar male: female ratio. Mean age of patients who had open Tendo Achilles rupture was 28 years while closed rupture patient was 47 years. A.G.Jennings et al<sup>[14]</sup> study population had a similar age interval like our study.

We had around 47% of closet mode type of injuries. The closet injuries were the commonest injuries in our series. While western literature had sports injuries as the commonest Achilles tendo injury, Nicola Maffuli et al have reported around 83% of sports related injuries as the commonest injury. Forty percentage of Achilles tendon rupture in our series were neglected while Nicola had only 20% of neglected rupture. The poor results in our series were due to the late presentation of open injuries. End to end repair by modified Kesslers method gave good results in our series.

Surgical Treatment of acute rupture of TendoAchilles led to fewer reruptures and better

patient generated ratings than did non-surgical treatment.<sup>[15]</sup> The period of postoperative regimen varies between each individual and none of the patients had pop more than three months. Mean immobilization period in open Achilles tendon rupture was 12 weeks while closed was 10 weeks. The rehabilitation progresses slowly into strengthening, gait and balancing activities. Early restricted motion with a brace instead of rigid immobilization reduced the incidence of scar adhesions and early return to normal activities.<sup>[16]</sup>

None of the patients in our series had deep vein thrombosis or rerupture while Arner and Lind Holm<sup>[17]</sup> reported 4 cases of re rupture and 2 cases of thrombosis as complications in a series of 86 patients. Five of our patients had calf atrophy, which gradually improved at the end of one year. When the leg is immobilized the muscle spindle relaxes and afferent impulses to type-1 fibers cease causing the calf muscle atrophy.

## CONCLUSION

Surgical management of tendo Achilles gives good and reliable results. Since Comprehensive SRMC scoring system was evolved after assessing the demerits of other available scoring system it has all the mandatory parameters. As comprehensive SRMC scoring is specific for Tendo Achilles it can be widely used.

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# STUDENT PERCEPTION ON STUDY GUIDES IN AN INTEGRATED PRECLINICAL CURRICULUM

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## ABSTRACT

**Objective:** This study aimed to evaluate the perception of preclinical students towards the usefulness of structured study guides in facilitating a guided discovery learning approach in an integrated curriculum.

**Methods:** Preclinical students experienced two integrated basic science modules on muscle and blood. The modules had a combination of student centric methods such as study guides, simulated case studies along with traditional lectures and practical demonstrations. A validated questionnaire seeking student perceptions on the value of study guides in aiding prior learning preparation was obtained. The questionnaire sought perceptions on the time of provision of study guides, the planning of integration within the topics, statement of learning objectives, usefulness of references and motivation towards self directed learning. 218 students' had responded to the

questionnaire. The responses were obtained in a 5 point LIKERT Scale. The results were expressed as percentages.

**Results:** More than 90% of students had opined that study guides provided timely prior preparation, reflected proper integration of topics and had appropriate learning objectives. A lesser response [= 80%] for study guides in motivating self directed learning, suggest that guidance is required in the preclinical years.

**Conclusion:** These findings suggest that study guides along with traditional teaching methods integrated with a simulated case scenario, facilitate contextual study of basic sciences, leading to better learning outcomes.

**Key words:** study guide, integrated curriculum, guided discovery learning

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## INTRODUCTION

Medical education requires the ability to gather vast information, contextual application through critical reasoning and a commitment to lifelong learning. A new entrant to a medical school is faced with the challenge of shifting learning behavior from an instructor dependant, teacher-centric method to student centric methods. This problem is more felt in developing nations such as India where a school teacher is generally regarded as a "sage on stage".<sup>[1]</sup> Therefore the preclinical student requires guidance to achieve satisfactory learning, by timely and appropriate combination of instructor dependant and self directed learning methods.

Guided discovery learning is based upon the discovery learning model proposed by Jerome Bruner an American psychologist. The principles of discovery learning were given as 1. Problem solving, 2. Learner Management, 3. Integrating and Connecting, 4.

Information Analysis and Interpretation, 5. Learning from failure and feedback from instructors.<sup>[2,3]</sup>

Guided discovery learning is a mix of didactic instruction with student participatory, task-based approaches.<sup>[4]</sup> Main components include (1) developing a framework for student learning, (2) making students responsible for deeper rather than surface learning (3) provision of study guides, and (4) application to clinical or experimental problems.<sup>[5]</sup>

Though strategies like problem-based learning [PBL], simulation-based learning and case-based learning, terms appear similar to guided discovery learning but are not exactly identical.<sup>[6]</sup> PBL is an open inquiry method requiring students to explore tangents and set their own learning objectives.<sup>[7]</sup> This is often not suitable for students from developing nations like India, where students enter medicine without any premedical course.<sup>[8,9]</sup> Classical problem based approaches use minimal guidance and require the learner to be equipped with vast prior knowledge.<sup>[10]</sup> Hence guided discovery approaches which combine traditional teaching methods with problem or case based studies are gaining better acceptance. This mixed economy approach can be further strengthened by study guides. Similar findings have been reported from a medical school in the middle east which had been

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following an integrated organ system block based curriculum with more emphasis on problem based learning. The students who had been accustomed to receiving instructions from tutors, found the study guide to aid day to day learning and further motivate active learning.<sup>[11]</sup>

A study guide is a comprehensive document which specifies the overall course framework, individual learning objectives and learning resources. It is particularly valuable in planning and executing an integrated curriculum.<sup>[12]</sup>

In this study, first year medical students went through an integrated learning program in basic sciences, where learning was guided by multiple strategies such as study guides, simulated case scenarios, traditional lectures and small group discussions. Our study is aimed to seek students opinion on the role of study guides towards motivating self directed learning for an integrated understanding of basic sciences. As per Kirkpatrick's levels of evaluation, assessing the students reaction is indispensable to justify the intended curricular reform.<sup>[13]</sup>

## METHODOLOGY

The study was carried out among first year medical students of Sri Ramachandra Medical College & Research Institute [SRMC & RI], Chennai, India. SRMC & RI had been following a hybrid curriculum in the preclinical phase.

The salient features of this curriculum were :

1. Organization of all basic sciences as integrated organ system blocks,
2. Helping prior preparation and guiding knowledge acquisition through study guides,
3. Interactive lectures,
4. A simulated case scenario as anchor and link to all the sessions,
5. Relevant hospital and lab visits and
6. Small group discussions.

The study guide included the following contents:

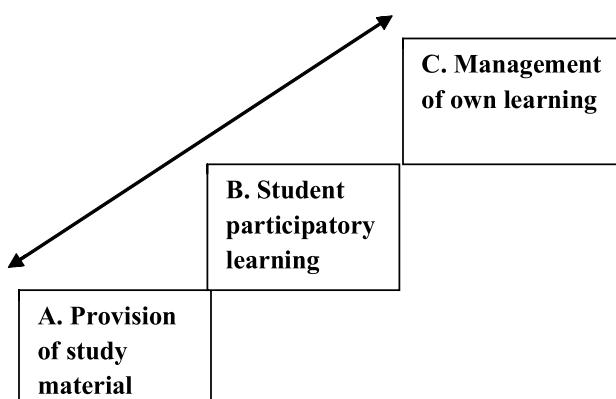
1. The overall objectives of the entire system.
2. The schedule of the sessions.
3. Specific learning objectives of the individual sessions.
4. A simulated case scenario, purposely designed to connect clinical findings with relevance to Anatomy, Physiology and Biochemistry
5. References from textbooks and web links for individual sessions.

A student questionnaire seeking their perceptions on the curricular reform was developed. The questionnaire was structured as 3 parts. The first part had questions about study materials provided for prior student preparation. The second part sought feedback on the actual conduct of the various teaching learning methods. The third part of the questionnaire sought opinions on small group discussion conducted at the end of the study. The 3 parts of the questionnaire showed good correlation [ $p < 0.01$ ] indicating good validity. Reliability of the questionnaire was also good with Cronbach's alpha 0.74. The internal consistency was checked by administering the questionnaire to 10 faculty.

After obtaining permission from the institutional ethics committee, MBBS students admitted into the 1<sup>st</sup> year where appraised of the curricular reforms such as integration of basic sciences and a relevant case study. The items in the questionnaire were explained & informed consent on their willingness to give their perceptions during the end of the block was obtained. After 3 weeks of study on muscle and other tissues, with Duchenne's muscular dystrophy as the case and another 4 weeks study on blood using a case report of aplastic anemia, questionnaire was administered. 218 students responded to the questionnaire. Analysis of the quantitative and qualitative data was done using appropriate statistical methods.

## RESULTS

218 students of the preclinical phase had responded to the questionnaire. The responses were obtained in a 5 point LIKERT Scale. The analysis was done in 5 headings strongly agree, agree, do not know, disagree and strongly disagree. The results were expressed as percentages (Table-1).



**Fig 1:** The study guide process

## DISCUSSION

An analysis of the students perception to the prior preparation of the block [refer table] more than [average 95%] had responded in the positive direction. They opined that the sufficient time was given to refer to the study guide, the learning objectives were clearly stated and the guide contained a proper teaching schedule reflecting integration of individual sessions. Defining learning objectives is the first step to ensure both for the instructor and learner to ensure that a curriculum is progressing towards intended outcomes.<sup>[14]</sup> A similar study guide template for an integrated cardiovascular module containing measurable objectives and clear

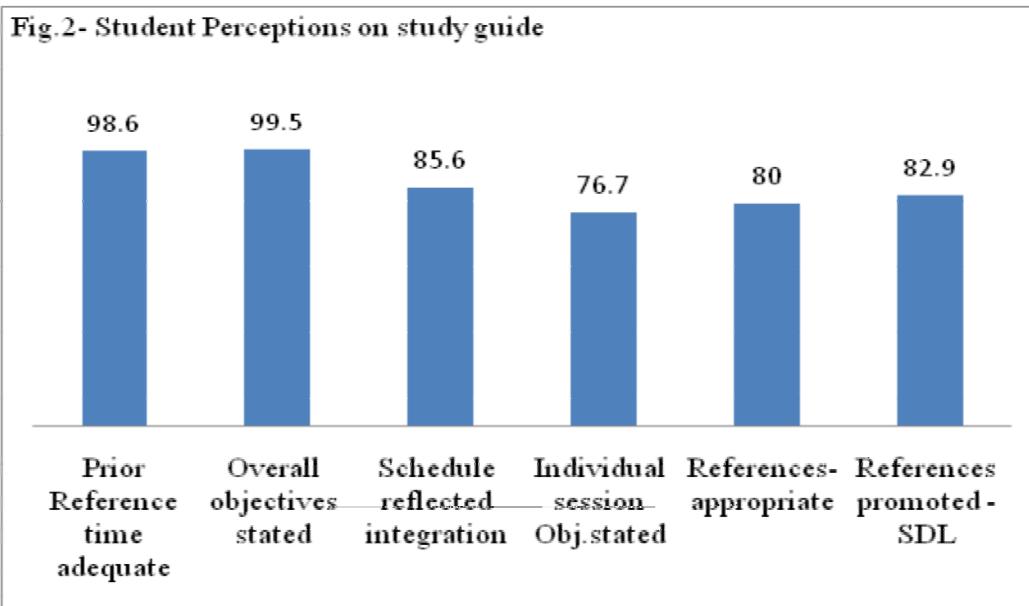
directions for study had been evaluated. The authors opined that it was beneficial for teachers and students in increasing productive time.<sup>[15]</sup>

The main focus of any study guide can be oriented towards any point in the figure (Fig.1) on the study guide process, similar to studies by Harden.R.M.<sup>[16]</sup> A represents a guide designed to just provide information, whereas B encourages student participation by suggesting learning resources and driving towards self directed learning. C is a guide of a more higher order where students' maintain records of learning activities in addition to self directed learning. The levels of learning are in an increasing order from A to C. These

**Table-1 : Students perception on Study Guide**

Sl. No	QUESTIONS	Strongly agree %	Agree %	Don't know %	Disagree %	Strongly disagree%
1.	The study guide was given for prior reference before the beginning of the module.	85.6	13	1.4	0	0
2.	The study guide was well structured & stated the overall objectives of the module.	90	9.5	0.5	0	0
3.	The teaching schedule reflected a proper planning, sequencing and integration of the individual sessions.	80.1	5.5	9.8	3	1.6
4.	The objectives of the individual sessions were well stated.	66.5	10.2	12.6	8.5	2.2
5.	The textbook, web- link references provided for the individual sessions were appropriate.	58	22	13	6	1
6.	The references in the study guide promoted self directed learning.	40	42.9	12.5	1	3.6

**Fig.2- Student Perceptions on study guide**



levels can also be correlated with the Millers pyramid of learning were point A represent 'knows' and B can indicate 'knows how'.<sup>[17]</sup> While many study guides usually follow the A mode, it is challenging for both teachers and learners to develop or use guides gravitating more towards self directed learning. Study guides provided in our curriculum was of the B type. It is the prerogative of any medical college to develop study guides having varying proportions of the above, based on individual curricular needs.

Eighty two percent (82%) (Fig.2) of students had felt that study guides contained references from text books and web links to encourage self directed learning. Though the students had answered in the positive direction, a decrease in response could be due to the individual study habits and less exposure to self directed learning in the school curriculum. This justifies our approach in using a hybrid curriculum containing traditional lectures integrated with case study instead of a classical PBL approach.<sup>[18]</sup> There is also evidence to suggest that students need guidance to develop self directed learning skills during their preclinical years.<sup>[19]</sup>

Provision of study guides is in tune with the principles of task based learning where efficiency of the curriculum is enhanced by giving an extended role to the teachers as providers of resource materials.<sup>[20,21]</sup>

## CONCLUSION

An integrated preclinical curriculum fosters better understanding of basic sciences and retention of knowledge, facilitating better comprehension of concepts of disease in the clinical years. A guided discovery approach embracing elements of traditional teaching methods and active learning, such as simulated case discussions is appropriate for such curricular reforms. This is particularly useful when students have not been completely weaned from teacher- centric methods. Inclusion of study guides serve as a catalyst to meet the above educational goals. Study guides provide learning objectives to achieve positive learning outcomes, sequencing of teaching learning sessions for an integrated learning and resources to motivate self directed learning. Feedback from student attitudes on study guides provides timely and valuable feedback on curricular changes.

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# DETERMINATION OF EFFECTIVE POLYHERBAL COMBINATION OF MEDICINAL PLANTS FOR ANTI-OXIDANT PROPERTIES

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## ABSTRACT

**Background and Objectives:** Plant based medicines are used by the people across the globe and are the mainstay to provide the leads for therapeutic remedies. Since the anti-oxidant leads are available in plenty from natural source, the present study is to focus on the determination of effective combination of plant based extracts for antioxidant property by adopting bio guided assisted methods.

**Methods:** Five medicinal plants commonly used in traditional and herbal formulations for various types of ailments were selected based on their efficacy on stress and anxiety. They are the roots of *Withania somnifera* (Solanaceae) and *Hemidesmus indicus* (Asclepiadaceae) fruits of *Aegle marmelos* (Rutaceae), *Emblica officinalis* (Euphorbiaceae) and aerial parts of *Ocimum sanctum* (Labiatae). All the samples were subjected to physico-chemical constant, pesticide content (HPLC method), aflatoxins (HPLC method), microbial load (I.P. 1996) and heavy metal content by atomic absorption spectroscopy, radioactive content (standard operating procedure) besides HPTLC quantification. Spray dried and lyophilized extracts were prepared and optimized and the proposed combinations were subjected to *in vitro* anti-oxidant activity by DPPH scavenging, lipid per oxidation, nitric

oxide scavenging and superoxide dismutase activity methods. The  $IC_{50}$  concentration was derived by linear method and Pearson's coefficient regression technique to arrive at the best combination exhibiting anti-oxidant activity. The phyto chemical estimation was also performed as a prelude to the anti-oxidant study.

**Results:** The diagnostic features of the selected medicinal plants complied with the official limits. Biology guided fractionation of the spray dried/ lyophilized extracts with significant  $IC_{50}$  values by linear regression has enabled to arrive for the best combination of the fractions suitable for anti-oxidant activity.

**Conclusion:** The HPTLC quantification of the raw materials identified and documented may be of help to the industries for authentication and standardization of raw materials wherein batch to batch variation that occurs during manufacturing process can be minimized and avoided. The anti-oxidant activity of the bio guided combination of the extracts enables us to derive the effective polyherbal combination for the activity.

**Keywords:** HPTLC quantification, heavy metal content, *in vitro* anti-oxidant studies, physico-chemical parameters, phytochemical estimation, radioactive content.

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## INTRODUCTION

In the current context, it is revised by FDA that the purified extracts either alone or in combination can be formulated as a lead to drug development. In the process of developing a formulation from the herbal source, one has to be sure of maintaining the quality, right from the raw material till the finished product i.e. quality by design ensuring that uniform standards are maintained and do not suffer from batch to batch variation. Raw material standardization is the only way for the entire drug manufacturer's to produce the drugs of same quality and to maintain the uniformity.<sup>[1]</sup> In case of raw material resources, it is mandatory to follow the

guidelines of GAP (Good Agricultural Practices), GHP (Good Harvesting Practices) and GLP (Good Laboratory Practices).

Stress and anxiety are the disorders caused due to behavioral changes in living organisms. In a challenging situation, the brain prepares the body for defensive action by releasing stress hormones viz. cortisone and adrenaline. Chronically high levels of stress hormones deplete both nutrient and energy reserves, creating an overall state of exhaustion. Even though there are various systems such as Allopathy, Naturopathy, Yoga, Reiki, and Ayurveda, Siddha and Unani etc. the natural systems has proven encouraging results with relatively few side effects.<sup>[2]</sup>

In the present study, five plants were selected namely roots of *Withania somnifera* (Solanaceae), aerial parts of *Ocimum sanctum* (Labiatae), fruits of *Aegle marmelos* (Rutaceae), Roots of *Hemidesmus indicus* (Asclepiadaceae) and *Emblica officinalis*

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(Euphorbiaceae) based on their effect on tridosa proven to possess anti oxidant<sup>[3,4]</sup> anti-stress<sup>[5-9]</sup> and anti-anxiety effects. Effective bio guided combinations of the above mentioned plants were attempted to reveal the highest anti-oxidant potential of the plant extracts as a prelude to anti-stress and anti-anxiety studies.

## MATERIALS AND METHODS

### Collection of specimen

The plant specimens for the proposed study viz. *Withania somnifera* roots were collected in September 2009 from wild source of Dindugal district, Tamil Nadu, *Hemidesmus indicus* roots from Thuthukkudi district, Tamil Nadu, fruits of *Aegle marmelos* collected in August 2009 from Tirunelveli district, Tamil Nadu and *Emblica officinalis* from Tirunelveli district in the month of August 2009, besides aerial parts of *Ocimum sanctum* collected in the month of August 2009, from Sri Ramachandra herbal garden, Chennai. The plant drugs were authenticated by Dr. Chelladurai, Research officer (Retd), Botany, CCRAS, Government of India whereas *Ocimum* by Dr. P. Jayaraman Director, National Institute of Science, Tambaram, Chennai.

### Quality Control of the selected plant drugs

The specimens were subjected to determine total ash, water soluble ash, acid insoluble ash and extractive values as per AYUSH guidelines.<sup>[17-19]</sup> Quantitative microscopy to determine the leaf constants (stomatal index, and vein islet number and vein termination number) in case of *Ocimum sanctum* according to the method adopted by Kokate, 1994.<sup>[20]</sup> The dried samples were subjected to pesticide analysis<sup>[21]</sup> and aflatoxins by TLC, microbial load, mineral and heavy metal content<sup>[22]</sup> by atomic absorption spectroscopy and radioactive content as per the standard operating procedures.

### HPTLC Quantification of the plant extracts

#### Chromatographic conditions

**HPTLC plates:** 20×10 cm<sup>2</sup>, 0.2mm thickness pre-coated with silica gel 60 F254:E. Merck KGaA, Cat. no. 1.05554, (Darmstadt, Germany).

**Syringe:** 100 $\mu$ l Camag (Switzerland)

**Applicator:** Linomat V sample applicator; CAMAG (Muttenz, Switzerland)

**Developing chamber:** TLC Chamber: Glass twin trough chamber (20 x 10 x 4cm); CAMAG

**Densitometer:** TLC CAMAG Scanner linked to WINCATS software III

#### Solvent Systems:

- Ethanolic extract of *Withania somniferum*: Chloroform: Methanol (90:10)

- Ethanolic extract of *Ocimum sanctum*: Toluene: Ethyl Acetate (70:30)
- Ethanolic extract of *Aegle marmelos*: Toluene: Ethyl Acetate (90:10)
- Ethanolic extract of *Hemidesmus indicus*: Chloroform: Methanol: Formic acid (7:2:1)
- Ethanolic extract of *Emblica officinalis* : Toluene: Ethyl Acetate: Acetic Acid: Formic Acid (20:45:20:5.0)
- Formulation with combined polyherbal extracts: Toluene: Ethyl Acetate: Formic Acid: Methanol (3.0: 3.0: 0.8:0.2)

### Preparation of standard solution

The stock solutions of withaferin A, eugenol, marmelosin,  $\alpha$ -amyrin and gallic acid of 1000 ppm each were prepared in ethanol. The stock solutions were quantitatively transferred to give a solution of appropriate concentration range of withaferin A (300, 400, 500 ng /spot), eugenol (500, 250, 125, 62.5, 31.25, 15.62, 7.81 and 3.9  $\mu$ g/ml), marmelosin (25, 50,100 ng /spot),  $\alpha$ -amyrin (100  $\mu$ g/ml) and gallic acid (1, 2, 4  $\mu$ g/spot) and made up to the final volume with ethanol. Standard solutions were prepared by dilution of the stock solution.

### Preparation of the extracts

#### Plant materials:

The samples were coded with numbers (1-5) in the order of *Withania somnifera* (1), *Ocimum sanctum* (2), *Aegle marmelos* (3), *Hemidesmus indicus* (4) and *Emblica officinalis* (5) respectively. The air dried and coarsely powdered roots of *Hemidesmus*, fruits of *Emblica officinalis* and *Aegle marmelos* were subjected to exhaustive maceration using hydro alcohol (ethanol:water) in the ratio of 60:40 and *Withania* in the ratio of 50:50 for 72 hours followed by 48 and 24 hours. The extracts were homogenized with 12% maltodextrin and aspirated into the spray drier (LU 20 model procured from LABULTIMA, Mumbai) as per standard conditions. Similarly, the aerial parts of *Ocimum sanctum* were freshly collected crushed into juice, filtered over muslin cloth. The filtrate was homogenized with 12% maltodextrin at 24,000 rpm for 20 minutes and subjected to lyophilization technique as per standard protocols.

#### Spotting of samples:

The chromatographic estimation was performed by streaking the extracts (15 $\mu$ l of 1% various ethanol extracts) in the form of narrow bands of 6 mm length on a pre coated silica gel 60 F 254 TLC plate (5 cm x 10 cm), at a flow rate of 150  $\mu$ l/s with a gas flow of

10s/ $\mu$ l employed by 100  $\mu$ l syringe connected to a Nitrogen tank using Camag Linomat V.

#### **Preparation of test solution:**

The stock solutions of the plant extracts (1-5) each were prepared in ethanol, which were quantitatively transferred to give a solution of appropriate concentration range of 25 mg/ml, 20 mg/ml, 50 mg/ml, 50 mg/ml and 20 mg/ml respectively and made up to the final volume with ethanol. Test solutions were prepared by dilution of the stock solution.

#### **Plate development:**

After spotting the plate, it was subjected to linear ascending development up to a distance of about 90 mm in a solvent system saturated with the same solvent system at room temperature just 10 minutes prior to development. The active principles were quantified in the extracts.

#### **Phytochemical estimation:**

The extracts were subjected to determine the phenolic content, tannin content, vitamin C, vitamin E contents and the values tabulated.

#### **In vitro anti-oxidant studies:**

The spray dried and lyophilized extracts of the five selected medicinal plants were mixed in various fractions based on their properties such as phenolic content, tannin content, Vitamin C and Vitamin E content respectively. 15 combinations were made and they were studied for *in vitro* anti oxidant properties by using DPPH scavenging assay<sup>[23]</sup>, lipid per oxidation<sup>[24]</sup>, super oxide dismutase activity<sup>[25]</sup> and nitric oxide scavenging activity<sup>[26]</sup> using bio

guided combination method. The percentage inhibition was calculated and IC<sub>50</sub> analysis was arrived for all the combinations (F-1 to F-15) by linear method. Regression analysis was carried out by using SPSS 15.0 version software to arrive at the effective combination.

## **RESULTS**

Plants serve as vast source for varied phytoconstituents exhibiting several pharmacological properties. Identifying such potential plants is of significance in medicine. So, it is significant to determine the effective poly herbal combination having anti-oxidant property.

#### **Physico chemical constants:**

##### **Ash values**

The total ash and water soluble ash of all the raw drugs are found to be within the limits.<sup>[27]</sup> A slight increase in acid insoluble ash of *Ocimum sanctum* (2.7%w/w) shows presence of more silica in it as indicated by the presence of trichomes which adheres to the fine soil particles and are difficult to remove even after thorough washing. The results are tabulated in Table-1.

##### **Extractive values:**

The high water soluble extractive values<sup>[27]</sup> of *Aegle marmelos*, *Ocimum sanctum*, *Hemidesmus* and *Embllica officinalis* may be due to the presence of aqueous soluble phytoconstituents like tannins, vitamins, amino acids, coumarins and flavanoids in the raw drug. The alcohol soluble extractive was found to be maximum with *Withania somnifera* roots

**Table 1: Determination of Ash values of selected plant drugs**

S. No	Name of the raw material	Water soluble ash (%w/w)	Total ash	Acid insoluble ash (%w/w)
			(%w/w)	
1	<i>Withania somnifera</i> roots	4.3±0.02	12±0.03 (NMT 7)	1.98±0.04 (NMT 1)
2	<i>Ocimum sanctum</i> aerial parts	7.0±0.03	12.6±0.03 (NMT 16)	2.7±0.03 (NMT 6)
3	<i>Aegle Marmelos</i> fruit	1.06±0.01	5±0.02 (NMT 4)	0.13±0.02 (NMT 1)
4	<i>Hemidesmus indicus</i> roots	6.3±0.02	5.67±0.02 (NMT 7)	0.167±0.01 (NMT 1)
5	<i>Embllica officinalis</i> pericarp	6.6±0.01	5.33±0.01 (NMT 7)	1.33±0.02 (NMT 2)

n = Mean±Standard deviation in triplicates

showing the presence of alkaloids and lactones in it, the order being *Withania* > *Hemidesmus* > *Aegle* > *Ocimum* > *Embllica*. The values are given in Table- 2.

#### **Estimation of heavy metal and mineral content by AAS:**

The micro nutrients such as iron and magnesium were found to be maximum in *Hemidesmus indicus* and copper found to be present only in *Embllica officinalis*

there by proving its antioxidant potential. The heavy metals like arsenic and chromium are not detected in all the 5 plant drugs. Lead and cobalt are detected in very

**Table 2: Extractive values of selected plant drugs**

S. No.	Name of the raw material	Water soluble	Alcohol soluble
1.	<i>Withania somnifera</i> roots	9.4±0.03 (NIL)	47.2±0.02 (NLT 20)
2.	<i>Ocimum sanctum</i> aerial parts	36±0.02 (NLT 1)	8.8±0.01 (NLT 2)
3.	<i>Aegle Marmelos</i> fruit	32±0.01 (NLT 50)	12.8±0.02 (NLT 6)
4.	<i>Hemidesmus indicus</i> roots	22±0.04 (NLT 10)	20±0.01 (NLT 18)
5.	<i>Emblica officinalis</i> pericarp	70±0.02 (NLT 50)	4±0.01 (NLT 40)

n = Mean ± Standard deviation in triplicates

low concentrations within the permissible limits as per the guidelines of AYUSH. The elements like Fe, Cu, Mn, Mg, Zn, Co besides heavy metals such as nickel (Ni), chromium (Cr), lead (Pb), mercury (Hg) and arsenic (As) were quantified by atomic absorption spectro photometer. The results are summarized in Table-3.

#### Estimation of Aflatoxins and pesticides:

All the five plants under study are found to be

**Table 4: Quantitative Secondary metabolite estimation**

S. No	Name of the raw material	Total phenol content (%w/w)	Tannin content (%w/w)	Vitamin C (% w/w)	Vitamin E (% w/w)
1	<i>Aegle marmelos</i> fruit	20.2	19.3	8.33	0.059
2	<i>Withania somnifera</i> root	86.18	15.2	7.96	0.02
3	<i>Ocimum sanctum</i> aerial parts	6	11.5	8.33	0.147
4	<i>Hemidesmus indicus</i> roots	6.2	19.8	0.72	0.032
5	<i>Emblica officinalis</i> pericarp	15.2	16.8	1.62	0.066

#### Estimation of microbial content:

The results indicated that all the five plant materials : were devoid of the pathogens viz. *E-coli*, *Pseudomonas aerugenosa* and *S.typhi* as specified by WHO, there by proving their safety. However, in *Ocimum sanctum*, some of the Enterobacteria was

**Table -3: Estimation of Metal and mineral content in the selected plant drugs by Atomic Absorption Spectroscopy method**

S. No	Metals/ Mineral (ppm/g sample)	<i>Withania somnifera</i> roots	<i>Ocimum sanctum</i> aerial parts	<i>Aegle Marmelos</i> fruit	<i>Hemidesmus indicus</i> roots	<i>Emblica officinalis</i> pericarp
1	Fe	0.2	0.25	0.21	1.5	0.56
2	Mg	1.12	0.97	0.91	1.14	0.55
3	Zn	0.05	0.07	0.05	0.21	0.06
4	Ni	0.13	0.12	0.1	0.13	0.14
5	Co	0.08	0.08	0.07	0.08	0.09
6	Mn	0.04	0.06	0.04	0.11	0.05
7	Pb	0.08	0.08	0.07	0.08	0.07
8	Cr	ND	ND	ND	ND	ND
9	Cu	ND	ND	ND	ND	0.56
10	As	ND	ND	ND	ND	ND

ND- Not detected

devoid of aflatoxins and pesticides.

#### Phytochemical estimation:

The selected medicinal plants estimated for quantitative secondary metabolite contents (phenolic, tannin, vitamin C and vitamin E) are tabulated in Table-4.

reported which was only about 102/gm and are not harmful to the system.

#### HPTLC Quantification of the active principles:

The spray dried *Withania somniferum*, *Aegle marmelos*, *Hemidesmus indicus*, *Emblica officinalis* and the lyophilized *Ocimum sanctum* were subjected

Fig. 1a

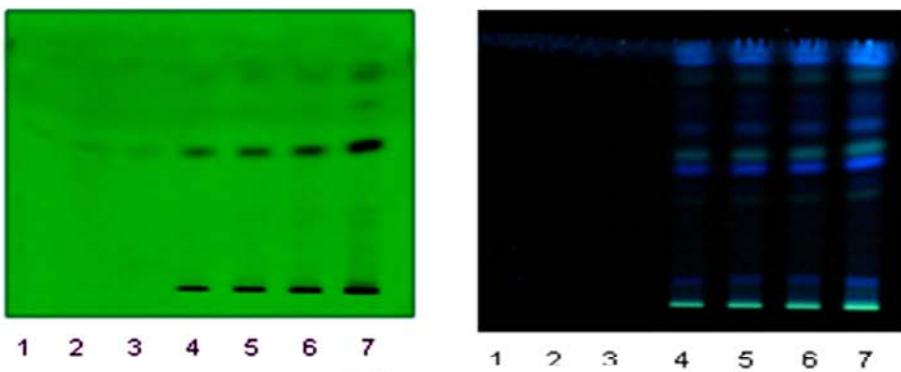


Fig. 1b

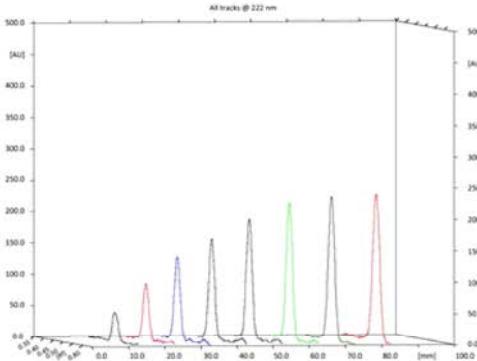


Fig. 1. Chromatographic plate of ethanolic extract of *Withania somnifera* and reference standard (withaferin A) at scanning wavelength of 222 nm.

a). Track 1-3: applied volume of 6, 8 and 10  $\mu$ l of withaferin A captured at 254 and 366 nm; Track 4-7: applied volume of 6, 8, 10, and 20  $\mu$ l of *Withania somnifera* methanolic extract captured at 254 and 366 nm.

b). Densitometry analysis of standard.

Fig.2a

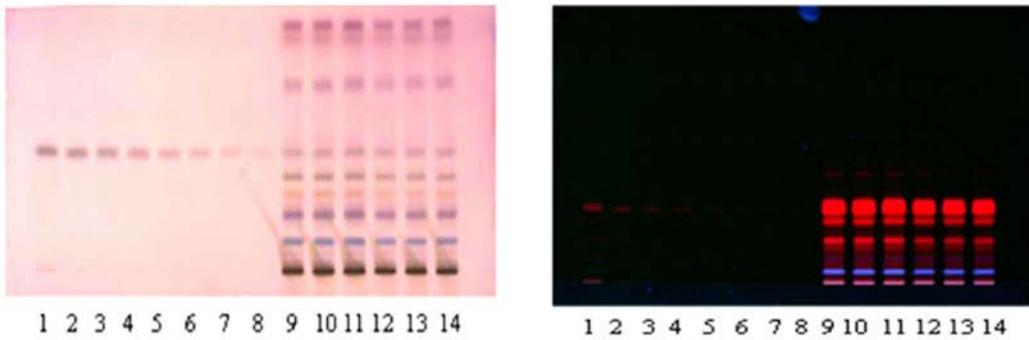


Fig.2b

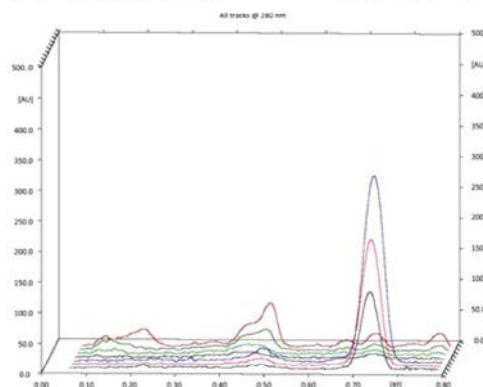


Fig. 2. Chromatographic plate of ethanolic extract of *Ocimum sanctum* and reference standard (eugenol) at scanning wavelength of 280 nm.

a). Track 1-8: applied volume of 5  $\mu$ l of eugenol captured at 254 and 366 nm; Track 9-14: applied volume of 5  $\mu$ l of *Ocimum sanctum* ethanolic extract captured at 254 and 366 nm.

b). Densitometry analysis of standard and sample.

Fig. 3a

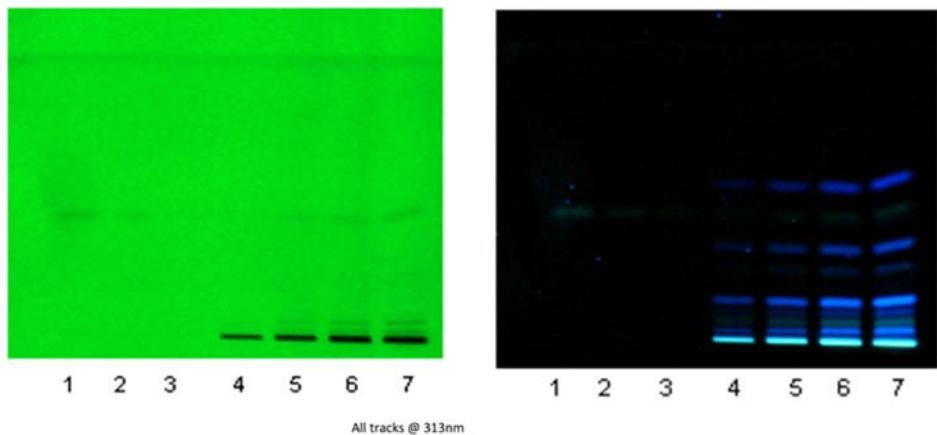


Fig. 3b

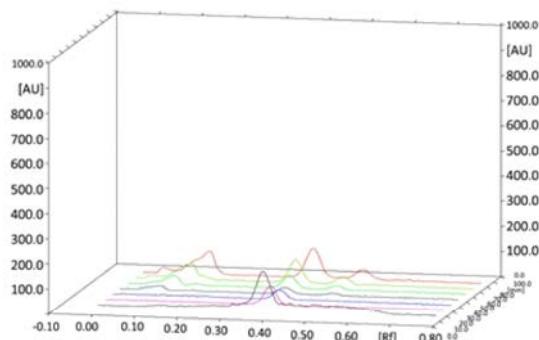


Fig. 3. Chromatographic plate of ethanolic extract of *Aegle marmelos* and reference standard (marmelosin) at scanning wavelength 313 nm.

- Track 1-3: applied volume of 2, 4, and 8  $\mu$ l of marmelosin captured at 254 and 366 nm; Track 4-7: applied volume of 5,10,20,30  $\mu$ l of *Aegle marmelos* ethanolic extract captured at 254 and 366 nm.
- Densitometry analysis of standard and sample.

Fig. 4a

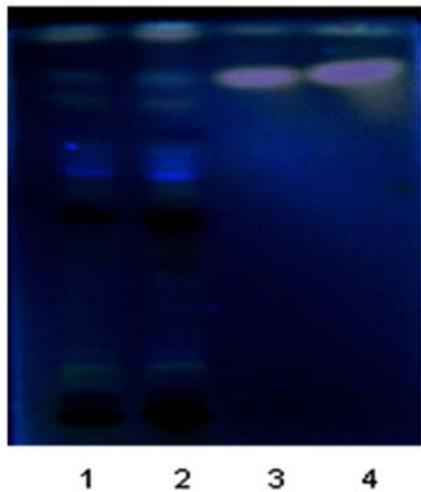


Fig. 4b

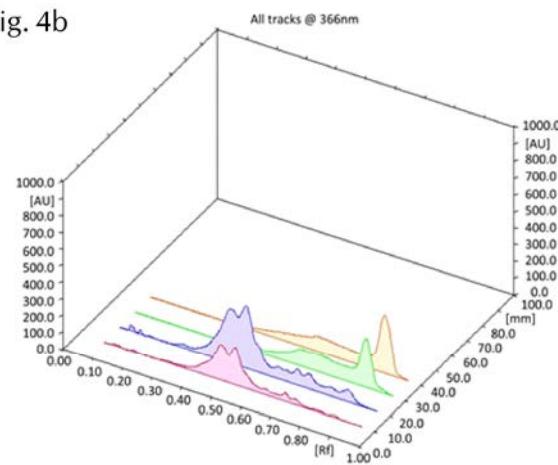


Fig. 4. Chromatographic plate of ethanolic extract of *Hemidesmus indicus* and reference standard ( $\alpha$ -amyrin) at scanning wavelength of 360 nm.

- Track 1 and 2: applied volume of 2 and 5  $\mu$ l of *Hemidesmus indicus* ethanolic extract captured at 366 nm; Track 3 and 4: applied volume of 5 and 10  $\mu$ l  $\alpha$ -amyrin captured at 366 nm.
- Densitometry analysis of standard and sample.

Fig. 5a

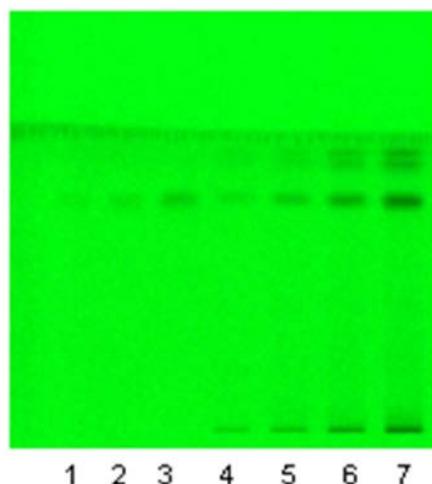


Fig. 5b

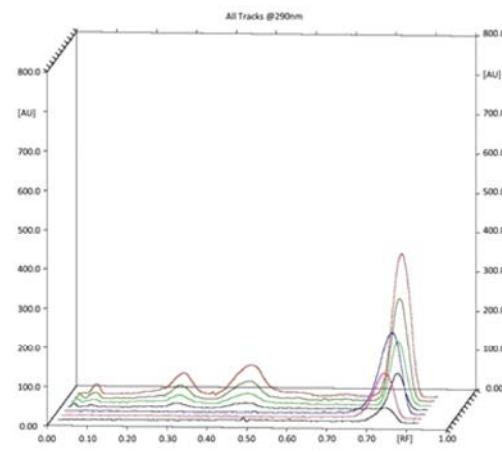


Fig. 5. Chromatographic plate of ethanolic extract of *Emblica officinalis* and reference standard (gallic acid) at scanning wavelength of 290 nm.

a). Track 1, 2 and 3: applied volume of 2, 4, 6, 10 $\mu$ l of Gallic acid captured at 254 nm; Track 4-7: applied volume of 2, 4 and 6  $\mu$ l of *Emblica officinalis* ethanolic extract captured at 254 nm.

b). Densitometry analysis of standard and sample.

Fig. 6a

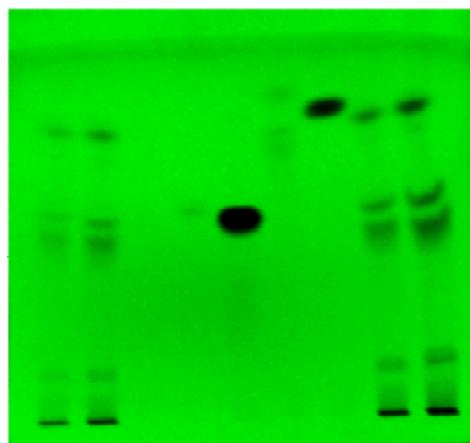


Fig. 6b

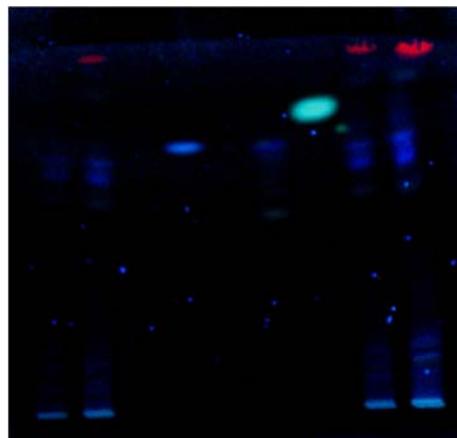


Fig. 6. Chromatographic plate of the polyherbal formulation at scanning wavelength of 254 nm and 366 nm.

a. & b. Track 1 & 2: applied volume of 2.5 & 5 $\mu$ l of polyherbal formulation captured at 254 nm and 366 nm; Track 3-5: applied volume of 5 $\mu$ l of standards, Gallic Acid, Withaferin, Marmelosin, captured at 254 nm and 366 nm.

to HPTLC analysis and by spectral matching, spectral specificity and linearity method the active principles were estimated by comparing with standard phytomarkers as shown in figures 1-5.

The composition of the active principles viz., withaferin A in *Withania*, eugenol in *Ocimum sanctum*, marmelosin in *Aegle marmelos*,  $\alpha$ -amyrin in *Hemidesmus indicus*, gallic acid in *Emblica officinalis*, quantified by HPTLC technique are as follows:

- ◆ 1 mg of spray dried *Withania* contained 1.90  $\mu$ g of withaferin A.
- ◆ 1 mg of lyophilized dried *Ocimum sanctum* contained 225 ng of eugenol.

- ◆ 1 mg of spray dried *Aegle marmelos* contained 5.23 ng of marmelosin.
- ◆ 1 mg of spray dried *Hemidesmus* contained 1.40  $\mu$ g of  $\alpha$ -amyrin.
- ◆ 1mg of spray dried *Emblica officinalis* powder contained 83.44  $\mu$ g of gallic acid.

#### In vitro anti-oxidant studies of the spray dried and lyophilized extracts:

The combination F-3 was found to have the least IC<sub>50</sub> values in all the parameters tested viz. DPPH scavenging, nitric oxide scavenging, lipid per oxidation and superoxide dismutase activity with values of 65.64, 77.77, 271.16, and 77.29  $\mu$ g respectively as evident from Table 5. Further, it was confirmed by linear

**Table -5: IC<sub>50</sub> values of combinations F-1 to F-15 in micrograms**

Formulations	DPPH	NOS	LPO	SOD
F1	270.75	325	406.32	442.9
F2	422.3	151.9	388.47	1410
F3	65.64	77.77	271.16	77.29
F4	508.23	1538	367.17	1092.22
F5	355.36	1581	529.09	1517.64
F6	477.98	1471	472.53	530.81
F7	1201.14	1048	356.29	866.52
F8	766.04	771.79	879	895.8
F9	326.04	575	400.83	542.83
F10	618.21	918.28	406.55	599.68
F11	924.05	1113.28	713.33	985.11
F12	975.05	1507.27	1011.12	915.56
F13	1526.36	1213.86	1052.5	1161.18
F14	882.02	1017.36	858.88	917.5
F15	842.03	876.9	553.71	748.54

**Note:**

DPPH : 2, 2-Diphenyl 1-picryl hydrazyl

LPO : Lipid per oxidation

NOS : Nitric oxide scavenging

SOD : Super oxide dismutase

**Table- 6: Linear Regression Analysis of F-1 to F-15 formulations at 500 µg**

Formulation	DPPH	NOS	LPO	SOD
F1	51.84	53.59	50.59	53.14
F2	9.02	25.41	55.63	26.84
F3	71.3	65.6	59.84	66.14
F4	-40.9	24.45	57.04	26.31
F5	-28.12	26.69	49.22	32.7
F6	22.08	26.69	93.9	40.18
F7	-15.64	21.4	58.26	35.9
F8	40.67	26.37	35.7	38.23
F9	41.58	39.9	56.45	47.43
F10	34.95	46	55.92	40.42
F11	28.37	31.32	40.9	28.67
F12	25.72	26.69	29.55	27.14
F13	34.13	20.78	30.61	24.87
F14	31.28	30.34	26.39	33.3
F15	37.85	31.65	45.91	36.07

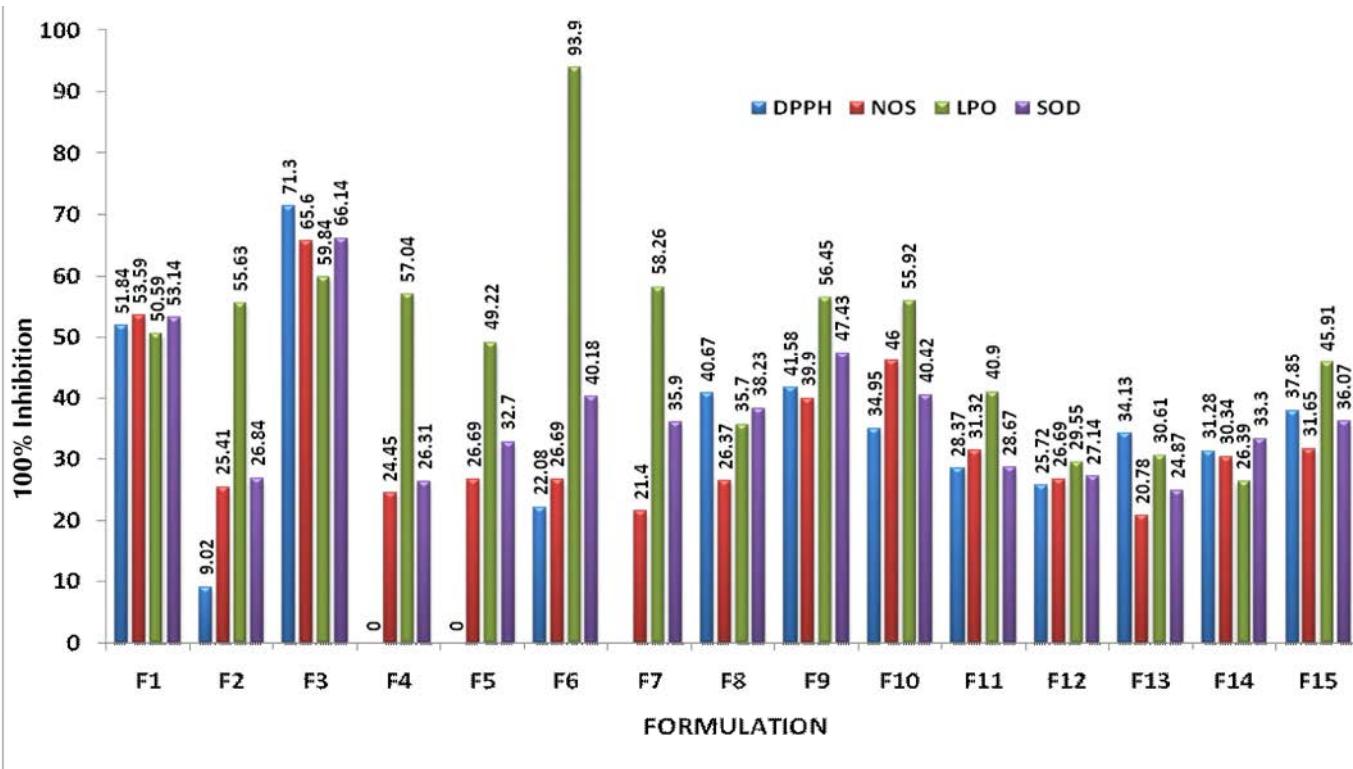
**Note:**

DPPH : 2, 2-Diphenyl 1-picryl hydrazyl

LPO : Lipid per oxidation

NOS : Nitric oxide scavenging

SOD : Super oxide dismutase



**Fig. 7. Linear regression analysis graph of F-1 to F-15 formulations at 500µg. (Blue - DPPH, Red - NOS, Green - LPO, Violet - SOD)**

regression analysis of all the combinations (F1-F15) at 500 $\mu$ g. F-3 possessed highest correlation coefficient proving its effective antioxidant activity as tabulated in Table 6. Hence, through regression analysis based on Pearson's correlation co-efficient, the combination viz., F-3 was found to be effective.

## DISCUSSION

The physiochemical standards developed for the selected plant drugs mentioned above, quantification of the active principles in their respective plant extracts using HPTLC technique besides, identifying the effective ratio of combination of the plant extracts for anti-oxidant property, paves way for the design of a suitable oral solid dosage form and serves as an effective quality control tool.

The HPTLC method developed for the simultaneous quantization of the biomarkers may be recommended for quality assurance and fingerprinting to establish the authenticity of the extracts under study and could be extended for the marker-based standardization of other herbal formulations containing the active principles of interest. The method may also prove to be useful, cheaper alternative as compared to the costlier HPLC and GC methods generally used in estimation of phytomarkers from formulations and plant extracts.

The physiochemical standards highlight the evaluation of plant raw materials used in the formulations/extracts to ensure batch to batch consistency in quality and efficacy.

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# FORENSIC ODONTOLOGY: A BRIEF REVIEW

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## ABSTRACT

Despite breakthrough in science and technology, natural calamities and crimes continue to persist in human life. Identification of human remains is essential for various reasons including legal, criminal, humanitarian and social grounds . Dental remains can be used for identification as using them is cost effective, reliable and fast. Forensic odontology is a branch of dentistry that analyzes dental evidence to overlap the dental and legal profession. This article reviews the recent advances in the field of forensic odontology

while highlighting the role of the dentist in identification of human, dental remains and crime investigation. Forensic information from soft tissues of the oral cavity, forensic methods of age estimation, therapeutic and molecular aspects of Forensic odontology have been elaborated. Scope for research, limitations challenges in Indian senario have also been discussed.

**Key words:** age estimation, forensic odontology, person identification, recent advances

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## INTRODUCTION

Despite breakthrough in science and technology, natural calamities and crimes continue to persist in human life. Identification of human remains is essential for various reasons including legal, criminal, humanitarian and social grounds. The human body becomes disfigured to a great extent in case of burns, accidents and mass disasters like earthquake so much so that identification of the individual becomes a challenge. However dental remains can be used for identification as using them is cost effective, reliable and fast. Forensic Odontology, or forensic dentistry was defined by Keiser-Neilson in 1970<sup>[1]</sup> as "that branch of forensic medicine which, in the interest of justice deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of the dental findings". This article sheds light on the role of the dentist in identification of human and dental remains along with recent advances in the field of forensic odontology.

## HISTORY OF FORENSIC ODONTOLOGY

The earliest recorded case of forensic dentistry concerns a female associated with Emperor Nero, who was identified after her death through the unique arrangement of her teeth. In the year 66 A.D, Nero's mistress Sabina got his wife killed by her soldiers and demanded to see the head of the victim in a dish. She recognized the head by a black anterior tooth.<sup>[2]</sup> Later in 1775, Paul Revere identified victims of a

revolutionary war by their teeth and dental work. He also identified the body of Joseph Warren by identifying a Walrus tusk used as a pontic for his missing maxillary canine.<sup>[3]</sup> In 1977, the body of Hitler and his wife Eva Brauma were identified using dental records with radiographs and prostheses.<sup>[4]</sup> Sansare K and Dayal PK in their review in 1995 have mentioned that according to Elphinstine, M. Raja Jayachandra Rathore of Canouj, died on the battle field in 1191. His body was identified by his false anterior teeth. This was probably the first case of identification using dentition from India.<sup>[2]</sup> Thousands of people lost their lives in the world trade center disaster in the U.S.A on September 11, 2001. Deoxyribo Nucleic Acid (DNA) extracts from tooth brushes of the victims were used in identification of some victims.<sup>[2]</sup>

## TOOTH AS A TOOL FOR FORENSIC EVIDENCE

### Use of teeth in identification of person:

Morphology and arrangement of teeth is unique for every individual. Comparative dental identification is a procedure by which dental evidence such as dental caries, missing teeth, restored teeth, prosthesis, alterations in shape of teeth such as taurodontism, talons cusp, developmental defects such as amelogenesis imperfecta, dentinogenesis imperfecta, changes in colour of teeth like dental fluorosis collected from human remains is compared with previous records for establishing identity of the decedent. This necessitates the importance of every dentist to maintain records of their patients. Acharya et al have demonstrated that dental profiling is another integral part of forensic dentistry which aids in person identification by identifying ethnicity, gender and age. Shovelling, cusp of carebelli, three cusped maxillary second molar, winging, cusp 5, 6, 7, mandibular

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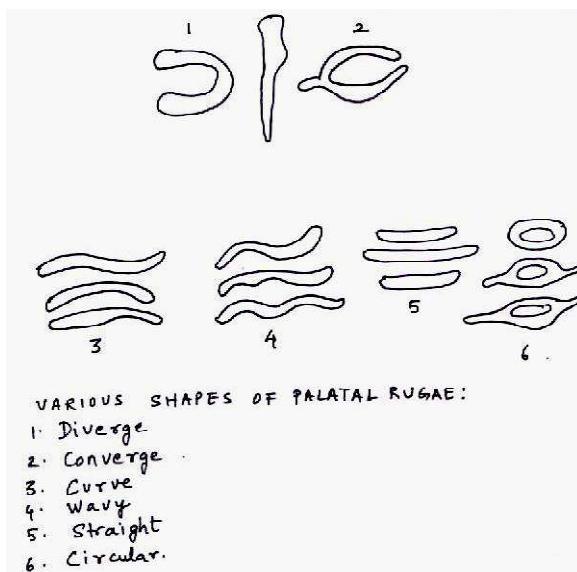
groove pattern, four cusped mandibular molars vary with ethnicity.<sup>[5]</sup> Morphologic features of the skull such as frontal, parietal eminence, forehead, supraorbital ridge, glabella, orbits, nasal aperture, zygomatic arch, occipital, mastoid process, glenoid fossa, foramen magnum, palate, condyles, shape of chin, gonial angle, height of mandible body are indicators of gender. Metric traits of teeth such as buccolingual and mesiodistal dimensions of teeth and morphology of canine and molars can also be used in gender identification.<sup>[6]</sup> Thus comparative dental identification and dental profiling can be used in identification of person.<sup>[7]</sup>

### **Ameloglyphics: A Recent advance in person identification:**

The term ameloglyphics is used to describe the study of enamel pattern. Manjunath et al<sup>[8]</sup> studied the enamel pattern of left maxillary canine and first premolar in 30 male and 30 female volunteers using cellulose acetate peel technique and the enamel rod patterns were obtained by subjecting photomicrographs to VeriFinger Standard SDK v5.0 software. Results of the biometric analysis revealed that enamel rod patterns was unique for every tooth and every individual and showed gender variations. It was observed that branched wavy subpattern was the most common type by visual analysis. Thus enamel rod pattern of teeth have been found to be unique for every individual and hence could be used as an aid in person identification. Manjunath et al in another study compared the efficacy of cellulose acetate film, cellophane tape, and light body impression material to record enamel rod pattern in thirty extracted teeth and observed that cellulose acetate film technique gave more accurate results in comparison with the other two techniques.<sup>[8]</sup>

### **Use of teeth in crime investigation:**

A bite mark is defined as 'a mark caused by teeth either alone or in combination with other mouth parts' and serves as a good source of forensic evidence in crime investigation. Bite marks can be human or animal and can be on human or on objects. Bite marks can also be used in investigation of child abuse. The Cameron and Sim classification of bite marks is based on agent and material. Based on etiology McDonald has classified bite marks as tooth pressure, tongue pressure and tooth scrape marks. Various steps in bite mark investigation include preliminary questions, evidence collection from the victim, case demographics, visual examination, photography, saliva swab, impression making, evidence collection from suspect, bite mark analysis, comparison and drawing.



**Fig. 1:** Schematic representation of the various types of palatal rugae

conclusion.<sup>[9]</sup> Vander Veldon. A et al have reported the use of image perception technology for bite mark analysis and investigation of crime. Advantages of this technique include additional colouring of images, pseudo three dimension imaging all of which improves accuracy.<sup>[10]</sup>

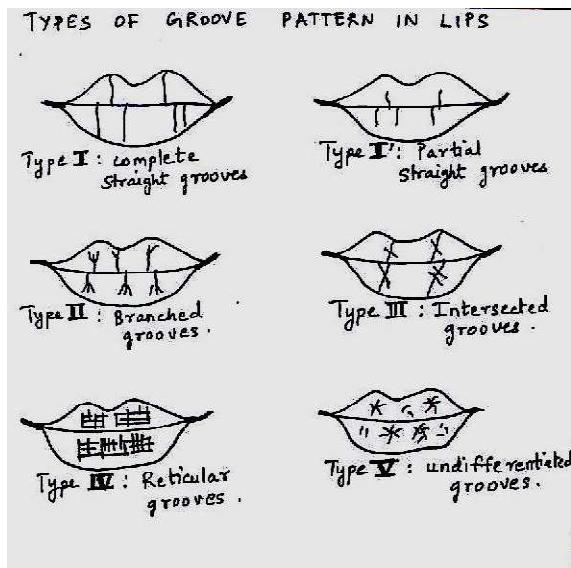
### **FORENSIC INFORMATION FROM OTHER ORAL TISSUES:**

#### **Palatal Rugae:**

The ridges present in the anterior part of the palate on either side of the midpalatal raphe are referred to as palatal rugae. Palatal rugae is well protected by teeth, buccal pad of fat, lips and hence is resistant to decomposition. Pattern of palatal rugae is unique for every individual similar to finger print and hence could provide forensic information. Thomas et al has classified rugae pattern as diverge, converge, curve, wavy, straight and circular (Figure 1).<sup>[11]</sup> Palatal rugae is a moderately accurate tool in establishing identity of Indians as proposed by V S K Shetti et al (2011).<sup>[12]</sup> M Ohtani et al (2008)<sup>[13]</sup> analysed the limitations of using palatal rugae for person identification in edentulous patients. In their study, although 90% of the 48 cases analysed were matched correctly the three misleading factors were found to be (i) poor demarcation of palatal rugae (ii) non complex pattern of rugae (iii) changes in palatal height. Palatal rugae patterns can also aid in gender differentiation and race differentiation.<sup>[14,15]</sup>

#### **Lip prints:**

Lip prints are an important forensic evidence in the scene of crime similar to finger prints. The study of lip prints is called cheiloscopy. Tsuchihashi et al<sup>[16]</sup> have



**Fig. 2:** Schematic representation of the various types of groove pattern in lips.

proposed six different types of groove patterns in the lip which could be useful in crime investigation (Figure 2). They are Type 1- with clear-cut vertical grooves that run across entire lip; Type I' similar to type I but not covering entire lip; Type II with branched grooves; Type III with intersected grooves; Type IV with reticular grooves and Type V with grooves that are not morphologically differentiated. Rachna V Prabhu et al, 2012<sup>[17]</sup> have conducted a study of various patterns of lip prints in dental students. They observed type V pattern to be the most common in occurrence and have recorded trifurcation, Bridge or H pattern, horizontal lines, cartwheel, pineapple skin and multiple branching appearances for the first time. This emphasises the need for sub classification of type V for better accuracy.

#### FORENSIC METHODS OF AGE ESTIMATION

Age estimation can be done from dentition by clinical methods such as eruption sequence; radiographic features like appearance of tooth germs, commencement of mineralisation, degree of mineralisation of various teeth, degree of crown and root completion, degree of root resorption of deciduous teeth, open apices, pulp to tooth ratio, volume of pulp chambers and root canals, third molar eruption, digitization of available radiographs; histologic features like neonatal line, incremental lines of cementum, dentin translucency, dentin predentin interface using scanning electron microscopy and biochemical characteristics like C14 levels and racemification of dentin. Dentition is used to estimate age in three groups namely prenatal, natal and post natal period; children and adolescents and adults. In neonatal period age

estimation is done for legal purpose like infanticide. Maceration,<sup>[18]</sup> CT scan,<sup>[19]</sup> dry weight of teeth<sup>[20]</sup> and Neonatal line are used in estimating age of neonates. In children and young adults, age estimation is important for legal aspects as judgement varies for minors under the age of eighteen. Clinical methods such as eruption sequence, radiographic methods such as degree of calcification are used in age estimation for the above mentioned group.<sup>[21]</sup> Third molar eruption is of great importance to distinguish juveniles and adults.<sup>[22]</sup> In adults, regressive changes like attrition; periodontal status like attachment loss, root resorption, dentin translucency, cementum apposition at the apex; histologic changes such as incremental lines of cementum; radiographic assessment of pulp to tooth ratio are used in age estimation.

A radiograph can reveal information about the age of a person and is vital in forensic odontology as the teeth and the bony complex of the face are structures amenable to radiographic examination. Saunders was the earliest dental surgeon to publish information about age estimation from radiographs and human dentition.<sup>[23]</sup>

Since 1982, there has been a revolution in this regard and radiographs have been used for age estimation on a daily basis.<sup>[24]</sup> The age assessment methods are simple and mostly based on identification of the stages of mineralisation on radiographic images followed by comparison with standardised data.

The radiographic projection techniques useful for age estimation are, intraoral periapical radiographs, lateral oblique radiographs, panoramic radiographs and cephalograms. The techniques of age estimation from the above mentioned radiographs require that the X ray images should include some developing teeth. Digitalized images of the radiographs are first obtained with the help of scanner or video camera. Following this, the image is processed through appropriate software with adjustments for image rotations, translations and scaling. Using the processed image, the forensic radiologist estimates the age of a person. The odontological age estimation triad can be enumerated as follows:

- 1) the subject for age estimation,
- 2) the chosen dental development survey and
- 3) legal considerations which together are considered important for age estimation .

Panchbai A.S has undertaken a Pubmed Medline search of articles from 1932 to 2009 related to age estimation from dental radiographs.<sup>[25]</sup> The results of

his study have shown that various researchers have used the following features as aid to radiological age estimation. They are:

1. Prenatal appearance of jaw bone
2. Tooth germ appearance
3. Earliest mineralisation events
4. Degree and nature of crown completion
5. Crown eruption into oral cavity
6. Root completion
7. Resorption of roots of deciduous teeth
8. Presence of open apices
9. Volumes and dimensions of pulp chambers and root canals
10. Third molar development and eruption.

Radiological methods of age estimation in children and adolescents.

There are four established methods of age estimation as mentioned below

- 1. Schouler and Masslers method<sup>[24]</sup>:** In 1941 Shouler and Massler have analyzed development of deciduous dentition and have evolved 21 chronological steps from 4 months to 21 years. They have published the data in the form of charts which have been updated from time to time by the American Dental Association.
- 2. Moorer, Fanning and Hunt method<sup>[24]</sup>:** In this technique 14 stages of mineralisation for developing single and multirooted teeth are used for age estimation.
- 3. Demirjian, Goldstein and Tanners method<sup>[24]</sup>:** rated seven mandibular permanent teeth in an orderly manner ie second molar (M2), first molar(M1), Second premolar (PM2), first premolar (PM1), Canine(C), Lateral incisor(I2), Central incisor (I1) . They determined 8 stages of mineralisation from A-M using the above data and correlating the two, they determined the age of the individual.
- 4. Nollas technique<sup>[26]</sup>:** This technique evaluated the mineralization of permanent teeth in ten stages and can be used to assess development of each tooth in the maxilla and mandible.

## RADIOLOGIC METHODS OF AGE ESTIMATION IN ADULTS:

The accepted methods of age estimation using radiographs are as follows:

1. Volume assessment of teeth: this method is based on the reduction of the size of pulp cavity due to

secondary dentin deposition which is directly proportional to age. A method by Kvaal et al used pulp to tooth ratio with volume values to calculate age.<sup>[27,26]</sup>

2. Third molar development: Technique by Harris MPJ and Nortje CJ gives five stages of third molar development. Based on the root formation of third molar one can estimate the individual's age.<sup>[28]</sup>

Based on the above mentioned radiological methods of age estimation we can conclude that age measurement from X rays is simple and easy. Also with more advancements in the field of investigative and diagnostic radiology we can expect newer methods of age estimation from radiographs.

## RECENT ADVANCES IN AGE ESTIMATION

Although there are several methods to estimate age of the individual as reviewed, research in the field of forensic odontology has led to the discovery of biochemical changes in teeth that could be used to estimate age of the individual. These include racemisation of aminoacids in dentin and carbon 14 levels in enamel.

Halfman P.M. et al<sup>[29]</sup> have shown that racemisation of L aspartic acid to D aspartic acid in dentin takes place as aging occurs. This can be used as a basis for age estimation. Meissner et al<sup>[30]</sup> have suggested that racemisation of aspartic acid could represent the pinnacle of age estimation at a molecular level because of standardization of sampling and methodology and an evaluation of different variables in many published studies with accurate results. Rajkumari et al<sup>[31]</sup> in their study have reported an accuracy of  $\pm$  3 years in estimating age of 36 individuals in six different age groups and equal gender and jaw distribution by determination of D/L ration of aspartic acid in dentin of non carious teeth using High performance liquid chromatography.

Another biochemical indicator of age is the level of Carbon 14 in enamel. The basis for age estimation is that Carbon 14 levels in atmosphere have increased over the years after the cold war between 1955 and 1963 due to ground test of nuclear weapons. This is referred to as bomb pulse. The C14 levels in enamel is unaltered. Hence Carbon 14 levels in enamel represent C14 levels in atmosphere at the time of enamel formation. Alkass. K et al<sup>[32]</sup> performed a study to estimate age of individuals by assessing Carbon 14 levels in enamel of 95 extracted teeth from various geographic origins. In their study, C13 levels indicated geographic origin of the individual with good accuracy.

Thus it can be understood that C14 and C13 levels can be used in age estimation and geographic origin determination respectively. In another study in Swedish individuals, Alkass.K et al<sup>[33]</sup> assessed age using both racemisation and carbon 14 level estimation and observed a correlation between the two techniques ( $R^2 = 0.66$ ,  $p < 0.05$ ). The absolute error for radiocarbon analysis was  $1.0 \pm 0.6$  years and for racemisation was  $5.4 \pm 4.2$  years. Thus racemisation gives information about chronological age and radiocarbon method gives information about time of birth. The accumulation of the 4977 base pairs, deletion of mitochondrial DNA, attrition of telomeres and accumulation of advanced glycation end products occurs with ageing. Hence these parameters could also be used in age estimation. However standardisation of procedures needs to be carried out which gives scope for future research.

### **MOLECULAR ASPECTS IN FORENSIC ODONTOLOGY**

Using DNA as a forensic aid can be credited to its discovery by Watson and Crick in 1953.<sup>[34]</sup> This discovery was responsible for identifying a person based on his unique DNA sequence. Thirty years later, Jeffrey et al in 1985<sup>[35]</sup> developed radioactive molecular probes to detect variations in DNA sequence and also invented the term DNA finger prints in order to find specific patterns of DNA in an individual.

To isolate and identify DNA, several biological samples can be used such as bone, hair bulb, biopsy and other body tissues. Finger prints have been used to identify victims of fire accidents, trauma and mass disaster. For all these techniques DNA sample harvest is important. Since dental pulp has a quantifiable DNA its use can be exploited to identify the deceased individual. Schwatz et al 1991<sup>[36]</sup> have demonstrated the environmental influences on concentration and recovery of DNA from dental pulp. Tsuchimochi et al 2002<sup>[37]</sup> tested the efficacy of chelating resins to extract DNA from dental pulp for PCR analysis. In order to evaluate different dental tissues as DNA sources for forensic analysis, Malaver and Yunis<sup>[38]</sup> conducted a study of twenty teeth providing 45 DNA samples from the pulp, cementum and dentin. The results of their study showed that the harvest of DNA was best from the pulp while cementum and dentin provided similar signals.

Potsch et al 1991<sup>[39]</sup> showed that the amount of DNA from a dental sample ranged from 6 micro gram to 50 microgram. In forensic samples the usually performed analysis is short tandem repeats (STR) which can identify hypervariable regions of DNA that present consecutive repetitions of fragments having 2-7 base

pairs.

The Polymerase Chain Reaction (PCR) technique is the usual choice for investigation of the frequency of STRs. The technique allows for amplification of the restricted regions of the human genome. Sweet et al 2001<sup>[40]</sup> have shown that the polymerase chain reaction is the most ideal to differentiate an individual from another.

The most important observation on the use of DNA from dead bodies and ancient material is that the DNA sample can be degraded by time, temperature, humidity and light. However teeth still represent the best source for DNA as a molecular tool for forensic odontology.

### **THERAPEUTIC ASPECTS OF FORENSIC ODONTOLOGY**

It is now accepted that the world frequently encounters mass disasters like typhoons, tsunami, fire and famine. So many scores of individuals die on a day to day range. The role of dentist in identifying individuals who have lost their lives in mass disasters or medico legal cases becomes extremely important.

The previous sections have shown how the teeth and DNA from pulp can be used as forensic aids. However it has been shown that even prosthesis like dentures can be used to identify deceased individuals. This has been achieved by a technique termed denture marking.<sup>[41]</sup> For this the identification mark should be specific, technique should be simple and the mark should be fire and solvent resistant.

Denture marking can be divided broadly into two categories namely surface marking and inclusion methods. Surface marking simply means scratching or engraving an identity mark onto the surface of the denture using a spirit based pen before covering them with a denture based acrylic. Inclusion methods involve the use of paper, onion skin or label which are included into the denture base prior to polymerisation. Also lead foils marked with patient details can be sandwiched in the denture base to enhance person identification. In conclusion the prosthodontist has a major role in person identification.

### **SCOPE FOR RESEARCH IN FORENSIC ODONTOLOGY:**

The use of molecular techniques and ameloglyphics for person identification, biochemical methods of age estimation requires standardisation. Moreover very few studies have been carried out in the Indian population. This warrants further studies to be carried out in our country so that regional variations could be assessed.

## LIMITATIONS OF RESEARCH IN FORENSIC ODONTOLOGY

Limitations of research include

- \* Ethical issues in sample collection in crime investigation.
- \* The application of molecular and biochemical techniques in Forensic Odontology are expensive.

## CHALLENGES IN THE INDIAN SCENARIO

- \* In our country awareness about the importance of record maintainence among Dental Professionals is not satisfactory which poses a great challenge for person identification both in crime investigation and mass disasters.
- \* Most of the dental professionals in our country do not pursue research or career in this field due to social and cultural reasons.

## CONCLUSION

Forensic dentistry is not a recent branch of dentistry. It has always been a part of forensic science. The use of teeth, biopsy samples, saliva, dentures and other aids have revolutionised forensic science. The teeth and facial bones are resistant to fire, burns and can be still recovered from disaster sites for use. DNA recovery from dental samples has become easy with advancements in PCR techniques; hence genetic identification of individuals has become easy. Ameloglyphics and age estimation using biochemical markers are some of the recent advances in this field that gives scope for future research and standardisation. Last but not the least even dentures and bridges marked with identity numbers and initials can be used in person identification in mass disasters. Forensic odontology can therefore be regarded as one of the most important areas of forensic science as far as "person identification" is concerned.

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# ADRENAL INCIDENTALOMA - A CASE REPORT

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## ABSTRACT

The prevalence of adrenal incidentaloma ranges between 1 to 9 percent. Such an entity is attributed to the technological advances in the current decade. A 41 yr old female presented with a right adrenal incidentaloma which was surgically managed owing

to the massive size. An approach to the evaluation and management of adrenal incidentaloma is emphasized in order to optimize the outcome in such patients.

**Keywords:** Adrenal, Adrenal cyst, Functional tumors, Incidentaloma.

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## INTRODUCTION

Incidentalomas are incidental radiographical findings with mass lesions of varying sizes.<sup>1</sup> The incidence of adrenal incidentaloma is 8.7%<sup>2</sup> and adrenal cysts vary between 0.064% and 0.18%<sup>3</sup> in autopsied patients. They vary from the benign cysts to truly malignant tumors and may occur at any age group, they are however, more common in the 4<sup>th</sup> decade. They can be either a functioning or a non functioning tumor. This incidental finding occurs in 0.4% of all the computed tomography (CT) scans<sup>4</sup> and 4.4% of higher resolution scanners with a relatively higher prevalence of 10% in older patients.

## CASE REPORT

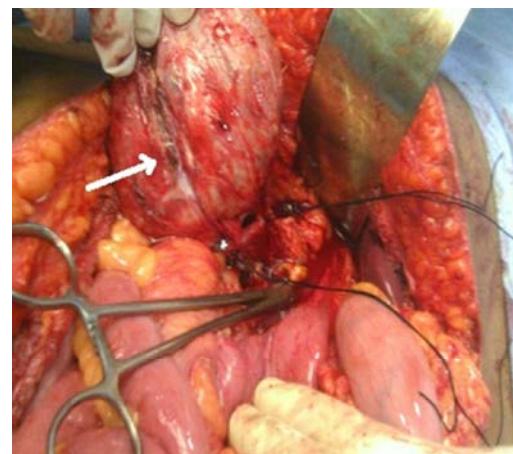
A 41 yr old female visited the hospital with complaints of dyspepsia for the past 3 months. She was investigated elsewhere for the dyspeptic symptoms and was found to have a right adrenal cyst on screening ultrasound. Her menstrual history was unremarkable. Systemic examination was normal with stable vitals. She was normotensive with no significant fluctuations in the blood pressure charting. Abdomen examination showed no evidence of organomegaly.

All the laboratory parameters were within normal limits including electrolytes. Her 24 hour urinary Vanillylmandelic acid (VMA) was 2.3 (ref. value < 13.6mg/day) and serum metanephrine was 20.8 (ref.value < 90pg/ml). Upper gastrointestinal endoscopy showed a lax lower esophageal sphincter tone. A repeat ultrasound confirmed a cystic mass of 15x16 cm occupying the right supra renal region suggestive of adrenal origin. Contrast enhanced computed tomography confirmed the sonological findings [Fig.1]



**Fig.1:** Contrast enhanced computed tomography Abdomen (Coronal view)

with CT washout value of >60% and HU of 8. The patient was taken up for surgery even though it was a non functioning tumor, in view of the large size. Laparoscopic approach was deferred owing to the large



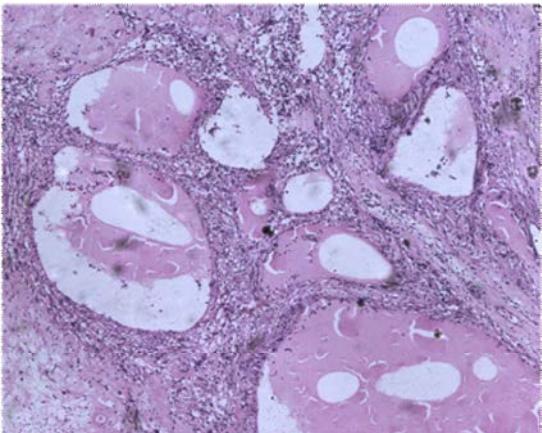
**Fig. 2 :** Intra operative - Adrenal Mass

size of the mass. Thus patient underwent a laparotomy through a Cheveron incision and right adrenalectomy was done [Fig.2]. Specimen was sent for histopathological study, which revealed a benign adrenal

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**Fig. 3:** Histopathology slide - Adrenal Cyst-Low power view of the adrenal cyst composed of multilocular spaces filled with eosinophilic material.

cyst of lymphatic origin composed of multilocular spaces filled with eosinophilic material [Fig.3]. The patient was discharged on the 7<sup>th</sup> post operative day and is doing well on her review, three months later.

## DISCUSSION

Adrenal incidentaloma is a mass lesion greater than 1cm in diameter which is an incidental radiographical finding.<sup>1</sup> They may be either functioning or non-functioning in type. Most of the patients are asymptomatic; however it is important to look for subtle signs of functioning, malignant and metastatic tumors.

Adrenal incidentaloma can be of different pathology, ranging from the non-functioning type which constitutes 78%, Cushing's adenoma (7%), adrenocortical tumor (4%), pheochromocytoma (4%), Myelolipoma (2%), cysts (2%), metastasis (2%) and Conn's adenoma (1%).<sup>4</sup> A detailed history is necessary to differentiate between the types, such as history of headaches, weight loss, anxiety attacks, sweating, cardiac arrhythmias, or palpitations for patients with pheochromocytoma; history of weight gain, easy bruising, severe hypertension, diabetes, virilization, proximal muscle weakness, or fatigue for Cushing's adenoma; presence of hypertension, fluid retention, or a history of hypokalemia for aldosteronism; history of recent weight loss, and a smoking history for metastatic lesions.

Histological classification of adrenal cysts includes four groups namely parasitic, epithelial, endothelial and pseudo cystic types. True cysts of the adrenal gland usually present with epithelial or endothelial lining unlike the pseudocysts. Endothelial cysts commonly manifest as lymphatic type which is secondary to developmental anomaly.

The actual frequency of primary adrenal carcinoma in patients with adrenal incidentaloma is approximately

2 to 5 percent and another 0.7 to 2.5% has non adrenal metastases to the adrenal gland.<sup>5</sup> There was a significant correlation between the size and occurrence of adrenocortical carcinoma, with 90% being more than 4cm in diameter when discovered. A 4cm cut off had a 93% sensitivity of detecting adrenocortical carcinomas.<sup>6</sup> However adrenal mass size alone should not be used as the parameter for guiding treatment. If an adrenal mass measures <10HU on plain computed tomography, the likelihood that it is a benign adenoma is nearly 100%.<sup>7</sup> The usefulness of fine needle aspiration biopsy to distinguish a benign adrenal mass from adrenal carcinoma is limited, however, can differentiate a metastatic lesion from primary adrenal tumour.<sup>8</sup>

The indication for operative intervention of adrenal cysts are size (cysts with 6 cm in diameter or more), symptomatic cysts, functional cysts and cysts that are suspicious for malignancy (as determined by imaging studies). Adrenalectomy may be done laparoscopically, endoscopically via the posterior approach, or as an open procedure.

The anatomical location of the adrenal gland has led to a number of laparoscopic approaches, including posterior or lateral retroperitoneal, transthoracic, and lateral transperitoneal. Contraindications for laparoscopic approach include tumors larger than 12 cm likely containing malignancy and local tumor invasion into adjacent structures. Debate still remains regarding the utility of laparoscopic approach for metastatic adrenal disease and adrenocortical carcinoma.<sup>9</sup> Transabdominal route is recommended in cases of large (> 10 cm) adrenal masses, including those benign imaging features, as the adrenal mass may be diagnosed as malignant on a definitive histologic review.<sup>10</sup>

## CONCLUSION

Adrenal incidentaloma are relatively uncommon but still have to be kept in mind in the differential diagnosis of abdominal masses. Ultrasound imaging is a valuable tool in the diagnosis of the cyst although CT imaging studies are of definitive value. Functional cysts of the adrenal gland require screening for the serum catecholamines and urinary VMA to arrive at the diagnosis. Patients with adrenal incidentalomas who do not fulfill the criteria for surgical resection need to have radiographic reevaluation at 3 to 6 months and then annually for 1 to 2 years. For all adrenal masses more than 10cm, including those masses with benign imaging phenotypes, open adrenalectomy is preferred to laparoscopic approach.

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# AUTO H ANTIBODIES, A RARE ETIOLOGY FOR ATYPICAL HEMOLYTIC UREMIC SYNDROME-A CASE REPORT

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## ABSTRACT

Atypical (non-diarrhea-associated) hemolytic-uremic syndrome is one of the diverse subset of hemolytic-uremic syndrome. The presence of auto antibodies directed against Factor H has been linked to as one of the aetiology for atypical HUS. Herewith we present a 7 year old boy with the above aetiology. To the best of our knowledge this is the first case report from South

India. In cases of atypical HUS, presenting in late childhood we propose the performance of anti-FH auto antibodies screening at the very onset of the disease as timely diagnosis and early initiation of therapy will extrapolate to a good prognosis.

**Keywords:** Atypical haemolytic uremic syndrome, anti FH auto antibodies, plasmapheresis

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## INTRODUCTION

Hemolytic Uremic syndrome presents as a triad of hemolytic anemia, thrombocytopenia and renal impairment. About 10% of overall HUS patients presents with the atypical form of the disease. The annual incidence is 2:1,000,000 population.<sup>[1]</sup> The pathogenesis is a dysregulation of complement alternate pathway. Auto antibodies to complement factor H is one of such rare etiology.<sup>[2]</sup>

## CASE REPORT

A 7 year old male, presented with fever, abdominal pain, vomiting and reddish discolouration of urine for three days. There was a significant history of an unevaluated death in his paternal uncle secondary to some renal disease.

On examination he had pallor, icterus, moderate splenomegaly and his blood pressure was above the 95<sup>th</sup> centile for in his age (132/94 mmHg).

On investigation, his hemoglobin was 9 mg/dL, Total bilirubin 50.78 micromol/L, Direct bilirubin was 14.19 micromol/L. LDH - 2907 U/L, CPK - 583 U/L, serum haptoglobin level was 1.5 micromol/L (normal range 0.33-2 micromol/L) and reticulocyte count (20%) was elevated. He had thrombocytopenia (platelet 60,000/L) and normal total leukocyte counts (11270 x 10<sup>9</sup>/L). Peripheral smear showed microangiopathic hemolysis with fragmented cells and helmet cells. Urine analysis showed gross hematuria with no proteinuria. He had elevated serum urea (21.42 mmol/

L), serum creatinine (150.28 micromol/L) and protein/creatinine ratio (31). USG abdomen showed poorly maintained cortico-medullary differentiation. Complement assay done showed low C3 levels (0.55 g/L) (normal range 0.9-2.07 g/L) and normal C4 level (0.33 g/L) (normal range 0.15-0.55 g/L). He was further worked up for autoantibody Ig G to complement factor H which was elevated 6726.4 AU/ml (normal < 150 AU/ml).

He was started on plasmapheresis, calcium channel blockers and ACE inhibitors, hemodialysis along with Fresh frozen plasma and packed cell transfusion. Child required 5 cycles of plasmapheresis and 2 cycles of hemodialysis. By the 2<sup>nd</sup> week he had become asymptomatic, his blood pressure stabilized, hematuria had cleared. He was discharged on a daily dose of prednisolone (1mg/kg/day) for 4 week.

On regular follow-up, his blood parameters including complete blood count, urine routine, serum urea, creatinine and LDH normalized, hence was prescribed the same dose of steroids on alternate days for 4 weeks. Repeat antibody assay to complement factor H, done after 3 months were within normal limits, hence prednisolone was tapered and later stopped. Currently the child is clinically stable not on any medication, on regular follow up once in 6 months. As the renal parameters had normalized and continued to be normal on follow up, repeat ultrasound was not done.

## DISCUSSION

One should consider the possibility of atypical HUS if any one of the atypical features like no recent diarrhoea, insidious onset, HUS in relapse, presence of a positive family history, age < 6 months or anaemia which could not be explained in the past, is present.<sup>[1]</sup>

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Atypical HUS (aHUS) is sub classified into genetic, acquired and idiopathic forms.<sup>[1]</sup> Among which 6-10 percent of cases have auto antibodies to factor H as the aetiology.<sup>[2]</sup> These antibodies prevent the binding of factor H to C3 convertase thereby affecting the functioning of the complement pathway.

As with our case, C3 concentration was found to be low in 40 to 60% of patients with anti-CFH (Complement Factor H) antibodies whereas C4 levels were normal in previous studies. [4] Any adolescent or preadolescent presenting as atypical HUS, especially in the setting of a low C3, anti-CFH antibodies are to investigated for first, before screening for other etiologies as it is the most common age group of presentation.<sup>[4]</sup>

Early plasmapheresis is the treatment of choice and as studies have shown good response in almost 75% of the episodes.<sup>[5]</sup> Further studies have shown benefits from treatment with steroids or other immuno suppressants as cyclophosphamide, Rituximab, Mycophenolate, in conjunction with plasma exchange.<sup>[5,6]</sup> The duration of plasmapheresis and the choice of the immunosuppressant are not presently standardized. Eculizumab a C5 inhibitor has shown promising results in recent times.<sup>[6]</sup>

As per recommendations the hemoglobin, platelet count, creatinine, LDH, C3,C4 and auto H antibody titers are to be monitored every month in the first year, and then on then every six months.<sup>[5]</sup> So even though our child became asymptomatic, with normal antibodies titers he still under regular follow up.

High antibody titers (over 8000AU/ml), low C3 and delay in plasma exchanges correlate with the risk of relapses and renal sequelae<sup>[6]</sup> In previous case reports from India,<sup>[5]</sup> these types of HUS presented late, and had a relatively severe illness with prolonged oligoanuria, severe hypertension and prominent extra renal manifestations and majority required renal replacement therapy and overall adverse outcome in

33.6% of patients. There are reports of successful renal transplants in patients with HUS.<sup>[5]</sup>

Our case presented early in the course of the disease, without extra renal manifestations and treatment was initiated early and so probably had a favourable outcome.

Recent progress in diagnosis of Anti-factor H antibodies-HUS and therapeutic options, including early aggressive plasma exchange along with various immunosuppressive modalities will help in betterment of prognosis in the future.

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## TORSION OF ADNEXA DURING PREGNANCY: A CASE REPORT

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### ABSTRACT

This is a case report of a 29-year-old Gravida 3, Para 1, Live 1, Abortion 1 with 24 weeks of gestation presenting with torsion of the adnexa. She presented to the causality with acute pain abdomen. With a differential diagnosis of torsion adnexa, ureteric colic, laparoscopy was done. Laparoscopy revealed a twisted and enlarged ovary and fallopian tube which

was detorted. Her pregnancy was followed up. She delivered a healthy female baby at term. Laparoscopy, instead of laparotomy and detorsion of the twisted ovary, instead of its removal helped the patient to recoup faster.

**Keywords:** Laparoscopic detorsion, Pregnancy, Torsion of ovary

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### INTRODUCTION

Torsion of adnexa has been reported to be more common in pregnancy than in the non pregnant state. The physiological enlargement of the ovary in pregnancy and the dynamics of the rapid changes in the size of the gravid uterus, contribute to it. The misconception of the occurrence of thromboembolism following detorsion has been disproved with growing evidence of viable ovaries following detorsion of twisted adnexae which looked blue black at the time of diagnosis. Laparoscopy in pregnancy has also become safe.

### CASE REPORT

A 29 year old G2P1L1A1 with 24 weeks of pregnancy came to the emergency with complaints of pain abdomen for one day. On examination, patient was conscious, coherent. Her pulse rate was 102 beats per minute, blood pressure 130/70 mm of Hg, temperature was normal, cardio vascular and respiratory systems were normal. Abdominal examination revealed fundal height corresponding to 24 weeks of gestation, fetal parts were felt. Fetal heart rate was regular. There was tenderness in left lumbar and left iliac fossa. Laboratory data on admission showed a white blood cell count of 13800, hemoglobin concentration of 9.1 g/dl, with a normal blood platelet level (390,000/mm<sup>3</sup>), a blood urea of 11 mg/dL and a creatinine level of 0.8 mg/dL. Ultra sonography revealed bilateral renal calculi and enlarged left ovary with suspicion of torsion. Obstetric scan revealed a single

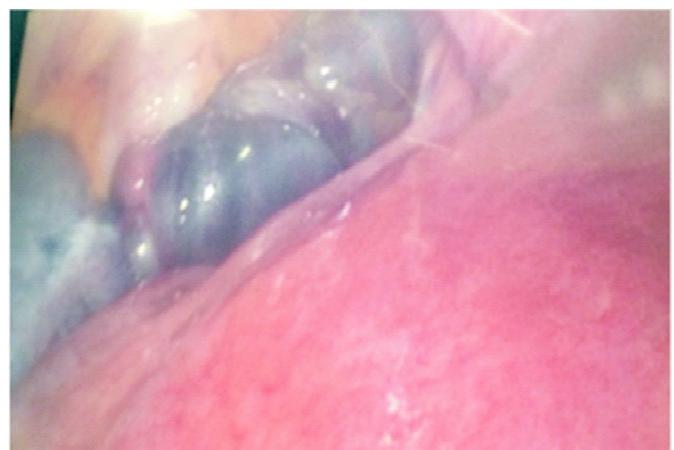
live intra uterine gestation corresponding to 23-24 weeks, amniotic fluid was adequate with a posterior placenta which was not low lying. There was no evidence of free fluid in the abdomen. Urology opinion was obtained, advised IV antibiotics and hydration.

In view of persistent pain and suspicion of twisted adnexa, emergency laparoscopy was done under general anaesthesia after 24 hours of admission. Supra umbilical entry, by Hasson's method in view of gravid

**Fig. A**



**Fig. B**

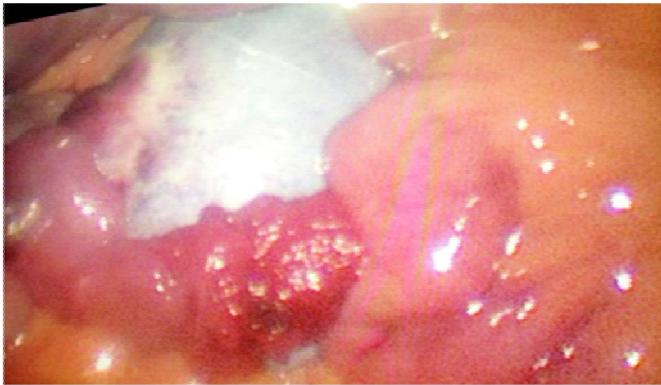


**Fig A & B Torsion of ovary in Pregnancy.**

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**Fig . C:** Normal colour of ovary after detorsion

uterus was used. Left ovary was enlarged to 8 cm size and was hyperaemic with torsion (Fig. A& B). Site of torsion was tubo-ovarian ligament which was twisted  $2\frac{1}{2}$  times over its pedicle. After untwisting the ovarian pedicle, the ovary returned to its normal colour and showed no signs of haemorrhage or necrosis (Fig. C). Foetal heart was checked after the procedure and found to be normal. Patient recovered with an uneventful post operative period and was discharged on post operative day 3. She was followed up, her rest of pregnancy was unremarkable and she delivered a live healthy female baby of birth weight 2.55 kg at term gestation by spontaneous vaginal delivery. Postnatally Transvaginal scan done showed enlarged left ovary. Patient consented for puerperal sterilization and insisted on removal of the left adnexa. Bilateral tubal sterilization was done along with left salpingo-oophorectomy. Intra operatively left ovary was found to be enlarged to 6 x 6 cm size. Histopathology revealed enlarged ovary with haemorrhage.

## DISCUSSION

Diagnosis of torsion of the adnexa especially in pregnancy can be difficult. The use of Doppler does not establish torsion. Torsion had been found even when Doppler shows flow in the ovarian vessels.<sup>[1,2,3]</sup> Most decisions to operate are based on clinical acumen.

Removal of the adnexae in torsion was the established practise. Detorsion of the pedicle was previously avoided to prevent emboli and toxic substances related to hypoxia, from entering peripheral circulation.<sup>[7]</sup>

With many reports of successful detorsion of the pedicle of twisted adnexae, detorsion has come to stay as the treatment of twisted adnexae.<sup>[4,5,6]</sup> Twisted adnexa with a blue black colour have shown viability after detorsion. These ovaries on follow up have shown folliculogenesis, which is the ultimate feature of viability. Untwisting of the affected adnexa was carried

out by simply pushing the ovary contralaterally in the direction of the torsion. This was done using two probes without grasping the tissue, to avoid bleeding. Following detorsion aspiration of cyst contents if any is advised

Laparotomy versus laparoscopy as the route of surgery is no longer a debatable issue in pregnancy. Laparoscopic detorsion is the favoured route in the non pregnant state. In a well selected case, laparoscopy is safe and recovery quick. The primary trocar entry may be modified depending on the size of the uterus. The Hassons open entry may be used in the face of an enlarged uterus.<sup>[10,11]</sup> Laparoscopy has the added advantage of minimal handling of the uterus, which reduces the possibility of preterm labour, and lessens morbidity secondary to infections. Healing of the lap scars is faster. Laparotomy scars made closer to term pose the problems of weakening during straining at labour.

The safety of laparoscopy in pregnancy depends upon the duration of pregnancy, general condition of the patient and associated medical complications if any.<sup>[9,10,11]</sup> The risks of laparoscopy in pregnancy include possible injury to the enlarged uterus, and cardiovascular and respiratory alterations during the pneumoperitoneum and Trendelenburg position.

## SUMMARY

Adnexal torsion is comparatively uncommon in the second trimester of pregnancy. Diagnosis can frequently be made on the basis of the characteristic clinical presentation in conjunction with ultrasound evidence of a unilaterally enlarged adnexal mass. Treatment options may be limited to surgery, either by laparoscopy or laparotomy.

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#### **TABLES:**

- (i) Each table should be typed double-spaced on a separate sheet.
- (ii) The total number of tables should be not more than 3.
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### **GUIDELINES FOR REVIEW ARTICLE:**

Articles addressing a theme of current interest is welcome in this category. Articles should not exceed 4000 words. The manuscript should be prepared as title page, abstract and keywords,

introduction followed by discussion, acknowledgement, reference, tables and figures. Each of the above mentioned should begin in a fresh page

#### I. ABSTRACT AND KEYWORDS:

- (i) In an unstructured format not more than 250 words.
- (ii) It should describe the background and summary of the discussion related to the topic of interest.
- (iii) Minimum of three Mesh words to be mentioned at the bottom of the abstract. Upto 50 references may be included in these articles.

**II. INTRODUCTION:** It should commence on separate page and should briefly explain the reason for the review. This should be a brief overview about what is already known on the topic of the article. This should be followed by a statement on the method of review of literature. A systematic explanation of the methods followed to search the literature on the topic of interest is desirable.

**III. DISCUSSION:** Topic being reviewed in the article should be extensively researched and it should be arranged in a logical manner with relevant subheadings. illustrations, flow charts and tables should be used to explain the text. It should conclude with a brief statement on current opinion on the topic of discussion and future of the same. A hypothesis for future research may also be generated.

Title page, acknowledgement, references, tables and figures should be prepared as per instructions already mentioned under guidelines for original article.

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**ABSTRACT:** It should be no more than 200 words. It should highlight the clinical importance and salient features of the case. 3 Mesh words should be provided.

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Thoughtful discussions of current topics are welcome in this category. Should be no more than 500-1000 words, no tables or figures and references to a maximum of 10. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms, text of the manuscript which may be self styled followed by references

#### GUIDELINES FOR RECENT ADVANCES:

Summary of recent advances in health sciences and education are welcome in this category. Should be no more than 500-1000 words, not more than 2 tables/2 figures and no more than 10 references. The manuscript should be prepared as title page, abstract of 150 words with 3 Mesh terms, text of the manuscript which may be self styled followed by references

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Interesting images which is of practical importance are welcome for this section. It should not exceed 300-500 words. Should be prepared as title page, text which briefly explains the details of the image with a short relevant discussion, images which are appropriately labeled and accompanied by legend and references no more than 5. Images should be as per our instructions mentioned under original article. Abstract is not required.

#### LETTER TO THE EDITOR:

Correspondence with comments on a recently published journal article or scientific content not related to a journal article [ case report, research protocol, original research, etc] is welcome. Word limit is 300 words and 5 references. Abstract is not required. Title page should be as already described.

The submission should be accompanied by

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