

SRI RAMACHANDRA JOURNAL OF MEDICINE

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GUIDELINES FOR AUTHORS

Scope of the Journal

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

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

Submission of Manuscript :

Each manuscript submission should include the following documents.

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

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

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

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

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

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

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

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

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

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

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

5. Other articles: The limit of word count for these articles are as followed:

Article type	Word Count
i) Journal scan	1000
ii) Good practice guidelines	4000
iii) Student experience	1000
iv) Human experience	1000
v) Health care management	1500
vi) Rehabilitation corner	1500
vii) Quality corner	1000
viii) Medical education	1500
ix) Scientific Fillers Quotes	20 - 50
x) Book review	1000

Address for correspondence: Office of the Editor, Sri Ramachandra Journal of Medicine, Sri Ramachandra Medical College & Research Institute (Deemed University) Porur, Chennai - 600 116. www.srmc.edu/srjm

V.R. Venkataachalam

Chancellor
SRMC&RI (DU)

Message from Chancellor's Desk

The publishing of a journal that captures the essence of scientific work done inside an institution is not only an important landmark in the growth of the institution but is also, a yard stick, by which the maturity of an institution is measured. I am delighted to note that "Sri Ramachandra" has reached this important milestone through the publication of its scientific journal "Sri Ramachandra Journal of Medicine".

I am sure that the journal will serve as a vehicle through which Sri Ramachandra communicates its scientific mettle to the rest of the world. I also hope that it will be the launch pad for the careers of many young scholar - scientists. It is my wish that the editors showcase not only the esoteric

and academic but also provide sufficient encouragement to "outcomes based" research on patient-care-quality and safety. I also would like to see the journal become a bridge that helps Translate Research into Practise (TRIP).

I hope that the editors will take a broad based balanced policy that advances scientific temper and the core values of the medical profession and represent the finest and best of our deemed university and academic medical center.

I wish the journal a speedy growth to the status of an indexed journal and quick ascent to a high impact factor. I wish the publication all success.

OUR FOUNDER – THE LEADER

S. Thanikachalam

Chairman and Director, Cardiac Care Center

‘Every job is a self portrait of the person who did it. Autograph your work with excellence.’

I cannot forget that particularly sunny January day, this year. It was the day I received a call from the Dean of Faculties, Sri Ramachandra Medical College & Research Institute, asking me to write an article on our beloved Founder, commemorating the fondest feelings that’s enshrined in my heart for a great soul, who never ceased to fascinate me ever since I first met him in 1992.

At that point in time, I never imagined in my wildest dreams that one day I will work for this great man.

Two years later, when I received a call from the great man himself asking me to come on board the management in a senior administrative position, my joy knew no bounds.

Later, when my friends and colleagues called up and asked what made me choose SRMC from amidst others, I simply said, ‘It is a privilege and an honor to work for someone of an extraordinarily high calibre as Shri. Ramasamy Udayar!’

Even as a boy, he must have been different. While his friends would have been devising ways and means to cut classes to watch the local matinee, he was probably dreaming about how, like young Alexander of Macedonia-before he went out and conquered Asia Minor -he would cut the Gordian knot of a commonplace farm life existence in one fearless and determined stroke of his genius!

Memorizing mundane verses was not his forte. Conquering the Everest of his own limitations certainly was. And once he was perched firmly on the peak, he began to build this eyrie- an eagle’s nest- from which, thousands of young eaglets flutter and rise today. Energised by his vision. To catch the thermals of success from their own little crannies.

For him the world was not a place to acquire education. Rather, the world itself was an education. Creativity infused his thoughts, perseverance powered his actions, our beloved Founder, the Late Shri NPV Ramasamy Udayar, was different from his parents as day was different from night.

His dreams were driven by divinity, failure naturally failed to fail him. Robust from his commercial successes, he could have gone on an endless expansion drive, as is the norm and wont of many today. Mergers & acquisitions,

Leveraged buy outs,

and some such caviar to the Generals.

But being an Ulysses in thought as much as Herculean in his efforts, his gaze turned towards medical education.

The quest against the darkness of the soul and decay of the diseased body. Through an odyssey of enlightenment, beckoned by the beacons of knowledge, founded SRMC & RI in 1985.

Soon it became an institution of excellence that has never ceased to amaze visitors from the west and the east alike for the world class educational and clinical standards. In 1994 it was awarded the distinguished status of a deemed university. In 1997, SRMC created history when an alliance was made with Harvard Medical International, Massachusetts, USA

Skilled exponents in the art of medicine, grounded on the solid foundations of science, of practicality, and the needs of the patient, today, the fruits of Shri. N.P.V. Ramasamy Udayar’s toil are here in front of us. A world class Medical University with its own state of the art Medical Centre, run by outstanding faculty who take the ever changing and evolving science of another sphere altogether.

Where Life is their life’s work.

He was a man of words sparsely spoken.

Yet fittingly spoken.

Words of wisdom.

Words of power and persuasion.

A mighty creative power that’s fed by the deepest calm within.

Sparking others to perform.

Beaming his vision on their collective Consciousness.

His uncanny common sense and intellectual prowess was legendary. There was nothing that could resist his will.

Which was of a caliber that would simply stake its very existence in pursuit of its stated purpose.

Try stopping that,

to him, overwhelming odds exist only for one reason.

To be overcome!

Like Shakespeare said,“... the elements so mix’ d in him that Nature might stand up and say to all the world, “This was a Man!”

His simple and affectionate nature had its own sweet fragrance, endearing him instantly to people from all walks of life. He talked with crowds and kept his virtue. He walked with kings but didn’t lose the common touch.

The poor and the downtrodden always tugged at his heart.

That's perhaps why he went ahead and established the free ward section with 750 beds earmarked exclusively for the poor from some 140 villages that surround this campus.

Mother Teresa's visit to this institution touched something deep and sacred within him. He was not the same person anymore. Life took on another hue. It was as if a new and hitherto concealed dimension of his existence suddenly tumbled out in front of him. And he wanted to respond to that new awareness and new responsibilities quickly before his time on this earth ran out.

He worked with a purpose that was too noble for us to understand.

His faith in people was total. Once, when we were finalizing the paperwork for purchasing an expensive cathlab system for the Cardiac Center, he stunned me by ordering a recall of a letter of indent that was about to be dispatched to a firm simply because I had impressed upon him about the efficacy of buying the equipment from a different one.

His sense of gratitude never ceased to amaze me. Years ago, having recognized a patient at my department as the brother of a man who had helped him a long time ago, he called me up immediately and requested that I may not charge the patient for consultation treatment and other tests. Indeed, he vouchsafed that he himself would pay out of his pocket on the patient's behalf, while preferring that this charitable subterfuge may not be disclosed to the patient himself.

I have had the privilege of being privy to all of his greatness for merely 5 years out of the 11 years, I have served at this institution. But it feels like I have known him for many decades.

His quick wittedness.

His razor sharp decision making intellect.

His hate for procrastination.

His unfailing respect and regard for the human soul – irrespective of its worldly appendages and physical accoutrements. They continue to enchant me and educate me even in the evening of my life as I muse over a mentor whom I miss sorely every day, in the most inexplicable way, as I go about discharging my duties. Who said He's gone to be with the Gods?

If any thing, he's only gone to revive the gods within each one of us. I feel his transcendental presence right here in this huge campus – which is the actualization of his most ardent dream-a dream to serve mankind in the truest sense and leave behind a legacy that's lofty and laudable. This great institution that breathes his name collectively, in one great heaving breath of adulation. Indeed, in the twilight of many an evening, when I watch the sinking sun and the lengthening shadows of the wonderful edifices that he'd so lovingly built, it appears to me that those majestic shadows are his own- in a mystical and awesome way.

And, as a votary of the belief in reincarnation, I personally have no doubt that he'll come back to us in another avatar, in another time. In the generations to come, maybe decades after we pass on, posterity will think of us as those who have taken the cue from our great Founder, to align our individual goals along the lines of his own. To serve mankind at our fullest ability. By persevering. Never forgetting to fill the unforgiving minute, with sixty seconds' worth of distance run. Then ours will be this Earth and everything that's in it.

Let's be worthy of him

Thank you!

SRI RAMACHANDRA TOWARDS EXCELLENCE

Dr. SSK. Marthandam

Vice-Chancellor

Sri Ramachandra Medical College and Research Institute (Deemed University) has been a pace setter in many areas of medical education. Ours is one of the first private medical colleges started in Tamilnadu. It is also the first to get the Deemed University status in the medical field.

The above achievements as well as the many innovative programmes that followed have been possible because of the vision and the commitment to excellence of the management.

It has been our endeavour to make this great institution foremost and best in our country. The nine colleges and ninety one courses run by the University, share one thing in common i.e. Commitment to Excellence. We all know that a concept in management is as strong as its weakest link. That is the reason we do not want weak links. We all aim towards perfection in all our endeavours.

Our commitment to excellence makes us go beyond our shores to interact with the best of the Universities giving us an opportunity to adapt the best practices around the globe and make it relevant to India. This has enabled us to take a good look at our own curriculum, faculty development and also leadership development in this University. It has resulted in special focus groups being assigned to various areas of development.

We had an occasion to celebrate 2 decades of services in 2005. This has resulted in Twenty Point Programme viz. GATE (Growth and Advancement Towards Excellence) which was initiated by our Chief Executive Director.

Students of all colleges who have passed out of this institution have found employment all over the world and have always cherished the years they spent in this campus. This University has also thought of all other aspects of human development in addition to the formal curriculum. This is the reason you see a wonderful landscape and also all the facilities given for sports and games by our Chancellor.

The Hospital is 1675 bedded multi-speciality tertiary care hospital with all specialities under one roof. Infrastructure in the hospital is second to none and standard of care given is of the highest order. It is important to remember that the same care is given to the free patients also. In fact 750 beds have been allotted for this. We have the state of the art equipments in all specialities and an excellent faculty whose services are recognized by the peers and very much appreciated by the patients.

We also have done a lot of work in research and we have a group of people who are intensely interested in research and who are able to get grants not only from India but also from overseas.

Our institution has grown in leaps and bounds in the last 20 years. However this necessitated that we create forum for communication among faculty members. Towards this end we have a Newsletter. **Sri Ramachandra Journal of Medicine** will be another feather in the cap. This will enable us to publish many high quality articles from various departments. It will not only help all the faculty members to know what is going on, it will also set standard in publishing scientific articles.

From the Editor's Desk

It is a moment of privilege for me to pen down few of my thoughts on the eve of the maiden issue of our Medical Journal - SRJM. The University is two decades old and it is the desire of the Chancellor, Vice Chancellor, Registrar and the host of elders and well wishers to bring out our University Journal. Today you have the 1st Edition of this journal, because of the labour, sweat and toil of my colleagues of the editorial board. I will fail in my duty if I don't mention the role of Prof K.V. Somasundaram, Director - Medical Education who was responsible in constantly interacting and directing everybody in the Editorial board and the administration with a set goal to get the 1st Edition published on time. It is possible to bring out this venture because of generous monetary and material support apart from personal involvement of Chancellor, Thiru. V.R. Venkataachalam and the Registrar of the University Tmt. Radha Venkataachalam.

It is the desire of the Editorial Board to maintain best standards in the years to come also and be an icon for other Medical Universities to follow this University.

With the goodwill and support of everybody, we are sure it will be a regular feature from Sri Ramachandra Medical College and Research Institute (Deemed University).

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

Dr. J.S.N. MURTHY

Editor,
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University Building



Medical Centre



Shri. **N.P.V. Ramasamy Udayar**,
Founder-Chancellor with Mother Teresa



Thiru. **V.R. Venkataachalam**, Chancellor and Thirumathi **Radha Venkataachalam**,
Registrar of SRMC & RI (DU) with his
Excellency **Dr. A.P.J. Abdul Kalam**, President of India



Prof. **M.S. Swaminathan**, Chairman, M.S. Swaminathan Research Foundation, Chennai
with Thiru. **V.R. Venkataachalam**, Chancellor at the Founder's Day & University Decennial
Celebration, 19th September, 2004



Dr. Kanu Neuro Surgery, Japan with
Shri. **N.P.V. Ramasamy Udayar**, Founder Chancellor



Smt. **Rajashree Birla**, Director, Aditya Birla Group of Companies with
Dr. Joseph B Martin, Dean, Harvard Medical School at the 5th Convocation
14th February, 2003



Prof. H. Thomas Aretz, Vice President for Education,
Harvard Medical International with Thiru. **V.R. Venkataachalam**,
Chancellor, at the Workshop for Leaders in Medical Education,
26th January, 2005



Lecture Hall



Harvard Medical International & SRMC
- Leadership Training Program



Library



Active Learning Centre



University Auditorium



Hostel



Hostel Gym



Outdoor & Indoor



Indoor Games

COCHLEAR IMPLANTATION

A. Ravikumar*, S. B. Jothiramalingam, K. Senthil, Manu Vergis, Vivek Sasindran

Cochlear Implantation

In the past, there was little anyone could offer to alleviate severe to profound sensorineural hearing loss and the deaf person had to learn to cope as normally as possible in the absence of hearing. In time if the hearing faculties are not developed well, the child invariably becomes too. It was accepted by most otologists, audiologists, teachers and other professionals that the most practical means of educating deaf children was to teach them primarily using 'sign' language, utilizing the visual system. The cochlear implant has radically changed the outlook for profoundly deaf adults and children. The Cochlear Implant can provide sufficient hearing sensation to enable most deafened persons to continue communicating using speech and can provide the opportunity for children born deaf or deafened early in life to use speech as their primary means of communication. In this surgery an array of electrodes is placed within the cochlea to electrically stimulate the damaged hair cells.

January 17th, 2006 was a land mark in the history of Department of ENT, Head and Neck surgery in our institution as the first Cochlear Implantation surgery was carried out by Prof. A. Ravikumar and his team. This was the first time a Cochlear Implantation surgery was being performed in a medical college in Chennai. Our patient was a 3 yr old girl, daughter of a bank employee, who was diagnosed to have bilateral profound hearing loss from childhood. She was using hearing aids in both ears for the last one and half years with no hearing benefit. She was evaluated in detail with a battery of audiological tests like BERA, OAE, Tympanometry, ASSR, Speech evaluation and Behavioral observational audiometry in the Department of SLHS. All tests confirmed the diagnosis of bilateral profound hearing loss. Radiological evaluation with CT scan and MRI studies of the temporal bone revealed normal inner ear anatomy. After detailed counselling of the parents on several occasions she was selected to undergo cochlear implantation in the right ear. The surgery was carried out successfully and the performance of the implant was assessed after implantation in the operation theatre and found satisfactory. Patient was discharged from the hospital after one week. 3 weeks following surgery switch on of the implant was carried out on 10th February and child could appreciate sound for the first time in her life. She is on regular follow up at present. An intensive habilitation program has been started, which will be spread over a period of 6 months as it is an important and



integral part of the cochlear implantation. The mapping and habilitation program is under the supervision of Prof. Roopa Nagarajan, Head of the Dept of Speech and Audiology. SRMC & RI (DU) is the first medical college to have an exclusive Cochlear Implant Program (CLIPS). Cochlear Implant package is offered at a cost of rupees six lakhs (inclusive of investigations). Implant that was used for the surgery was a Nucleus C124, manufactured in Australia. The cost of this implant alone is 5.14 lakh rupees. At present we have a dozen candidates who are being evaluated for the same problem in our institution. India is the country with the highest number of potential candidates for cochlear implantation. We hope that we will continue to bring ray of hope into the lives of those million deaf children in our country.

Historical Background

The first suggestion that electricity could be used to help the deaf has been attributed to Benjamin Franklin. In 1957, Djourno published the first description of cochlear implants inserted into two totally deaf patients. This stimulated much interest especially in the USA. In 1961, House et al implanted a single electrode into the cochlea and soon afterwards Simmons et al (1964) inserted six stainless steel electrodes directly into the auditory nerve. Both devices were initially successful but met a howl of criticism as the scientific community did not feel sufficient basic research had been accomplished to justify human implantation. Several workers began some careful animal experimentation. It was William House who recommended human implantation in 1969. In 1972, the single wire intracochlear device was designed by House and Urban. Clark and coworkers developed a multielectrode intracochlear implant at the University of Melbourne. This device was developed further as a 22 electrode array by a commercial company called Nucleus Ltd. The Melbourne/Nucleus implant device was the first commercially available cochlear implant which was capable of providing recognition of speech without any contextual clues by listening alone. The device is now being produced commercially by Cochlear Ltd and since 1994 several improvements to the speech processing strategies have occurred. The nucleus is the first implant to get FDA approval. We have used the Nucleus 24 channel implant in our patient.

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INTERVENTIONAL RADIOLOGY – SPECIALITY OF THE CURRENT CENTURY: INITIAL EXPERIENCE AT SRMC & RI

Santhosh Joseph *, Deepak Bharathi, Senthilnathan

Keywords : Interventional Radiology

INTRODUCTION:

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

MATERIALS AND METHODS:

Invasive angiography and interventions performed by the interventional radiologist has been analyzed. A total of 2732 angiographic / interventional procedures were performed (M=1737: F=995; Age range=1day to 87years, N = 48years). The procedures were performed using Biplane DSA 3D rotational angiography system (LCN+ GE Milwaukee). The indications for interventional

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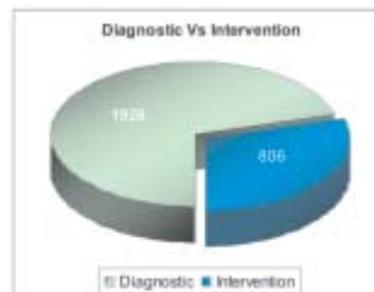


Chart 1: Diagnostic and Interventional procedures



Chart 2: Number of procedures

procedures included acute vascular emergencies, control of acute bleeding, acute stroke, acute limb, several difficult clinical problems such as AVM, aneurysms etc. and for preventive measures, to manage therapeutic complications, as an aid to palliative care, pain management and infertility. This article aims to highlight the variety and the scope of these procedures in patient care.

A) INTERVENTION IN ACUTE VASCULAR EMERGENCIES

A.1 Aortic dissection (Fig. 1)

Aortic dissection typically originates in the ascending aorta (Type A) or post left subclavian artery (Type B). Both are surgical emergencies and cause high degree of morbidity and mortality due to co-morbid conditions. Branch vessel occlusion with end organ ischemia complicates approximately 1/3 of cases of aortic dissection^(1, 2). Mortality for aortic dissection is 15-25% but exceeds 50% when the dissection is complicated by paraplegia due to renal or mesenteric ischemia^(1,2). Several percutaneous options for management of acute and chronic dissection are available. Placement of a stent – graft over the entry tear has been shown to lead to depressurization of the false lumen and restoration of normal flow dynamics in the true lumen. Percutaneous fenestration of the aortic flap (intentional creation of a large distal exit tear) can decompress the false lumen and relieve obstruction of the true lumen.



Fig.1 Aortic dissection

Aortic dissection. Patient presented with sudden onset of chest pain and hypotension. ECG and Troponin T was negative. **A:** 3D CT Reconstruction shows Type B aortic dissection. Stent graft was deployed to seal the entry point. **B:** 3D CT reconstruction after stent graft deployment site shows disappearance of the false lumen.

A.2 Aortic injury

Aortic injury may result from rapid deceleration and crush injuries, penetrating wounds or instrumentation during surgical or angiographic procedures. The majority of thoracic aortic injuries are due to blunt trauma. Transections of the ascending aorta are almost uniformly fatal. Survival beyond the initial injury occurs only when a pseudoaneurysm forms that is contained by adventitial or periadventitial mediastinal tissues. Mortality from untreated aneurysm rupture exceeds 90% within a month. The conventional therapy for aortic transection is surgery with placement of a short tube graft. This surgery has a 5-10% risk of spinal cord ischemia but otherwise has excellent long term results. Endovascular stent grafts can be used to exclude the traumatic aortic pseudoaneurysm. This may be the preferred initial management in a multitrauma patient, followed later by elective surgical repair.

A.3 Aortic aneurysm (Fig. 2)

Infra renal abdominal aortic aneurysm (AAA) is the

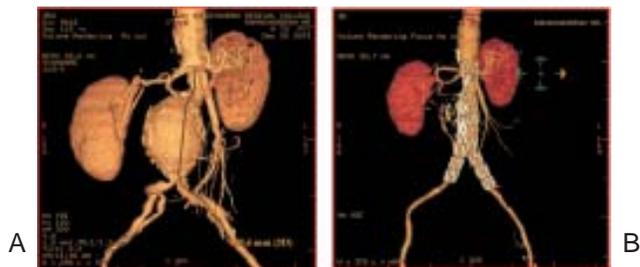


Fig.2 Aortic aneurysm

Infra renal aortic aneurysm with impending rupture. Patient presented with severe abdominal pain and hypotension. **A:** 3D CTA showing infra renal aortic aneurysm. **B:** CT Angiogram after stent graft deployment showing exclusion for the aneurysm from circulation with preservation of flow in the renal arteries. Patient had a remarkable recovery.

most common aneurysm of the aorta. The natural course of AAA is expansion and rupture. Estes reported that 1-, 3- and 5- year survival rates of untreated patients were 67%, 49.2% and 18.9% respectively⁽⁴⁾. In his series, the cause of death was aneurysm rupture in 63.3%. The current standard of treatment for AAA is open surgical repair, which carries a low overall risk mortality of 1.4 to 6.5%⁽³⁾. In high risk patients with co-morbid medical conditions or in patients with impending rupture, the operative mortality is considerably higher (5.7 to 31%)⁽⁵⁾. Endovascular stent graft repair may provide a suitable alternative for the high risk patient. Aortic stent grafts are available in three basic configurations. Patients must meet anatomic criteria involving the proximal and distal attachment sites, angulation and tortuosity of the aorta and pelvis, the presence of calcification and occlusive disease in the access arteries.

A.4 Acute Limb Ischemia (Fig. 3)

The acute, profoundly ischemic limb is a surgical emergency. Cell death begins after 4 hours of total ischemia and is irreversible after 6 hours. Hence, urgent revascularization is necessary. The mortality of patients with acute limb ischemia is almost 25% despite aggressive intervention, with amputation in 20% of those that survive. Surgical intervention remains the choice of therapy.

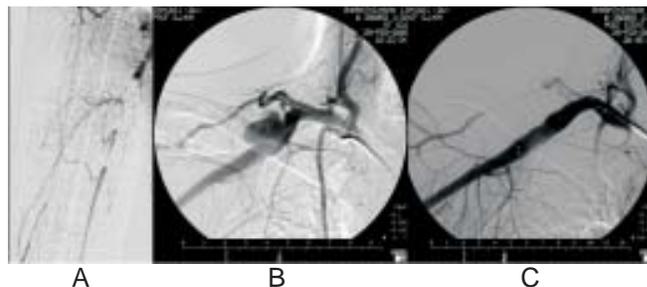


Fig.3 Acute Limb Ischemia

Acute right upper limb ischemia. 11 year old boy presented with history of acute pain and swelling of the right upper limb with previous history of cricket ball injury. **A:** Brachial angiogram shows embolic occlusion of brachial, ulnar and radial arteries. **B:** Traumatic pseudo aneurysm of right subclavian artery. **C:** Angiogram following stent graft placement reveals complete exclusion of the pseudoaneurysm from circulation. Patient underwent surgical embolectomy through the brachial arteriotomy. Limb function improved and became normal.

Percutaneous interventions in appropriate patients are indicated when emergent revascularization is not required. Pharmacologic, mechanical and aspiration thrombectomy are useful techniques in both embolic and thrombotic occlusion. Occlusions less than 14 days old are amenable to pharmacologic and mechanical thrombolysis^(6,7). Aspiration thrombectomy should be considered for acute (<48 hours) occlusions. Thrombolysis and mechanical thrombectomy successfully restore antegrade flow in over 95% of patients provided that the occlusion can be crossed

with an infusion catheter or thrombectomy device. Often a combination of intervention and surgical procedures may be helpful to achieve the optimal results.

B) CONTROL OF HEMORRHAGE

B.1 Bronchial arterial embolisation (Fig. 4)



Fig.4 Bronchial arterial embolisation

Hemoptysis. 65 year old patient presented with intractable hemoptysis. **A:** Right bronchial angiogram shows hypertrophied right bronchial artery with abnormal lung vascularity. **B:** Post embolisation angiogram shows reduction of flow. Patient had no hemoptysis on follow up.

The most common cause of hemoptysis in our country is infective lung disease such as Tuberculosis or Bronchiectasis; malignant disease, sequestration and AVM being the other causes. Massive hemoptysis is defined as hemorrhage > 300mL in 24 hours. A single episode of hemoptysis carries the risk of asphyxiation if it approaches the volume of the tracheo-bronchial tree (approximately 150ml)⁽⁶⁾. Massive hemoptysis has a high mortality rate (> 50%) when treated conservatively. Surgery has been considered the standard therapy in patients with adequate lung function and localized hemorrhage. However it may not be feasible in patients with poor pulmonary reserve and carries high morbidity and mortality in an emergent situation^(9,10,11). BAE is a well accepted procedure in the management of massive or recurrent hemoptysis in a patient who is a non surgical candidate. The purpose of BAE is defined as: first, to achieve immediate control of bleeding in all patients, second to obtain continuous bleeding control in patients without surgery; and third, to improve clinical conditions for a prospective surgery.⁽¹⁰⁾

B.2 Upper GI bleed (Fig. 5 & 6)

The causes for GI bleed include gastritis, bleeding ulcer, benign or malignant tumors or angiodysplasias. Most of the patients are in an unstable state and surgical correction carries significant morbidity and mortality. Because the risk of post embolic ischemia is minimal due to rich collateral circulation in the upper GI tract, transcatheter embolotherapy is more preferred method compared to pharmacotherapy. The goal of embolotherapy is to reduce the pressure in the bleeding artery while maintaining enough collateral flow to preserve viability. Bleeding gastric and duodenal ulcers and hemobilia can be effectively managed by embolotherapy with gelfoam, polyvinyl alcohol particles

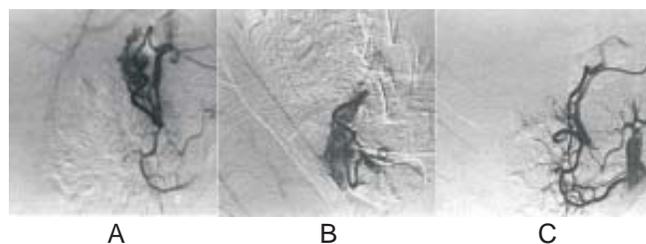


Fig.5 Upper GI bleed

Upper GI Bleed. 25 year old male presented with severe hematemesis with drop in hemoglobin (Hb – 4gm%). **A:** Selective gastro duodenal angiogram showing angioma in the first part of the duodenum. **B:** Catheter advanced selectively into the feeder and embolised using particulate agents. **C:** Post embolisation angiogram shows disappearance of angioma and the patient did not have further hematemesis

(Ivalon) and coils. Gelfoam powder, extremely small Ivalon particles and absolute alcohol should not be used because of the risk of mucosal ischemia or necrosis.⁽¹²⁾ In patients with upper GI bleed due to varices, the TIPS procedure is of documented benefit in case of failed medical management. The transjugular Intrahepatic portosystemic shunt (TIPS) procedure decompresses the portal venous system by the percutaneous creation of a low resistance tract in the liver between the portal and hepatic venous systems. The availability of stent grafts has vastly improved the primary patency of these shunts. TIPS is also of documented benefit in intractable ascites due to portal hypertension. The technical success rate for TIPS for variceal bleeding averages about 97% with hemostasis obtained in approximately 98%.

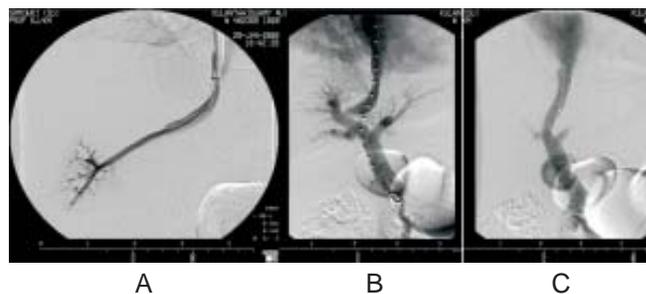


Fig.6 TIPSS

TIPS. 48year old gentleman presented with intractable ascites due to hepatitis B related cirrhosis. **A:** Right hepatic venogram. **B:** Right portal vein access obtained through the liver parenchyma. **C:** Following successful stent graft placement forward flow noted from the portal vein into the IVC through the stent. Following stent placement portal pressure gradient drop from 40 to 22mm. Significant reduction in ascites after TIPSS

B.3 Lower GI bleed (Fig. 7)

The common causes of major lower GI bleeding are diverticular disease, angiodysplasia tumors, ischemic enteritis, Meckel's diverticulum, inflammatory bowel disease and post polypectomy bleed.^(13,14) Embolotherapy is safer in

the small intestine than in the colon because of relative spare vascularity and poorer collateral pathways in the colon.⁽¹⁵⁾ Embolisation should be performed at a level distal enough to maintain some collateral perfusion with a caution of preserving vasa recta.⁽¹⁶⁾ Although embolotherapy for colonic bleeding presents a considerable risk of acute mucosal necrosis, the risk is less than for surgery.

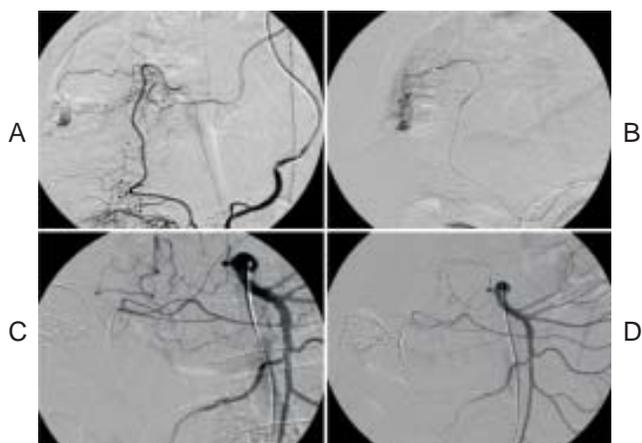


Fig.7 Lower GI bleed

Lower GI Bleed. 64 year old male known CAD underwent CABG on anticoagulants presented with intractable bleeding per rectum. **A:** Selective superior mesenteric artery angiogram revealed bleeding angioma in the ascending colon. **B:** Microcatheter placed just proximal to the bleeding point and embolised with NBCA. **C & D:** Post embolisation shows complete occlusion of the bleeding point. Patient hematocrit improved with no further episodes of bleeding

B.4 Trauma

The diagnostic angiographic examination in trauma patients is focused on the injured limb segment. The range of vascular injuries include spasm, intimal tears, pseudoaneurysm, extravasation, occlusion and arteriovenous fistula. Branch vessel pseudoaneurysms and arteriovenous fistulas can be easily treated by transcatheter embolisation. Similar injuries to the major arteries can be effectively stabilized with stent grafts.

B.5 Postpartum and gynaecologic bleeding (Fig. 8)

Pelvic embolisation is sometimes required in women with vaginal bleeding following vaginal delivery, obstetric or gynecologic surgery or from unresectable gynaecologic tumors. A pelvic angiogram followed by selective internal iliac injections should be obtained. Post partum bleeding from uterine atony or placental abnormalities can be managed with selective catheterization of the uterine artery and embolisation with PVA particles or gel foam. Small permanent particles, such as 300-500mm are usually used to devascularize tumors. The procedure is simple and can preserve the uterus and reduce the number of blood transfusions. Post partum hemorrhage may at times be resistant to medical management and destructive

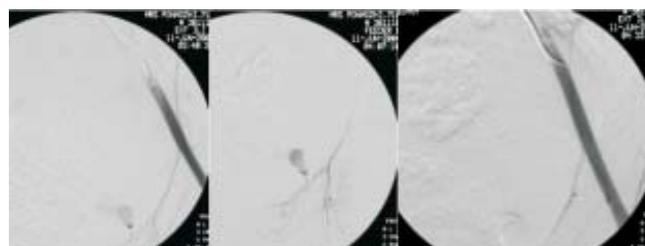


Fig.8 Postpartum and gynaecologic bleeding

Post partum haemorrhage. 27 year old lady presented with profuse bleeding following delivery. **A:** Selective angiogram of the external iliac artery shows pseudoaneurysm from a small tip. **B:** Micro catheter placed distally within the pseudoaneurysm, embolised with NBCA. **C:** Post embolisation angiogram showing total embolisation of the pseudoaneurysm

surgical techniques such as hysterectomy or internal iliac artery ligation with its complications may have to be performed.

B.6 Epistaxis (Fig. 9)

Causes of nose bleed include spontaneous anterior or posterior epistaxis conventionally treated by nasal packing. Bleeding may be intractable when it is due to defective vascular disease such as carotid aneurysm or AVM. Nose bleeds are common and usually due to source in the anterior portion of the nose. These bleeds can be controlled easily by surgical packing. Posterior epistaxis is much more difficult to control by post nasal packing. In cases where conventional management fails, surgical ligation or clipping

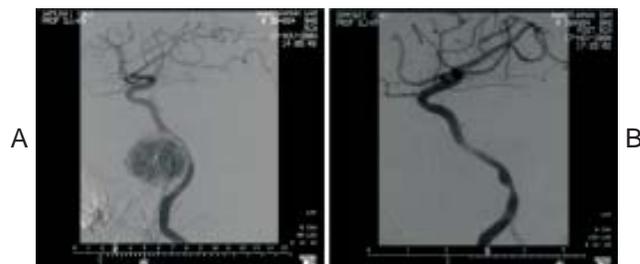


Fig.9 Epistaxis

Epistaxis. This 54 year old male presented with intractable epistaxis. **A:** Right internal carotid angiogram shows pseudoaneurysm in the petrous segment of ICA. **B:** Following stent graft placement the pseudoaneurysm was completely excluded from the circulation. Patient recovered with no episodes of bleeding

of internal maxillary artery is done which has a 10-30% failure rate. Selective catheterization of the internal maxillary artery and embolisation with particles (Ivalon) and gel foam successfully controls bleeding in 75-95% of the cases. Adjunctive coil embolisation can be considered in patients with intractable epistaxis.

C) PRIMARY INTERVENTIONS IN STROKE

Stroke is the third leading cause of death in the United States and perhaps the greatest cause of morbidity. There

are 150,000 deaths from stroke per year, and about 550,000 new strokes per year. Lingering terminal existences and morbidity is the rule and causes a high disease burden for the community.

C.1 Ischemic Stroke (Fig 10)

In most ischemic insults, there is an area of irreversible tissue damage surrounded by a region that may be viable and teetering on the brink of cell death - the ischemic penumbra.^(17,18) It is this potentially salvageable ischemic penumbra that results in clinical improvement in many cases and that is the true target of therapy. The choice of treatment of an intracerebral arterial occlusion is revascularization by intra-arterial thrombolysis by superselective catheterization of the involved vessel. The occluded vessel can be opened

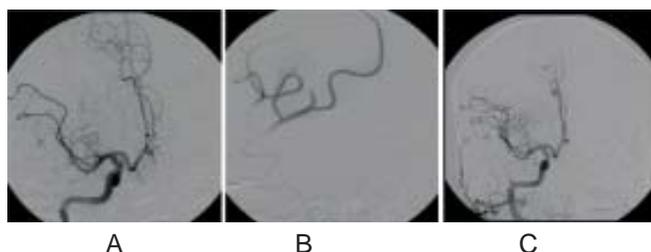


Fig.10 Ischemic Stroke

Ischemic Stroke. 40 year old lady presented with sudden onset of left hemiplegia. On examination, power on the left side was 0/5. **A:** Right ICA angiogram shows occlusion of the superior trunk of right MCA. **B:** Micro catheter was placed distally in the occluded artery and thrombolysis was performed. **C:** Post thrombolysis ICA angiogram shows restoration of flow in the superior trunk of MCA. Following thrombolysis, her power improved to 4/5.

using varying strategies which include infusion of pharmacologic agents, aspiration of thrombus and mechanical thrombolysis with catheters. Several recent studies give encouraging results using these techniques.

C.2 Aneurysmal hemorrhage (Fig 11 & 12)

The incidence of intracranial aneurysm in the general population is between 1.5 to 8%.^(19,20) The peak age for rupture is 40-70 years. The mean being 50 years.⁽²¹⁾ Patients with acute sub-arachnoid haemorrhage secondary to aneurysm had a 36.2% mortality rates and an additional 17.9% morbidity rate; only 46% of patients had a favorable outcome at 90 days.⁽²²⁾ The ideal treatment of an aneurysm is total exclusion from the circulation by open surgical clipping. As an alternative, endovascular non-operative techniques with platinum micro coils are becoming increasingly popular. In addition complex aneurysm with irregular/wide neck can be treated by stent assisted / balloon assisted coiling technique making a new opportunity for treatment in several situations. The GDC (Guglielmi detachable coil) is a platinum coil soldered to the end of an insulated stainless steel introducing guidewire. A low voltage current employs electrolysis to detach the coil (dissolve the solder) when the coil is accurately positioned within the aneurysm. Regardless of aneurysm location, GDC coiling is today applicable for most intracranial

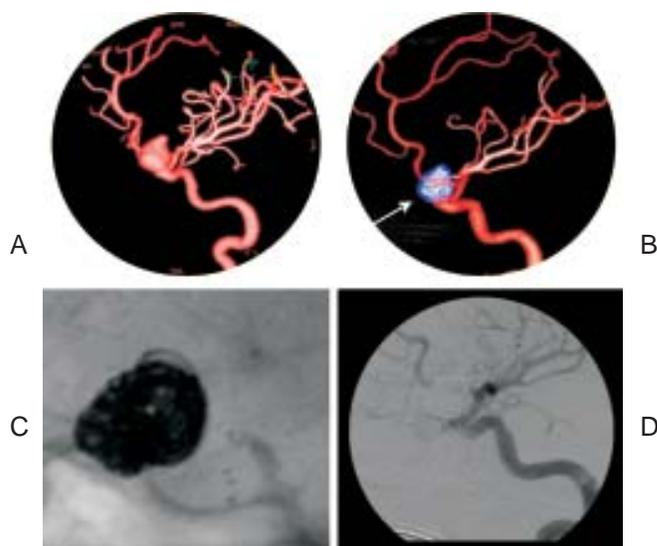


Fig.11 Aneurysmal hemorrhage - Case 1

Aneurysmal hemorrhage. Patient presented with headache, vomiting and unconsciousness, CT scan showed Subarachnoid hemorrhage. **A:** Left carotid angiogram 3D DSA – VR image, showing large left carotid ophthalmic segment aneurysm. **B:** Stent assisted coiling done, 3D DSA VR shows exclusion of the aneurysm from circulation. **C:** Coiling was performed through the stent struts. **D:** Post procedure DSA Angiogram showing exclusion of the aneurysm from circulation

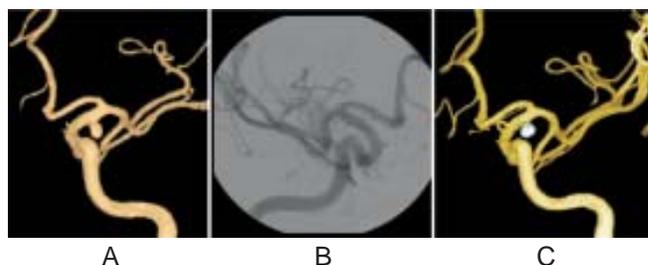


Fig.12 Aneurysmal hemorrhage - Case 2

Aneurysmal hemorrhage. 43 year old female presented with severe headache and unconsciousness. CT scan showed Subarachnoid hemorrhage. **A:** Right carotid angiogram 3D DSA – VR image, showing small PCom aneurysm. **B:** Post coil embolisation angiogram shows complete exclusion of the aneurysm from circulation. **C:** 3D DSA VR image shows coils in situ. Patient recovered without any neurological deficits

aneurysms.⁽²³⁾ Aneurysm size and RSN (Ratio – Sac / Neck) are the main factors critical to the success of endovascular therapy. Further, aneurysms that are difficult for surgical access such as basilar top, carotid ophthalmic or infraclinoid locations can be effectively managed using endovascular technique.

D) INTERVENTION AS A PREVENTIVE MEASURE

D.1 Stroke Prevention - Carotid Angioplasty and Stenting (Fig. 13)

Patients who have 70% or greater stenosis involving the ICA have a 30% risk of stroke during the next two years.

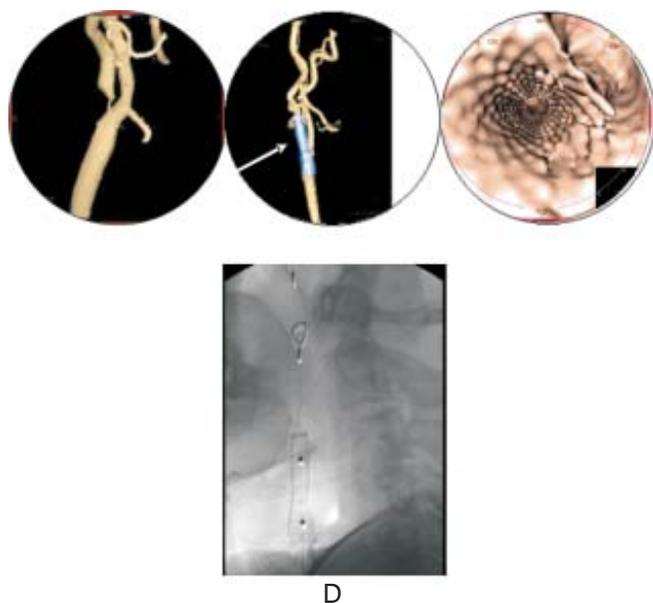


Fig.13- Carotid PTA and Stenting

Carotid PTA and Stenting. Senior Insurance Officer presented with transient loss of consciousness. **A:** Angiogram revealed significant narrowing of left carotid artery. Conventionally treated by surgical endarterectomy. This patient was treated by angioplasty and stenting with distal protection with filter. **B:** Stent in situ. **C:** 3D Rotational Angiogram with endoscopic view showing the opened stenosis and stent struts. **D:** Distal EPI filter was placed prior to deployment of the stent

Ulceration of the plaque increases the subsequent risk of stroke to about 7.5% per year.⁽²⁴⁾ Carotid angioplasty has the following advantages over carotid endarterectomy- a) there is no cervical incision. Cranial nerve palsies by manipulation are uncommon.⁽²⁵⁾ Cerebral ischemia due to clamp occlusion of the carotid artery is not a problem with angioplasty. Lesions that are surgically inaccessible can be treated with angioplasty. With angioplasty, there is no need for general anaesthesia and the patient's clinical status during the procedure can be monitored. Post operative recuperation is shorter and less intensive than for endarterectomy. Cerebral protection during angioplasty can be achieved by two mechanisms: mechanical or pharmacologic. Mechanical means are intended to prevent emboli from arising at the site of the angioplasty and can be achieved using embolic protection filters. Pharmacologic cerebral protection can be achieved by using agents such as Nimodipine. The NASCET Trial has conclusively proved the superiority of endarterectomy in preventing stroke and reducing the stroke rate to 19%. As an alternative to endarterectomy, carotid PTA and stenting is increasingly being employed to prevent stroke.

D.2 Pulmonary embolism prevention - Vena cava filter placement (Fig. 14)

Pulmonary embolism due to Deep venous thrombosis(DVT) is a dreaded complication of prolonged immobilization.

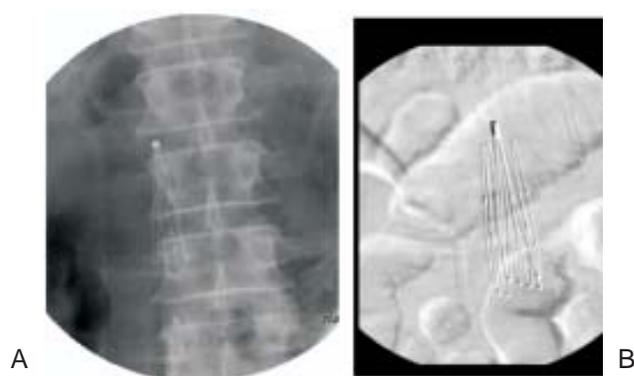


Fig.14 Vena Cava filter placement

Vena Cava filter placement. **A:** Patient presented with recurrent episodes of pulmonary embolism due to DVT. Greenfield filter in the IVC. **B:** Known case of recurrent DVT underwent Venatech filter placement in the IVC

Vena cava filters prevent thrombus from embolising to the pulmonary circulation by trapping the thrombus in the vena cava. When patients with thrombo-embolic disease cannot be anticoagulated or patients at high risk of developing DVT cannot be screened, monitored or receive prophylaxis, filters are indicated. There are a large number of permanent vena cava filters available. Temporary filters are designed to be removed once the venous thrombus resolves in patients with high risk disease.

To be continued in the next issue...

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BYSTANDER RESPONSE: A NON-DNA TARGETED EFFECT OF ALPHA-PARTICLE RADIATION

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ABSTRACT

Exposure of cell populations to low level ionizing radiation results in biological effects in both the irradiated and non-irradiated cells in the population. This phenomenon, termed 'the bystander effect', has been investigated both *in vitro* and *in vivo*. The occurrence of such a non-targeted effect is a major concern for the general public and the radiation protection agencies. There has been a recent upsurge of interest in the contribution of indirect and delayed effects of low dose exposures to the ultimate response to ionizing radiation due to the development of facilities for targeted irradiation of cells. Using different experimental systems and multiple biological endpoints, data from several laboratories indicate that radiation traversal through the nucleus of a

cell is not a prerequisite to produce genetic damage or a biological response. While previous studies reported the existence of bystander response in cell populations exposed to low-fluence of alpha particle irradiation at a dose of 0.3 cGy, the present study showed induction of bystander response in cells exposed to low-fluence of alpha particle as low as 0.03 cGy. The bystander response was also studied using MN and gene expression changes as a measure of DNA damage. Furthermore gene expression studies performed support the involvement of intercellular communication through gap-junctions.

Key Words: Ionizing radiation, Bystander effects, Micronucleus, Gene expression

Mesh Words: Bystander effects; Biological phenomena; Cellular communication.

1. INTRODUCTION:

The paradigm of genetic alterations being restricted to direct DNA damage after exposure to ionizing radiation has been challenged by observations in which effects of ionizing radiation arise in cells that in themselves receive no radiation exposure (1,2). These effects are demonstrated in cells that are in contact with irradiated cells or receive certain signals from irradiated cells (radiation-induced bystander effects) and in cells that are the descendants of irradiated cells (radiation-induced genomic instability). A large volume of laboratory and human epidemiological studies indicate that high doses of ionizing radiation are mutagenic and carcinogenic (3). While, the cellular effects and underlying mechanisms of high doses are fairly well elucidated, the health risks of low-level radiation exposures remain ambiguous and are a source of concern to the public and scientific community. The occurrence of bystander effect in a cell population exposed to low-level radiation could have significant impact on the concepts adopted in radiation protection whereby linear extrapolation of risks to very low doses is based. There has been a recent upsurge of interest in the contribution of indirect and delayed effects of low dose exposures to the ultimate response to ionizing radiation. This is partly due to the availability of tools such as low fluence alpha irradiators (4), the microbeam (5) and

advanced cell culture model system. However, limited facilities for targeted irradiation and laborious experimental procedures restrict the ability to study endpoints such as gene or protein expression at low doses which were previously difficult to study.

The potential application of radionuclides in radiotherapy is based on the classical dogma of radiation biology, which asserts that all effects of radiation on cells are due to its direct, immediate actions. However recent studies with DNA damage measurement using micronuclei (MN), Sister Chromatid Exchanges (SCE) (6), chromosomal aberrations (7), clonogenic survival (8) cell transformation (9) mutations (10) and cell proliferation (11) provide concrete evidence that biological response to ionizing radiation has a contribution from unirradiated "bystander" cells that respond to signals emitted by irradiated cells. The bulk of studies concerning radiation induced bystander effect indicated that such an effect has been detected in numerous cell types after exposure to both high and low LET ionizing radiations. The mechanical studies showed that signals can be passed either by cell to cell communication through inter-cellular junctions (12) or by culture medium (13) depending upon the type of radiation. Though the bystander effect has been observed in a wide variety of *in-vitro* as well as *in-vivo* systems, a dose-response has not been well established. While soft X-rays at doses as low as 50 mGy are known to induce bystander response (14), a single ion track of high LET radiation delivered to a single cell triggers a response throughout the population, which does not increase even when further radiation is given (15). This suggests that high LET radiation is more effective at inducing bystander response than low LET radiation. Considering the magnitude of response evoked by high LET alpha radiation and its risk in

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the human population, the bystander effect may be of particular importance for lung cancer-associated exposure to alpha-emitting radon gas and its decay products. It is now known that a large component of the background exposure dose equivalent received by the general public results from α -particles emitted by radon and its progeny decay products. Hence, the present study was aimed at investigating the bystander effect and dose-response in AG1522 human lung fibroblast cells exposed to low fluence of alpha particles using DNA damage and gene expression changes as end points.

2. MATERIALS AND METHODS

2.1 Maintenance of AG1522 Cells:

AG1522 normal human diploid skin fibroblasts were obtained from the Genetic Cell Repository at the Coriell Institute for Medical Research (Camden, NJ). The cells were grown in stainless steel dishes with 1.5- μ m-thick replaceable mylar bottoms at a seeding density of about 1.2×10^5 cells/dish. The cells were subsequently fed on days 5, 7 and 9 with Eagle's MEM supplemented with 15% (v/v) heat-inactivated FCS, 50 units/ml penicillin, and 50 μ g/ml streptomycin. Experiments were started 48 h after the last feeding. At this juncture, 95–98% of the cells were in G_0 - G_1 as determined by labeling with [3 H] thymidine and/or flow cytometry. As cellular radiation sensitivity changes at different phases of the cell cycle, the cells were synchronized in G_0 - G_1 by confluent, density inhibition of growth to eliminate complications in the interpretation of the results. Passage 10 or 11 cells maintained in a 37°C humidified incubator (atmosphere = 5% CO_2 in air) were used. Control cells were sham-treated and handled in parallel with the test cells.

2.1 Alpha Particle Irradiation:

Cells were exposed to α -particles from a ^{241}Am -collimated source at a dose rate of 2 cGy/min. Irradiation was carried out from below with α -particles of 3.65 MeV average energy at the cell layer with a dose range of 0.03 cGy to 10 cGy. The fraction of cells whose nucleus was traversed by an α -particle was derived from Poisson statistics and estimates involving cell geometry, α -particle fluence, and energy loss were calculated as described previously (16).

2.2 Micronucleus Assay :

The frequency of micronucleus was measured in binucleated cells as an index of DNA damage by cytokinesis block MN technique using Cytochalasin-B. Three hours after treatment, cultures were dissociated by trypsinization, and approximately 3×10^4 cells were seeded in chamber flaskettes (Nunc) in the presence of 2.5 μ g/ml cytochalasin B (Sigma) and incubated at 37°C. After 72 h, the cells were rinsed in PBS, fixed in methanol, stained with Hoechst 33342 solution (1 μ g/ml), and viewed under a fluorescence microscope. At least 1000 binucleated cells were examined, and only micronuclei in binucleate cells were considered for analysis.

Binomial statistics were applied to the analysis of data, whereby a certain number of cells were found to be micronucleated in a population of binucleated cells. The frequency of micronucleus formation (r_0) was calculated as: $r_0 = a/b$, where a is the total number of micronucleated cells scored, and b is the total of binucleate cells examined. The error associated with r_0 is given by the following formula: $r_0 = [(a/b) (1 - a/b)]^{1/2}$. Paired t-test (a linear regression analysis) was applied to compare the differences in the MN frequencies of different treatment groups using the INSTAT programme.

2.3 Western Analysis:

Sham and α -irradiated cultures were held at 37°C in 5% CO_2 atmosphere for 3 hours prior to harvesting for analysis. The cells were washed in PBS and lysed in chilled RIPA buffer [50 mM Tris-Cl (pH 7.5), 150 mM NaCl, 50 mM NaF, 5 mM EDTA, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS] supplemented with protease inhibitor cocktail (Sigma) and sodium orthovanadate (1 mM). Anti-p21^{Waf1} (Ab-6), anti-p53^{Ser15} (Ab-1) and anti-cx43 (Ab-3) were obtained from Oncogene Research Products and reaction with non-specific antibodies was used to verify whether equal amounts of sample were fractionated. Secondary antibodies conjugated with horseradish peroxidase and the enhanced chemiluminescence system from New England Nuclear was used to detect the various proteins.

3. RESULTS:

The number of cells present during the time of irradiation was around 1000×10^3 in each dish. The cultures were exposed to low fluence alpha particle of a dose range between 0.02 - 10 cGy at a dose rate of 2 cGy per minute. The fraction of cells expected to be traversed by at least one alpha particle for various doses are shown in Table-1. At this fluence used, 0.2 to 48.7% of cell nuclei were hit by an alpha particle through the nucleus.

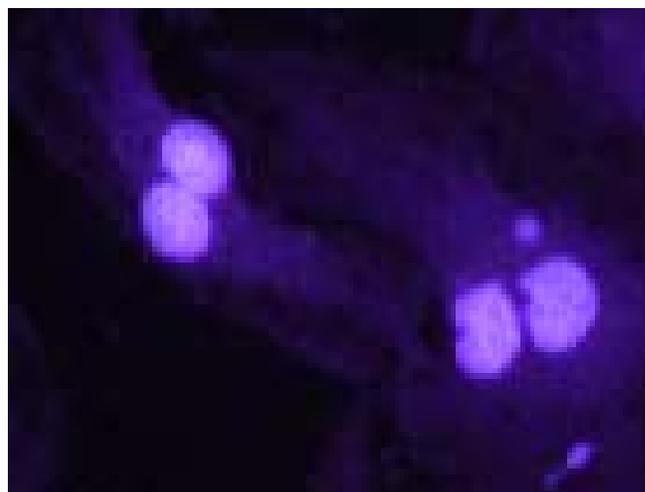
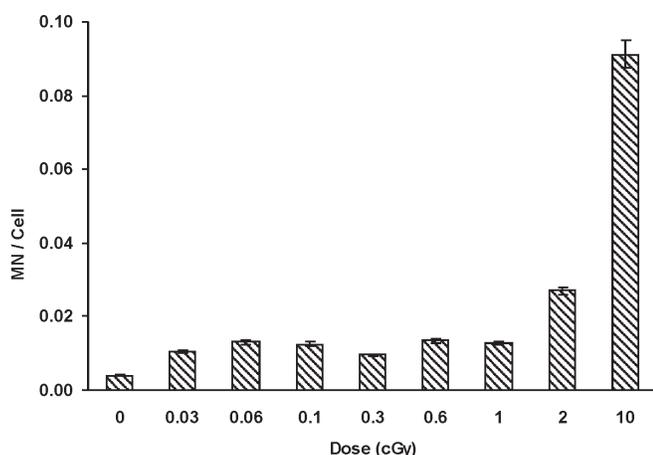
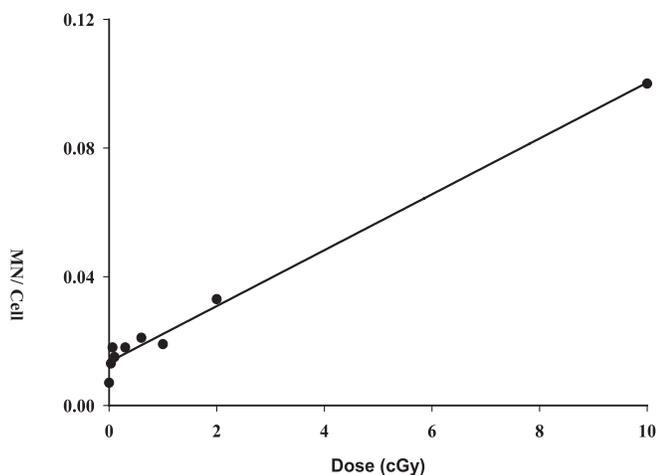


Figure-1: Cytokinesis blocked binucleated Human lung fibroblast cells (AG-1522) with and without micronucleus (arrow indicates MN)

Table-1: Fraction of cells exposed to alpha particle and the relative fold increase in Micronucleus frequency

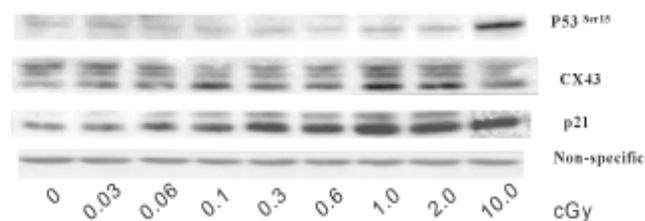
Mean Dose (cGy)	Fraction of cells traversed by a α -particle (%)	Relative increase in MN over control (Fold)	Value of significance (p-value)
0.0	0.0		
0.03	0.2	2.63	0.008
0.06	0.4	3.25	0.030
0.1	0.7	3.13	0.005
0.3	2.0	2.40	0.070
0.6	3.9	3.36	0.020
1.0	6.4	3.08	0.005
2.0	12.5	6.75	0.001
10.0	48.7	22.75	0.001

**Figure-2:** MN frequency obtained from AG-1522 cells exposed various doses alpha particle radiation.**Figure-3:** Dose response for micronucleus frequency obtained from AG-1522 cells exposed various doses alpha particle radiation.

The induction of micronucleus (MN) in bystander cells was used as a surrogate measure of DNA damage. The confluent density inhibited AG1522 human fibroblast cells were exposed to low-fluence of α -radiation at different doses and held at 37°C for three hours. Then the cultures were

trypsinised and plated for the MN assay. The MN frequency was calculated by scoring 1000 binucleated cells as shown in Figure-1. The obtained result showed a relative 2 - 4 fold increase in the MN frequency compared to the control even when only 0.2 % of cells were traversed by an alpha particle of dose 0.03 cGy (Table-1 and Figure-2). The relative fold increase in the remaining doses is comparable up to 2 cGy where 12.6% of the cell population is traversed by an alpha particle. Further, only a small increase was noted with 10 cGy where 48% of cells were hit. It is presumed that the excess MN are a result of the residual DNA damage occurring in neighboring bystander cells. The dose – response obtained for the yield of MN frequency is shown in Figure-3 (r value - 0.938).

In order to explore the molecular changes associated with excess DNA damage in the bystander cells, changes in expression level of various proteins were examined using Western Blot. Consistent with the occurrence of excess MN frequency in a higher percentage of cells, a significant up-regulation of p53^{Ser15} and p21^{WAF1} was observed in cultures exposed to a mean dose of 0.03 cGy (0.2 % cell nuclei were traversed by one alpha particle). The accumulation of p53 protein phosphorylated at Ser15 position and its downstream effector p21^{WAF1} supports the DNA damage response in bystander cells. Furthermore, the up-regulation of connexin-43, at the same dose level highlights the significance of involvement of gap junction and its intercellular communication role in the spreading of signals to the surrounding bystander cells (Figure – 4).

**Figure-4** Gene expression changes in AG-1522 cells exposed various doses alpha particle radiation.

4. DISCUSSION:

The majority of the literature concerning the bystander effects is in the field of gene therapy and toxicology. The gene product of the transfected DNA can travel through gap junctions from the transfected cells through neighboring cells and maximizes the cells that are affected, in a situation in which only a few cells in the population may be successfully transfected. The term is applied specifically to the death of unmodified tumor cells when in contact with ganciclovir exposed herpes simplex virus-thymidine kinase modified tumor cells. The term has been applied only recently in the field of radiation biology. The bystander effect in this contact refers to detection of responses in unirradiated cells that can reasonably assumed to occur as a result of exposures of other cells to radiation.

The classical theory of radiation biology states that, the traversal of the cell nucleus by ionizing radiation is a pre-requisite for the manifestation of radiation signature in the exposed cell population. However, recent literature substantially demonstrates the occurrence of non-targeted effects like bystander response (17) and genomic instability (18). Of these, the existence of bystander effect is a major concern for the general public as well as the radiation regulatory agencies because of the magnified effect even though fewer cell populations are exposed.

The bystander effect has been observed in a wide variety of *in-vitro* (17) as well as *in-vivo* (19), systems but a dose-response has not been well established. While soft X-rays at doses as low as 50 mGy are sufficient enough to induce bystander response, a single ion track of high LET radiation (14) delivered to a single cell triggers a response throughout the population, which does not increase even when further radiation is given to the same or other cells (15). This suggests that high LET radiation is more effective at inducing bystander response than low LET radiation. In the present study we observed a bystander effect in a cell population where only 0.2% of the cells were traversed by an alpha particle. This is evident from the excess MN frequency observed in cells that were not actually traversed by an alpha particle. The relative fold increase in MN frequency is not statistically significant when the fraction of cells traversed is 0.2 or 6.5%. However, the highest dose studied, in which 48% of cells were traversed by alpha particles, resulted in a 23 fold relative increase in MN frequency. The obtained results suggest that the bystander response operates even at lower doses and is saturated at high doses irrespective of the LET radiation as reported earlier for microbeam studies (15). Low fluence α -particle studies using microbeam irradiation have also provided evidence for a significantly enhanced frequency of micronucleus formation and apoptosis in bystander cells. The effect was found to be independent of dose and number of cells hit. Cellular traversal by α -particles was necessary to observe the effect, which was maximal after a single particle traversal through the nucleus. Targeting of α -particles outside the cells did not result in increased damage (8).

Experiments using gene expression as an endpoint have also indicated that stress effects are transmissible from irradiated to non-irradiated cells. It was found, by flow cytometry, that α -particle irradiation of cell cultures caused a dose-dependent increase in p53 levels at mean doses as low as 0.6 cGy (20). Importantly, these studies indicated increased expression of this stress responsive protein in a greater fraction of cells than were hit by the α -particle track. These initial observations were further developed and examined in a variety of cell types using western blotting and *in situ* immunofluorescence techniques in which 2% of cell nuclei were traversed by an alpha particle (12, 17). Consistent with earlier findings, in the present study, the accumulation of phosphorylated p53 protein at Ser15 loci (specific response to DNA damage) and its downstream effector p21^{WAF1} further support the induction of bystander response even though only 0.2 % of cell population was

traversed by an alpha particle. These data strongly support the concept that stress is inducible by mechanisms other than direct interaction of DNA with ionizing radiation. The up-regulation of p21^{Waf1} and induction of micronuclei by an α -particle mean dose of 1 cGy in bystander cells, was further supported by the observation that the G₁ checkpoint is induced in a greater number of cells than predicted based on dosimetric estimates (17). Hence, using multiple and related biological endpoints, data generated in different laboratories indicate that the expression of stressful effects in exposed populations of cells is not restricted to those cells that are directly irradiated. Multiple studies show that the effect of radiation dose no longer depends only on the amount of energy deposition; cross talk between irradiated and neighboring non-irradiated cells significantly modulates the overall cellular response to radiation exposure.

Homeostatic maintenance of cells in tissues depends on a complex network of communication modalities that allow coordinated interactions among themselves and with their environment. Among various structures, the gap-junction is one of the most widespread, specialized plasma membrane structures, which contains a low resistance channel linking adjacent cells. It consists of a complete cell-to-cell channel that spans two plasma membranes and results from the association of two half channels, or connexons, contributed separately by each of the two participating cells. Each connexon, in turn, is a multimeric assembly of protein subunits called connexins (21). Connexins are an extensive family of proteins comprising several members. Different connexins are expressed in different tissues and have different selectivity related to the size and charge of the communicated molecules. Chemical and genetic evidence for the participation of gap junction intracellular communication (GJIC) in the transmission of damage signals from irradiated to nonirradiated mammalian cells has been reported in human fibroblasts exposed to very low fluences of α -particles using *in-situ* immunofluorescence techniques (12). In the present study too, over expression of connexin-43 supports the involvement of gap-junctions in the transmission of signals from irradiated to non-irradiated cells.

5. CONCLUSION:

The occurrence of bystander effects in cell populations exposed to low level radiation, as described above, could have a significant impact on the concepts adopted in radiation protection whereby linear extrapolation of risks to very low doses is based (22). An understanding of the molecular/biochemical events involved in such effects would contribute to setting adequate radiation protection standards and may have implications in radiotherapy. Importantly, it could offer opportunities in ameliorating our understanding of the adverse effects of ionizing radiation.

6. ACKNOWLEDGEMENT:

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PREVALENCE OF EXTENDED SPECTRUM β -LACTAM RESISTANCE AMONG BACTERIA CAUSING HOSPITAL INFECTIONS - DETECTION USING PCR

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ABSTRACT

Resistance to contemporary broad spectrum β -lactams mediated by extended spectrum β -lactamases (ESBL) is an increasing problem world wide. The epidemiology of ESBL producing Enterobacteriaceae and the genotypic determinants in an ICU setting in India is largely unknown. The available phenotype tests can sometimes be misleading as more than one type of resistance can be detected in a single isolate or more than one gene is harbored by the same isolate. In the present

study, fourteen isolates of *E. coli* and *K. pneumoniae* that were antibiotic resistant in a E-strip test were selected. Presence of the *bla*_{TEM} and *bla*_{SHV} genotypes among these isolates was screened by PCR. While, four resistant isolates showed no specific amplicons for these two genes, four others carried both the genes. The others either carried the TEM or the SHV type only. This first report from India, clearly highlight the epidemiology of the prevalent genes among the isolates from our ICU.

Key words : beta lactamases, polymerase chain reaction

INTRODUCTION

Hospitals have always acted as a source of infection to patients admitted to them. The concept of asepsis and its application in hospital practice have reduced their incidence but hospital infections still cause considerable morbidity and mortality. It is much higher in crowded hospitals in the developing countries. Even when hospitalization does not lead to obvious infection, it causes a change in the patient's microbial flora. The normal flora is gradually replaced by the drug resistant micro-organisms typical of the hospital environment. In recent decades, the enteric gram negative bacilli *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus* & *Serratia* have become the most important group of hospital pathogens in addition to *Pseudomonas* species and the Gram positive *Staphylococci*.

Emergence of resistance to antibiotics among the Enterobacteriaceae has serious implications in hospitals and for therapy. Of the many types evident the resistance to β -lactam antibiotics is widespread. Routine methods to detect it include, isolation and culture of these bacteria from patient samples and determining their range of resistance by disc diffusion assays or MIC's. Multiple drug resistance is known in all these species. In most instances this resistance is carried by genes present on plasmids or transposons and on chromosomes [1-4] (1^{et al.}, 2002; Arlet, et al.,²1995; ³1990, ⁴2003). Plasmids and transposons being mobile are responsible for the spread of multiple drug resistance among bacteria. The mechanism of drug resistance encoded by these genes

includes decreased permeability of drugs, alternative metabolic pathways and production of enzymes that inactivate the drugs.

Of the three mechanisms known, the occurrence of enzymes e.g., β -lactamases (Medeiros, ⁵1984) that degrade the lactam ring of the antibiotics is the most common among gram negative bacteria. The first beta-lactamase was identified in an isolate of *Escherichia coli* in 1940. To date, there are >130 TEM-type and >50 sulfhydryl variable (SHV)-type beta-lactamases, mainly in *E. coli*, *K pneumoniae*, and *Proteus mirabilis* but also in other members of the Enterobacteriaceae family and in some nonenteric organisms, such as *Acinetobacter* species.^[6,7] With emergence of resistance to penicillins, use of broad spectrum antibiotics such as cephalosporins became widespread. But within 5 years of their use in 1983, transferable resistance to these antibiotics in clinical isolates of *K. pneumoniae* and *Serratia marcescens* was reported.^[8-10] Resistance to these drugs was a result of a new class of β -lactamases, the CTX-type that were similar in sequence to *bla*_{TEM} but with a few amino acid changes that extended its substrate range for hydrolysis.^[6,11] These β -lactamases differed from TEM types by hydrolysis of cefotaxime or ceftriaxone but were inhibited by clavulanate and cloxacillin. These new class of enzymes called as Expanded spectrum of β -lactamases (ESBLs) are enzymes that mediate resistance to β -lactam antibiotics such as penicillin, extended spectrum (third generation) cephalosporins and monobactams by catalyzing the hydrolysis of β -lactam and could inactivate antibiotics with oxyimino groups. Most ESBLs are derivatives of TEM type or SHV type enzymes encoded by ESBL plasmid.

ESBL-producing organisms frequently also possess resistance factors to other classes of antibiotics, such as aminoglycosides and fluoroquinolones, and possibly also piperacillin-tazobactam and cefepime. Different TEM variants were found to coexist within the same cells. Again,

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a patient could harbor two or three different strains that encoded the same enzyme or two indistinguishable isolates that produced distinct TEM, β - lactamases.^[3]

The incidence of expanded-spectrum beta-lactamases (ESBLs) varies; depending on which area of the globe the isolates originate from. ESBLs render the oxyimino-cephalosporins ineffective, and hence it is important to know the type of beta lactamase that is prevalent in the clinics. It is, therefore, imperative that microbiology laboratories should routinely test for the presence of antibiotic resistant strains among their isolates and determine the epidemiology of the prevalent types.

In many countries, ESBLs are expressed in 10%-40% of *E. coli* and *K pneumoniae* isolates. (ref) In Indian hospitals, several studies on drug resistance among Gram -ve bacteria have been made but most describe their phenotypic response. Recently studied drug resistance among *Staphylococcus aureus* (MRSA) isolates report the occurrence of both ESBL's and penicillin binding protein genes in these isolates by PCR.^[12]

Routine screening for drug resistance is carried out at the Microbiology department of SRMC and RI (DU) by disk diffusion assay, E-tests and MIC. However the molecular type of this resistance is largely unknown and has not been attempted so far except for the brief study by Sireesha (2005).

In this study, gram negative strains were collected from C4 ICU (SRMC&RI) -patient's sample and their ESBL production was determined using double disk method. The minimum inhibitory concentration (MICs) for various antibacterial agents was determined using E test strips. The presence of genes to confirm the production of ESBLs was carried out using gene specific primers. Results and the protocols to detect the TEM type or the SHV type ESBL's are presented here. The prevalence of the specific types of resistance in these isolates and their molecular genotypes are reported here for the first time.

2. MATERIALS AND METHODS

General molecular biology protocols as recommended in Sambrook et al., 2000^[15] were followed. All the general chemicals used for molecular techniques were purchased from Sigma or Himedia. DNA polymerase and dNTP's were purchased from Invitrogen, USA or Genie (Bangalore, India).

CULTURES: Bacterial strains used in the present study were collected from C4 - ICU (SRMC & RI) during 2004. First, samples of five different bacteria were chosen to optimize plasmid isolation protocols. They were:

Name of strain	Strain No.	Expected Plasmid
<i>E. coli</i>	SRMC-26	bla _{TEM} Plasmid (> 12kb)
<i>K. Pneumonia</i>	SRMC-20	bla _{SHV} Plasmid (> 12kb)
<i>E. coli</i>	SRMC-3	bla _{TEM} Plasmid (> 12kb)
<i>E. coli</i>	ATCC(25923)	No Plasmid
<i>K. Pneumonia</i>	ATCC(70603)	Plasmid (> 12kb)

Isolates obtained from the clinical specimens include the following:

Strain No.	Nature of isolates
Klebsiella	
U9316	Urine sample
E4177	Wound exudates
E4119	Wound exudates
R1738	Respiratory sample
R1756	Respiratory sample
E4146	Wound exudates
E4140	Wound exudates
E. coli	
R1966	Respiratory sample
R1703	Respiratory sample
U8192	Urine sample
R1759	Respiratory sample
U8688	Urine sample
E4159	Wound exudates
E4098	Wound exudates

They were tested for production of β -lactamases using strip E-tests based on the recommendation of the National Committee on Clinical Laboratory Standards (NCCLS^[14]; 2003;)^[1].

GROWTH AND MAINTENANCE: All isolates were routinely maintained on appropriate antibiotic containing medium. Luria Bertani media (LB; Sambrook; et al. 2000) amended with the antibiotic, ceftazidime (LCA) at a concentration of 5 mg/ml was used routinely to maintain the isolates. Agar at 15 g/L was used to solidify.

ISOLATION OF PLASMID (Alkaline Lysis Method;^[15] Briefly the method involved inoculating liquid cultures with single colony of the isolates in LB broth (50ml) containing ceftazidime (5ppm). They were grown overnight at 37°C in an orbital shaker and the OD was checked at 600nm. Cell lysis and denaturation involved an alkaline lysis buffer. DNA was separated from the solution with potassium acetate. After obtaining the plasmid DNA it was resuspended in 100 μ l of TE buffer. A sample (7ml) was then loaded on agarose gel (0.8%) and electrophoresis was carried out for 45 min at 70V and 50 mA and Tris

Acetic acid and EDTA buffer (pH 8.0). Spectrophotometric quantification of DNA (UV-Visible Spectrophotometer, Techcomp, India) at 260 and 280nm was done.

PCR for bla_{TEM} and bla_{SHV} genes: Plasmids isolated from the antibiotic resistant clinical isolates were used for amplification using TEM and SHV primers. The primers were synthesized from Microsynth, Switzerland. The details of the sequences are:

SHV-OS/F1 (20mer), 5'- TTATCTCCCTGTTAGCCACC-3' and SHV-OS/R1 (20mer)- 5'-GATTTGCTGATTTCCGCTCGG-3' for the SHV β -lactamase genes (Arlet, 1997).

For the TEM β -lactamase genes (Mabilat et al., 1990) the primers used were:

TEM/F1 (17mer)- 5'- ATAAAATTCTTGAAGAC-3' and TEM/R1 (17mer)- 5'- TTACCAATGCTTAATCA3'. The PCR reaction mix of 20 μ L contained Forward and Reverse primers 5 picomoles each; (1 μ L); 2 μ L of Buffer (10x; 15mM); dNTPs (2 μ L; 2.5mM); plasmid DNA (50 ng); 0.2 μ L of Taq Enzyme (1Unit) and the total volume was made up with 12.8 μ L of water. The PCR was run using the following conditions in a thermal cycler (MJ Research, USA). Initial denaturation -94°C for 5 minutes was followed by PCR of 35 cycles that included denaturation at 94°C for 1 minute; annealing at 41°C for 1 minute; extension at 72°C for 7 minutes; followed by a final extension at 72°C for 10minutes. After the completion of the amplification, the PCR products were checked on 0.8 % agarose gel run in TAE buffer.

RESULTS

Phenotypic characterization of antibiotic resistance of isolates

Seven isolates of *E. coli* and seven isolates of *Klebsiella* were tested for growth on antibiotics by disc diffusion and MIC. Their response to antibiotics Ceftazidime (ca), Cephatoxime (ce), Cefoperazone (cfs with sulbactam), Cefoperazone, (cs), Tazobactam with piperacillin (tzp) was done using E strips. Data presented in Table1 clearly confirmed that these isolates were resistant to the tested drugs. *Klebsiella* isolates showed a MIC as high as >2048 mg/ml when tested for cs. Among the *E. coli* isolates highest MIC of >2048 mg/ml was exhibited for ce and cs. These results confirm the existence of resistance among these clinical isolates.

Molecular analysis of drug resistant bacteria: Plasmids were obtained from these isolates (Fig. 1) and genotypes, TEM and SHV, were characterized using PCR technique. Yields of plasmid DNA ranged from as low as 1.8 μ g/ μ L to as high as 24.8 μ g/ μ L. All the clinical isolates showed one high molecular weight plasmid and 1-4 forms of the plasmid(s) moving faster on the gel. These plasmid preparations were then amplified by PCR using the primers for bla_{TEM} and bla_{SHV}. The PCR amplified products were resolved on a gel and isolates positive for bla_{TEM} showed an amplicon of ~ 1.2 Kb (Fig. 2; lane 3) while those

positive for bla_{SHV} produced an amplicon of ~ 0.9kb (Fig 2; lane 5).

The results from the PCR analysis for all the fourteen isolates are compared with their drug resistance profile in Table1. The seven *Klebsiella* isolates were tested with both sets of primers. The analysis presented in Table 1 and Fig. 3, 4 shows that out of the 7 isolates, one isolate (R1738) did not show any amplicon with either primer but was drug resistant. Three had both bla_{TEM} and bla_{SHV} (e.g., isolate E4119; Fig. 3a, lane 3- SHV +ve and Fig. 4 a; lane 5- TEM +ve) encoded in their plasmids. Two carried only the bla_{TEM} gene (Fig. 4a; lane 6) and one was positive for bla_{SHV} (Fig. 3b; lane 7). The known test *E. coli* strain SRMC-26 carrying the bla_{TEM} was positive (Fig. 4b). Out of the seven clinical *E. coli* strains, three were negative with both sets of primers; two were positive for only bla_{TEM} (Table 1 and Fig. 4a; lanes 3,4). One however, carried both genes bla_{TEM} and bla_{SHV} resistance (Fig. 3a; lane 8) and one was positive for bla_{SHV} (Fig. 3b; lane 4).

DISCUSSION

The prevalence of ESBL among clinical isolates varies from country to country. They can be seen in the community setting as well. Because of their complexity and substrate specificity their detection is a major challenge faced by laboratories.

ESBL producing bacteria are typically associated with multiple drug markers (because genes for other mechanisms of resistance often reside on the same plasmid as the ESBL genes.). Apart from resistance to β -lactams they can have resistance to quinolones, aminoglycosides and Trimethoprim sulphamethoxazole. Infection with ESBL producing bacteria can result in avoidable treatment factor with resultant increase in the care of patient case and prolonged hospital stay.

Earlier reports on evaluation of resistance to antibiotics also studied the presence of the β -lactamase and the occurrence of the responsible genes bla_{TEM} and bla_{SHV} either in plasmids or the chromosomes. In this study, we screened for the presence of a likely plasmid in these resistant strains as all were antibiotic resistant to a range of antibiotics [Ceftazidime (ca), Cephatoxime (ce), Cefoperazone (cfs with sulbactam), Cefoperazone, (cs), and Tazobactam with piperacillin (tzp)]. Among the 14 isolates tested for the presence of TEM or SHV type β -lactamases, four of them (one *Klebsiella* and three *E. coli*) did not show any PCR products but were however resistant to various antibiotics (Table 1). These isolates may have other mechanisms of resistance or it may reside in its chromosome.

A high MIC indicates that the isolate is resistant to the drug but resistance can be mediated by many mechanisms and ESBL is one such. Since it is a transferable, it is important to recognize the type. E-test helps us in knowing the MIC rapidly. Again it has to be

confirmed by agar dilution or broth dilution method which is very cumbersome and best done only in lots or batches.

There is no data on the prevalence of TEM, SHV type β -lactamase in India (except for the preliminary report by Sirashee, 2005). Incidence of ESBL reported from various centres in India is largely based on the phenotypic tests. A knowledge of the genotypic pattern is of importance in the molecular epidemiology of the resistant types and this study has helped us distinguish the occurrence of these genes in the clinical isolates from our ICU.

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Table1: Minimum Inhibitory Concentration (MIC) value of various clinical isolates and the corresponding amplification products with primers for bla_{TEM} and bla_{SHV}

Strain No.	MIC value (μ g/ml)					PCR results(this study)
	ca	ce	cfs	cs	tzp	
Klebsiella						
U9316	1024	2048	4	> 2048	128	P TEM (+)
E4177	128	> 1024	32	1024	256	P TEM(+)
E4119	512	> 1024	12	1024	512	P, α Both +ve
R1738	1024	1024	> 256	> 2048	1024	Both -ve
R1756	> 1024	> 1024	16	> 2048	512	P, α Both +ve
E4140	> 1024	> 1024	> 256	> 2048	512	P, α Both +ve
E4146	> 1024	> 1024	> 256	> 2048	512	α SHV(+)
E. coli						
R1759	512	1024	2	4	128	α SHV(+)
U8688	1024	> 1024	8	> 2048	256	Both -ve
E4159	1024	> 1024	16	> 1024	512	Both -ve
E4098	512	> 2048	32	> 2048	128	P TEM(+)
R1966	256	512	4	> 2048	64	P TEM(+)
R1703	512	> 1024	2	1024	128	Both -ve
U8192	128	> 1024	2	1024	32	P Both +ve

Both - TEM & SHV; +ve / -ve: Presence/ absence of an amplicon; Sizes of amplicon include ~ 1.2kb for bla_{TEM} (P) and ~0.9 kb for bla_{SHV} (α). Note that in four isolates both TEM and SHV could not be amplified. Antibiotics tested are:

ca - Ceftazidime; ce - Cephalosporin; cfs - Cefoperazone with sulbactam; cs - Cefoperazone

tzp - Tazobactam with piperacillin

FIG.1. Presence of a plasmid (s) seen in drug resistant clinical isolates of E. coli (EC) seen in panel (a)- lanes 2,6,10: SRMC 26; lanes 4,8- SRMC 3; and in panel (b)- lanes 4,5,6 - R1759, U8688 & E4159. Plasmid(s) of Klebsiella (KI) are shown in panel (a)- lanes 3,7 - SRMC 20; lane 5, 9 - ATCC 70603 (with plasmid); and in panel (b)- lane 2,

3 & 7 - R1738; R1756 & E4146. The lane 1 in both panels contained λ DNA / Hin dIII digest. Note the presence of more than one plasmid in some isolates. EC

FIG. 2: A comparison of the sizes of the PCR products on a 1.3% gel from both genes, bla_{SHV} and bla_{TEM} indicating that they are of expected lengths. The lanes were loaded with lane 1- 100bp ladder; lane 2- (TEM +ve; 1.2 kb); lane 3- (SHV +ve; 900 bp); lane 4 - negative control. EC- E. coli; KI- Klebsiella.

FIG. 3: Detection and identification of bla_{SHV(->)} genes by PCR was seen in isolates of E. coli (EC)-lane 8 (panel a) and lane 4 (panel b). Isolates of Klebsiella (KI) that were positive ca be seen in lane 9 (panel a); lane 3,6,7 (panel b). Lane 1- Lambda DNA/Hind III digest.

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

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DOSE DEPENDENT EFFECT OF *PIPER BETLE* LINN. LEAF EXTRACT ON ERYTHROCYTES OF EXPERIMENTAL MICE

S. Chitra*, N. Vidya

ABSTRACT

Background:

Objective: This study examined the dose – dependent effect of oral administration of *Piper betle* leaf extract on lipid peroxidation, antioxidants, antioxidant enzymes and membrane – bound ATPases in mice.

Method: Adult female mice weighing 30 ± 2 grams were administered different doses (0.2, 0.4, 0.6, 0.8, & 1.0 gm / day) of betel leaf extract orally for 15 days. Plasma, erythrocytes and erythrocyte membrane were separated and used for the assay of thiobarbituric acid reactive substances (TBARS), superoxide dismutase,

catalase in RBC hemolysate, ascorbic acid and vitamin E in plasma and membrane – bound ATPases (Na^+ / K^+ - ATPase, Ca^{2+} - ATPase, Mg^{2+} - ATPase) in erythrocyte membrane were measured.

Results: A significant reduction in TBARS and significant increase in ascorbic acid, vitamin E, super oxide dismutase, catalase and membrane – bound ATPases were observed in mice fed 0.2 gm / day. The extract of *Piper betle* leaf at the low dosage of 0.2 gm / day for 15 days provides better antioxidant potential as well as membrane stabilizing action in Swiss mice over controls.

Key words : *Piper betle*, antioxidants, membrane – bound ATPases, lipid peroxidation

INTRODUCTION

Reactive oxygen species (ROS) are responsible for oxidative damage of biological macromolecules such as DNA, carbohydrates and proteins [1]. These processes are discussed as pathobiochemical mechanisms involved in the initiation and progression phase of various diseases [2]. Some of the most relevant ROS are: peroxy radicals ($\text{ROO}\cdot$), the nitric oxide radical ($\text{NO}\cdot$), the superoxide anion radical ($\text{O}_2^{\cdot -}$), singlet oxygen ($^1\text{O}_2$), peroxy nitrite ($\text{ONOO}\cdot$), and hydrogen peroxide (H_2O_2). ROS are either radicals (molecules that contain at least one unpaired electron) or reactive non – radical compounds, capable of oxidizing biomolecules. Therefore, these intermediates are also called oxidants or pro - oxidants [3]. The superoxide radical anion appears to play a central role, since other reactive intermediates are formed in reaction sequences starting with $\text{O}_2^{\cdot -}$. H_2O_2 is a non – radical reactive species and can easily diffuse between living cells. It is efficiently converted to water by the enzyme catalase, a process which determines its half – life. The peroxy radical ($\text{ROO}\cdot$) is relatively long lived (seconds) with a considerable diffusion path length in biological systems. It can be generated in the process of lipid peroxidation which is initiated by the

abstraction of a hydrogen atom from poly unsaturated fatty acids (PUFA); the hydroxyl radical is capable of starting this reaction sequence [4, 5]. While *Piper* species are reported to have wide spectrum of biological activity but its antioxidant potential has not been established so far, making it important and interesting to analyze the antioxidant potential of *Piper betle* leaf extract in the present study.

Many unknown and lesser - known plants are used in folk and tribal medicinal practices in India, with their medicinal values not known much to the scientific world. *Piper betle* (Family - Piperaceae) is one such plant, which is commonly known as “pan” and often chewed by Indians resulting in habit formation of this practice. Medicinally *Piper betle* has been attributed with properties – like anti poisonous [6] and wound healing [7]. Extracts of *Piper betle* leaves also possess antimicrobial, antifungal, anti-inflammatory and antiplatelet activities [8, 9]. Ethanol extracts of *Piper betle* leaf exhibited gastrocytoprotective properties on experimentally induced gastric lesions [10] and betel nut along with the betel leaves significantly reduced the infiltration activity and abolished the surface anesthetic activity of betel leaf [11]. Studies on the physiological effects have shown that the initial effects of chewing betel with areca nut and other adjuncts can cause excitation of the salivary glands and also irritation to the mucous membrane of the mouth. Betel leaf alone apparently does not induce tumors and it seems to be the combined usage of betel nut and tobacco which provides the carcinogenic stimulus [12]. Prolonged consumption of betel leaves will cause cancer of the mouth, upper aero digestive tract and stomach [13].

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An earlier report on the anti – inflammatory and anti - diabetic activity of leaf extracts prompted us to evaluate its dose – dependent action. We therefore, studied the antioxidant and – membrane stabilizing action on experimental mice. Results from enzymatic and non – enzymatic antioxidant activities and membrane - bound ATPases are presented.

MATERIALS AND METHODS

Plant Material: *Piper betle* Linn (Syn: Chavica betle Miq.) popularly known as “vetrelei” in Tamil, “betel” in English and “Nagavalli” in Sanskrit was purchased from local market, Chennai, Tamil Nadu, India.

Chemicals: Chemicals used were of analytical grade.

Experimental animals: Adult female Swiss mice weighing approximately 30 ± 2 grams were obtained from the Central Animal Facility of Sri Ramachandra Medical College and Research Institute, Deemed University, Chennai were used in this study. The animals were maintained 12 hour light / 12 hour dark cycle and fed on a pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. The animals were maintained in their respective groups for 15 days. All studies were conducted in accordance with the National Institute of Health “Guide for the care and Use of Laboratory animals [14].

Experimental design: Thirty-six adult healthy female mice were divided into six groups of six animals each.

Group I - Control (2ml of saline / day)

Group II - 0.2 gms of betel leaf in 2 ml of saline / day.

Group III - 0.4 gms of betel leaf in 2 ml of saline / day.

Group IV - 0.6 gms of betel leaf in 2 ml of saline / day.

Group V - 0.8 gms of betel leaf in 2 ml of saline / day and

Group VI - 1.0 gms of betel leaf in 2 ml of saline / day.

Betle leaf extract was given orally for 15 days; the mice were fasted overnight and sacrificed by cervical decapitation. Plasma was separated using EDTA as an anti coagulant. The hemolysate and erythrocyte membrane was isolated according to the procedure of Dodge *et al* [15] with a change in buffer according to Quist [16]. Plasma was used for the analysis of ascorbic acid [17] and vitamin E [18]. RBC hemolysate was used for the estimation of TBARS [19], protein [20] and assay of superoxide dismutase [21], catalase [22], and erythrocyte membrane was used for the assay of $\text{Na}^+ / \text{K}^+ - \text{ATPase}$ [23], $\text{Ca}^{2+} - \text{ATPase}$ [24], $\text{Mg}^{2+} - \text{ATPase}$ [25].

Statistical analysis: Statistical analysis of the data was performed using student’s ‘t’ – test and $p < 0.05$ was considered as significant.

Results

Table 1 shows the level of TBARS, in erythrocyte membrane, ascorbic acid and vitamin E in plasma of experimental animals. Significant reduction ($p < 0.001$) in the level of TBARS was observed in group II mice (0.2 gm / day) when compared to control, whereas concomitant increase ($p < 0.001$) was observed in the levels of plasma ascorbic acid and vitamin E in group II animals. Significant increase in the levels of TBARS and significant decrease in the levels of plasma ascorbic acid and vitamin E was observed in all the other groups of experimental mice (i.e. group III to VI).

Table 2 shows the activities of superoxide dismutase and catalase in RBC hemolysate of experimental animals. Significant increase ($p < 0.01$) in the activities of superoxide dismutase and catalase was observed in group II mice (0.2 gm / day) when compared to control. Significant decrease was observed in all the other groups of experimental mice (i.e. group III to VI).

Table 3 shows the activities of $\text{Na}^+ / \text{K}^+ - \text{ATPase}$, $\text{Ca}^{2+} - \text{ATPase}$, $\text{Mg}^{2+} - \text{ATPase}$ in erythrocyte membrane of experimental animals. Increased activities ($p < 0.05$) were observed in group II mice (0.2 gm / day) when compared to control. Significant decrease was observed in all the other groups of experimental mice (i.e. group III to VI).

DISCUSSION

Oxidative stress is a state of imbalance between generation of reactive oxygen species (ROS) like hydroxyl and superoxide radicals, and the level of antioxidant defense systems. Oxidative stress results in the damage of macromolecules including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates. Lipid peroxidation is oxidative deterioration of polyunsaturated fatty acids and it involves reactive oxygen species and transition metal ions [26]. Peroxidation of membrane system are the foremost consequences of free radical damage and the efficiency of plant extracts in inhibiting lipid peroxidation *in vivo* is a very good measure of assessment of antioxidant potential. The lowest dose of 0.2 gm / day inhibited lipid peroxidation in erythrocytes, indicating anti-peroxidative effect of betel leaf extract. Lipid peroxide contents were increased in other groups (group III and group VI) which indicate high doses of betel leaf extract produce toxic effect in the experimental mice (Table 1). The increased lipid peroxidation by higher doses of betel leaf extract is due to fall in total radical – trapping capacity of blood plasma and marked reduction in plasma levels of antioxidants such as vitamin E and C was evident in the present study.

Table 1. Effect of different doses of *Piper betle* leaf extract on the levels of TBARS in erythrocytes, ascorbic acid and vitamin E in plasma of control and experimental animals after 15 days of treatment.

Statistically significant values were expressed as mean \pm S.D of 6 mice from each group.

Groups	TBARS (nmoles / ml)	Ascorbic acid (mg / dl)	Vitamin E (mg / dl)
Group-I-Control (2 ml saline / day)	1.78 \pm 0.14	1.6 \pm 0.17	1.9 \pm 0.10
Group-II- 0.2 gms / day)	1.50 \pm 0.1***	1.9 \pm 0.09***	2.1 \pm 0.07***
Group-III- 0.4 gms / day)	1.89 \pm 0.09*	1.4 \pm 0.10*	1.8 \pm 0.09 ^{NS}
Group-IV- 0.6 gms / day)	1.97 \pm 0.12 **	1.38 \pm 0.11**	1.8 \pm 0.11**
Group-V- 0.8 gms / day)	2.30 \pm 0.19***	1.30 \pm 0.08***	1.7 \pm 0.12***
Group-VI-1.0 gms / day)	2.81 \pm 0.23***	1.28 \pm 0.11***	1.5 \pm 0.16***

Group II to VI were compared with group I (Control). * - p < 0.05, ** - p < 0.01, *** - p < 0.001, ^{NS}- Non- significant

Table 2. Effect of different doses of *Piper betle* leaf extract on the activities of superoxide dismutase and catalase in erythrocytes of control and experimental animals after 15 days of treatment.

Statistically significant values were expressed as mean \pm S.D of 6 mice from each group.

Groups	Superoxide dismutase (Units / min / mg protein)	Catalase (mmoles of H ₂ O ₂ utilized / min/ mg Hb)
Group-I-Control (2 ml saline / day)	3.01 \pm 0.28	7.5 \pm 0.6
Group-II- 0.2 gms / day)	5.21 \pm 0.19***	8.3 \pm 0.4**
Group-III- 0.4 gms / day)	3.20 \pm 0.07 ^{NS}	7.9 \pm 0.7 ^{NS}
Group-IV- 0.6 gms / day)	2.69 \pm 0.12**	6.9 \pm 0.3**
Group-V- 0.8 gms / day)	2.06 \pm 0.08***	6.4 \pm 0.8**
Group-VI-1.0 gms / day)	1.88 \pm 0.14***	6.0 \pm 0.7***

Groups II to VI were compared with group I (Control). ^{NS}- Non significant, ** - p < 0.01, *** - p < 0.001

Table 3. Effect of different doses of *Piper betle* leaf extract on the activities of membrane – bound ATPases in erythrocyte membrane of control and experimental animals after 15 days of treatment.

Statistically significant values were expressed as mean \pm S.D of 6 mice from each group.

Groups	Na ⁺ /K ⁺ ATPase@	Ca ²⁺ ATPase@	Mg ²⁺ ATPase@
Group-I-Control (2 ml saline / day)	0.0549 \pm 0.003	0.0238 \pm 0.002	0.0779 \pm 0.004
Group-II- 0.2 gms / day)	0.0620 \pm 0.008*	0.0298 \pm 0.006*	0.0818 \pm 0.002*
Group-III- 0.4 gms / day)	0.0532 \pm 0.009 ^{NS}	0.0222 \pm 0.003 ^{NS}	0.0751 \pm 0.003 ^{NS}
Group-IV- 0.6 gms / day)	0.0501 \pm 0.004*	0.0201 \pm 0.004*	0.0746 \pm 0.005*
Group-V- 0.8 gms / day)	0.0498 \pm 0.005*	0.0198 \pm 0.003*	0.0732 \pm 0.002*
Group-VI-1.0 gms / day)	0.0490 \pm 0.003**	0.0188 \pm 0.005*	0.0711 \pm 0.004*

@ - μ moles of Pi liberated / hr / mg protein.

Groups II to VI were compared with group I (Control). * - p < 0.05, ** - p < 0.01, ^{NS}- Non significant

Superoxide anion (O_2^-) reacts with water to form hydrogen peroxide (H_2O_2), which in turn is responsible for the generation of hydroxyl radicals. Hydroxyl radicals attack membrane fatty acids inducing lipid peroxidation. Catalase is believed to be the most effective defensive agent against high concentration of H_2O_2 . Interestingly, in the present study, the higher doses of the drug decreased the activity of catalase in group III to VI and the lower doses in group II increased the catalase activity, suggesting that these dose dependent differential actions of the plant extract on lipid peroxidation are probably mediated by catalase (Table 2).

Apart from enzymatic antioxidants, non-enzymatic antioxidants namely vitamin A, E, C and glutathione are important for cellular system in curtailing reactive oxygen species. The most important antioxidants, ascorbic acid and vitamin E were studied to evaluate the antioxidant potential of different doses of betel leaf extract in experimental mice. Ascorbic acid is reported to be associated with better scavenging action in *in vivo* than the antioxidant enzymes, because it is present in both extracellular as well as in the intracellular fluids [27]. The low levels of ascorbic acid was found in group III to group VI animals might be due to high lipid peroxidation products formed in these groups or rapid removal of ascorbic acid and vitamin E in the body.

Vitamin E may protect cellular components against peroxidative damage via the free radical scavenging mechanism or as a constituent of the membrane [27]. The antioxidant properties of α -tocopherol result from its ability to quench both singlet oxygen and peroxides [28]. Within the membrane, vitamin E is the only protective agent that can act against the toxic effects of oxygen radicals [29]. In the present study a low dose of betel leaf extract has antioxidant potential but at higher doses vitamin E levels in group III to VI decreased dramatically (Table 1). This damage to the membranes was discernible in the slightly lower activity of membrane-bound ATPases in animals fed with higher doses of the extract.

Membranes are vital for biological system and their integrity is essential for normal functioning of cells and damage to membrane organization is an initial step in cell death. Peroxidation of membrane lipids initiates a loss of membrane-bound enzyme activity and cell lysis [30], alters membrane permeability and cell function [31]. Abnormal lipid peroxides affect membrane-bound ATPases activities and their levels were decreased due to the excessive production of thiobarbituric acid reactive substances [32]. The membrane bound enzymes such as Na^+ / K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase are responsible for the transport of sodium / potassium, magnesium and calcium ions respectively, across the cell membranes at the expense of ATP on hydrolysis [33]. Na^+ / K^+ ATPase, which is an integral membrane protein and it is responsible for a large part of energy consumption constituting the basic metabolic rate [34]. The reduced activity of Na^+ / K^+ ATPase indicate changes in the

membrane under a pathological conditions [35]. Na^+ / K^+ ATPase is reduced, whereas Mg^{2+} ATPase activity is inhibited in photosensitivity-induced damage in plasma membrane [36]. Ca^{2+} -ATPase, the enzyme responsible for active calcium transport, is extremely sensitive to hydroperoxides and this may lead to its inhibition. Hence high concentrations of betel leaf extract causes loss of membrane-bound enzymes activity (Table 3).

From the results it is evident that, betel leaf extract exhibits dose-dependent actions. At low concentrations it is anti-peroxidative, whereas at higher concentrations it induces oxidative damage to biological membranes as indicated by the elevated levels lipid peroxides in erythrocytes and lower activity of superoxide dismutase, catalase and membrane-bound ATPases. Low doses of betel leaf extract have beneficial effect rather than high doses of the extract. The result of this study shows that a daily administration of *Piper betle* at the dosage of 0.2 gm / day for 15 days provides better antioxidant potential as well as membrane stabilizing action. However further investigations are needed to assess the efficacy of *Piper betle* in oxidative stress induced pathological conditions.

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LAPAROSCOPY IN PAEDIATRIC SURGERY – OUR EXPERIENCE AT SRMC

Prakash Agarwal*, R.K. Bagdi, S. Balagopal, R. Madhu, P. Balamourougane

ABSTRACT

a) *Aim of the study:* To review the current status of laparoscopic surgery at Sri Ramachandra Medical College Hospital (SRMCH), a paediatric tertiary care centre in Chennai. We highlight our experience in terms of feasibility, safety cost effectiveness and effect on standard practices.

b) *Methodology:* It is a retrospective case series study. All patients who were admitted to Paediatric Surgery at SRMCH and underwent laparoscopic surgery were included in the study group. We reviewed the literature in comparison to our results.

c) *Results:* 25 cases were operated in a span of 9 months, majority of the cases were appendicectomies followed by diagnostic laparoscopy. We had only one case converted to open.

The mean operating time was 65 minutes, median post operative stay of 2.3 days for lap appendicectomies and there were no intra operative or post operative procedure

related complications. Duration of hospital stay was reduced compared to open appendicectomies due to less post operative pain and early recovery.

d) *Discussion:* Laparoscopic appendicectomy is still the most common emergency operation performed laparoscopically. Diagnostic laparoscopy was the second largest group in our series. Initial results at our institution are very encouraging and comparable to international literature meta-analysis. We feel laparoscopic procedures are safe for a wide range of indications in children. In our centre they account for all appendicectomies, diagnostic laparoscopies, female herniotomies and few advanced procedure. Although laparoscopic procedures have gained an integral place in paediatric surgery and are relatively safe, advanced laparoscopic procedures should be developed, practiced and evaluated in dedicated surgical units to ensure a broad base of experience on which to base future decisions and guidelines.

Key words : Laparoscopy, appendicitis, child

INTRODUCTION

One of the significant changes in medical practice that has evolved gradually during the last three decades is the reduction of the traumatic insult inevitable and incidental to surgical interventions.

Laparoscopic surgery in children is not new. Paediatric surgeons were among the pioneers of laparoscopic surgery in the early 1970s,¹ but the vast potential of this “minimally invasive” approach to treat children with surgical conditions has only recently begun to be realised. For over three decades, paediatric laparoscopy was restricted mainly to diagnostic use. In the early 1990s, an explosive expansion of laparoscopic surgery occurred in adults as a result of the success of laparoscopic cholecystectomy⁽¹⁾.

Nevertheless, interest in laparoscopic surgery in children remained confined to a few enthusiasts initially,²⁻⁴ while the rest of the paediatric surgical community adopted a “wait and see” attitude. More recently however, with increasing experience in paediatric laparoscopic procedures,⁵⁻⁷ and

advances in miniaturised instrumentation, laparoscopy’s place in the modern paediatric surgical armamentarium has finally become accepted.

In the USA, it is estimated that 82% of paediatric surgeons perform laparoscopic surgery.⁸ The question is no longer whether laparoscopic surgery should be done in children, but what conditions should be treated laparoscopically.

HOW IS LAPAROSCOPIC SURGERY DIFFERENT IN CHILDREN?

It is important to recognize that in infants and small children, the surface area for access is small, the abdominal wall is thin and compliant, the liver margin is below the rib cage, the bladder is largely an intra-abdominal structure, the viscera is close to the anterior abdominal wall and the abdominal cavity is small. In small infants, 400ml CO₂ may be required to establish, a pneumoperitoneum. The so-called obliterated structures, umbilical vein and arteries, and urachus remain relatively large and partially patent in infants.

These anatomical characteristics make access and manipulation in the younger age group a more demanding and difficult task when compared to grown up children or adults. On the other hand, young children have well-defined anatomical landmarks due to lack of excess fat, making recognition and dissection of structures a relatively easy task.

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OUR EXPERIENCE AT SRMC:

Keeping in pace with demand for Paediatric laparoscopy we started laparoscopic surgery in our department in 2005. With the available infrastructure, we made a modest start and within a span of 9 months had 26 cases of laparoscopy done in paediatric age group (Table-1). Our youngest child was 8 months old and oldest child was 18 years old. The cases ranged commonly from appendicectomies (73%) to diagnostic laparoscopy (19%), intrabdominal lymph node biopsies, ovarian cyst removal etc.

We usually prefer the open Hasson's technique of first umbilical port insertion in children as it is done under vision and considered safe in children.

We tend to follow the principle of "triangularisation"

in the placement of 3 ports for most of the laparoscopic surgeries in compliance with the ergonomics of laparoscopic surgery. Appendicectomy being the commonly performed procedure in our department (73%), we have standardized the procedure and follow the same principle in all cases.

We use 3 ports inserted at the infraumbilical, suprapubic and left iliac fossa. After skeletonising the appendix, we pass 2 endoloops for ligating the appendix at the base and do an appendicectomy in between the 2 endoloops. The appendix is usually removed by the infraumbilical port. The wound at the umbilicus is closed in layers and the rest of the wound are closed only at the skin level. Of all the cases we had to convert to open laparotomy in one case where the appendix had necrosed and the patient had fecal peritonitis as a result of sloughing of the appendix in entirety.

Table 1. shows an analysis of the children who underwent laparoscopic surgery for various reasons - at SRMC from 2005 till date.

TABLE -1. (n = 26)**ANALYSIS OF CHILDREN WHO UNDERWENT LAPAROSCOPIC SURGERY**

Sl.No	Name	Age	Sex	Diagnosis	Procedure
1.	M	12	F	AC APPENDICITIS	LAP APPENDIX
2.	RR	14	F	AC APPENDICITIS	LAP APPENDIX
3.	K	7	M	MESENTERIC LYMPHADENOPATHY	LAP LYMPH NODE BIOPSY
4.	SS	15	F	AC APPENDICITIS	LAP APPENDIX
5.	SS	10	F	AC APPENDICITIS	LAP APPENDIX
6.	G	14	M	AC APPENDICITIS	LAP APPENDIX
7.	B	1	M	LEFT NON PALPABLE TESTIS	DIAG LAP WITH LEFT ORCHIDECTOMY
8.	S	13	F	PERFORATED APPENDIX	LAP CONVERTED TO OPEN
9.	D	8	M	PERFORATED APPENDIX	LAP APPENDIX
10.	RN	10	M	AC APPENDICITIS	LAP APPENDIX
11.	JE	8	M	REC. APPENDICITIS	LAP APPENDIX
12.	S	8	F	REC APPENDICITIS	LAP APPENDIX
13.	P	16	F	AC APPENDICITIS	LAP APPENDIX
14.	SF	15	F	LEFT OVARIAN TUMOR	LEFT OVARIECTOMY
15.	T	8 MTHS	M	SUB HEPATIC CYST- CYSTIC DUCT PERF.	DIAG LAP – OPEN CHOLECYSTECTOMY
16.	G	12	M	ACUTE APPENDICITIS	LAP APPENDIX
17.	M	3	F	B/L INGUINAL HERNIA	B/L LAP HERNIOTOMY
18.	SS	15	F	ACUTE APPENDICITIS	LAP APPENDIX
19.	LA	18	F	ACUTE APPENDICITIS	LAP APPENDIX
20.	L	16	F	TB ABDOMEN	DIAGNOSTIC WITH BIOPSY
21	KV	1 ½	M	HIRSCHSPRUNG'S DISEASE	DIAGNOSTIC LAP
22	A	9	M	ACUTE APPENDICITIS	LAP APPENDIX
23	E	9	F	ACUTE APPENDICITIS	LAP APPENDIX
24	DP	10	F	ACUTE APPENDICITIS	LAP APPENDIX
25	AK	12	F	ACUTE APPENDICITIS	LAP APPENDIX
26	N	16	F	ACUTE APPENDICITIS	LAP APPENDIX

One of our patient who had fecal peritonitis and had to be converted to open appendicectomies had wound infection and fecal fistula. She had her total appendix sloughed out and stump blow out at the cecum. No other patient had wound infection. All the patients recovered early, had less post-operative pain and returned to school early compared to open appendicectomy group.

While the costs of performing laparoscopic surgery per se was found to be higher compared to open surgery however, all the parents expressed satisfaction post operatively.



Fig. 1 : Photograph showing Laparoscopic appendicectomy in progress

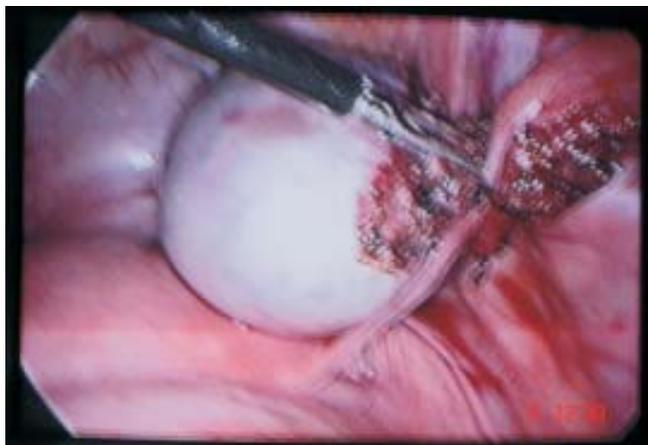


Fig. 2 : Photograph showing Laparoscopic ovariectomy in progress

DISCUSSION:

The traditional Hippocratic ethos provides the background of the idea that the less invasive the procedure, the better. In conditions in which the body wall itself is not affected, it is a pity that the body wall must be opened in a classic open surgery. Body wall related complications do occur, for example infection dehiscence and incisional hernia. Even in the absence of body wall complications, opening of the body wall results in morbidity. The larger the wound, the more fascia, muscles and nerves are transected and more morbidity can be expected. Pain related to access trauma may not only be limited to the immediate

post operative period, but also may become chronic. Hypertrophic scars also may become a source of annoyance causing pain, parasthesia and itching. Hypothermia and desiccation of tissues are other adverse effects more prominently noticed in open surgery.

Creation of pneumoperitoneum and extremes of patient position, pose challenges to paediatric anesthesiologists in laparoscopy. An ideal insufflating gas should have minimum physiologic effect, should be absorbed minimally, excretion should be rapid, should have high blood solubility and should be non-combustible. Rarely, complications arise from CO₂ insufflation for pneumoperitoneum during laparoscopy. These include gas embolism, cardiovascular compromise, hypercapnia. The risks are minimised by the use of low pressure CO₂ insufflation in children. Intraabdominal pressure (IAP) up to 12 mm Hg has minimal effects on cardiac output but IAP levels > 15 mm Hg are not well tolerated. Elevated IAP shifts the diaphragm upwards and reduces its excursions. Slight increases in end tidal CO₂ and peak airway pressures might be detectable intraoperatively. This can usually be compensated for by slight hyperventilation.⁹

Laparoscopy in children have been shown to have distinct advantage over the traditional open methods in causing less post-operative pain and wound complications, faster recovery as well as shorter hospital stay. This decrease in morbidity could be attributed to smaller access wound, hence less fascia, muscles, nerves being transected and reduced water and heat loss¹⁰⁻¹¹

In our study group though the cost of laparoscopic surgery is marginally higher than open surgery, but taking all the factors (e.g.: reduced analgesia requirement, less hospital stay, early return to school) into consideration, the cost may be same compared to open surgery.

Operating time was more than open surgeries. Comparatively we feel operating time may be reduced in cases of retrocecal or complicated appendicitis. Finding and dealing with a buried or retrocecal appendix will be less time consuming and less traumatic laparoscopically than by open method. We feel laparoscopy is a very important tool when the diagnosis is not certain.

In a Meta analysis report by Aziz O et al ¹², they compared studies published between 1992 and 2004 of laparoscopy versus open appendicectomy in children. 23 studies including 6477 children (43% laparoscopic and 57% open were included. They report suggests that laparoscopic appendicectomy in children is associated with less wound infection than open appendicectomy (1.5% Vs. 5%) Operative time was not significantly longer in the laparoscopic group, and post-operative stay was significantly shorter. This study results are comparable to ours.

Incidence of postoperative adhesions is also reduced¹³. Due to magnification and good illumination, endoscopic surgery gives a much better view of the operative field than open surgery. This is particularly true in the pelvis and cardiac end of the oesophagus.

Paediatric laparoscopy is here to stay. It has already invaded the surgical domain. Therefore it would be prudent for all of us not to be endoresistant. "Key hole surgery revolution" has begun. Laparoscopic cholecystectomy and, to an extent lap appendectomy have become patient demand operations and surgeons would lose patients if they cannot deliver the 'keyhole' approach.

LONG TERM OBJECTIVES:

1. Recognize the common pediatric conditions that may be treated with minimally invasive surgery
2. Prospective randomized clinical trials of laparoscopic surgery in infants with miniaturized instruments.

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CARDIAC REHABILITATION AND EXERCISE TRAINING - CHALLENGES AND FUTURE DIRECTIONS

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ABSTRACT

Cardiac Rehabilitation (CR) is an intradisciplinary program of education and exercise established to assist individuals with heart disease in achieving optimal physical, psychological and functional status within the limits of their disease.

As CVD is a multifactorial disease, the beneficial outcomes from CR are numerous. Possible outcomes include improvement in lifestyle, reduction in CVD risk factors, cost of care, disease progression, morbidity, and mortality. The prevention of subsequent coronary events and the maintenance of physical functioning in such patients are major challenges in preventive care.

Comprehensive rehabilitation offers physical training, nutritional advice, and psychological therapy as well as health education, social support and including assistance for return to work. But individual patient needs for constituent elements of the total programme vary widely.

Regular dynamic exercise as a rehabilitative measure is accepted as the major component of a cardiac rehabilitation programme.

Research and Clinical activities have reached the point where evidence, clinical practice and professional experience have recommended exercise training in CR. Non compliance in continuation of the programme should be focused and measures to make patients to continue as home based programme in INDIA should be implemented.

New models of CR need to be developed in our country. These new CR programs will need to address issues of promoting long-term adherence, improving accessibility, particularly for patients in rural community and addressing the growing need for CR in elderly. An individualized program should be developed in close collaboration with the patient's primary care and needs.

Key words : Cardiac Rehabilitation CR, Coronary Artery Bypass Graft CABG, Myocardial infarction MI, Cardio Vascular Diseases CVD

INTRODUCTION:

Rehabilitation is understood by most of us as that process enabling, encouraging and assisting patients to make the transition from a state of illness back to a state of health, as near as possible to normal.

Cardiac Rehabilitation CR is an intradisciplinary program of education and exercise established to assist individuals with heart disease in achieving optimal physical, psychological and functional status within the limits of their disease.

Cardiac rehabilitation programmes have developed in a number of Countries over the past twenty years. Initially most programmes concentrated on patients having acute Myocardial infarction. Programmes have been developed more recently for patients discharged after elective procedures (coronary artery bypass graft and percutaneous transluminal coronary angioplasty) and for patients with chronic stable angina. While the over - all objectives of rehabilitation, focus to facilitate the patient's return to as near normal possible; even though the protocols followed are common

to all these patient groups, the specific needs of these patients differ.

Rehabilitation therapists will appreciate the relevance of both treatment and prevention to rehabilitation in so far as they impinge as rehabilitation practice, and both are included.

Most rehabilitation programmes, whether predominantly exercise based, or predominantly psychological based, are comprehensive; include some patient education on the heart, heart disease, risk factors including life style modification during treatment and management in hospital. Rehabilitation therapists should have sound knowledge in managing these patients.

As the concept of comprehensive rehabilitation gained acceptance, revascularization of the myocardium by coronary artery bypass graft surgery or later by angioplasty became increasingly common. It is apparent that a poor functional outcome of surgery was the result of the same mixture of physical, psychological, social and economic factors [1]. So, during the '80s the indications for cardiac rehabilitation widened to include these patients. Those with stable angina,[2] following surgery for valvular heart disease [3] or after cardiac transplantation[4] were also shown to improve after rehabilitation. More recently, as evidence has accumulated cardiac rehabilitation benefits older people[5], those with poor left ventricular

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function[6] or with a low ischemic threshold[7]. Rehabilitations should also be considered in patients known to have coronary heart disease but who are free of symptoms and in those at high risk of developing the disease. Educational counseling and behavior modification are also important aspects.

Risk factors and primary preventions includes stopping smoking [8], having a controlled lipid levels [9, 10], increasing physical activity [11, 12], controlling hypertension [13], reducing alcohol [14], altering diet and reducing mental stress. Also hormonal replacement therapy reduces the risk of coronary disease in the post – menopausal woman. [15]. An overall life style modification is necessary. By becoming more physically active and doing small bouts of exercises, must be the first step towards life style modification.

The Role of Exercises Training In Cardiac Rehabilitation

The concept of exercise training as a therapy for patients suffering from coronary heart disease was emphasized by a English Physician, William Heberden, who was the first to describe the classical picture of effort- induced angina pectoris, also recorded the case of a patient **“Who set himself the task of sawing wood every day and was nearly cured”**. Irish physician William Stokes published his classic work, “The Diseases Of The Heart And Aorta” in which he wrote, “the symptoms of debility of the heart are often removable by a regulated course of gymnastics, or by pedestrian exercises.”

After Stokes’ death in 1878, Prolonged immobilization in bed became the mainstay of medical care for close to a century and seldom was it practiced more assiduously than after an acute myocardial infarction. The physician insisted that the heart attack survivor be nursed in bed for eight weeks or more, washed and fed, and not even allowed up to use the bedside commode. The time of hospital discharge may be often three to four months after acute event and the patient becomes severely deconditioned, weakened, demoralized, and permanently unemployable,often.

The annual meeting of the American Medical Association, held in Chicago in 1944 included a symposium on “ The abuse of rest in the treatment of disease” at which for the first time physicians collectively questioned the wisdom of prolonged immobilization.

In 1952, Levine and Lown introduced their innovative “armchair treatment”, in which they progressed their patients to sitting up in chair by the side of the bed a few days after admission[16].

Throughout the ‘50s and ‘60s there were number of reports on the beneficial effects of early ambulation and progressive graded activity.[17, 18]

From early mobilization to a formal inpatient exercise regimen was a natural progression. Pioneers in this area

were Wenger and Zohman[19] who encouraged low level self care activities to be commenced early in the coronary care unit, which was followed even after transfer to the general ward, by more strenuous activities of daily living and monitored upper and lower limb strengthening exercises.

Gottheiner of Israel was the first to embark upon a large scale post coronary **out patient** exercise training programme. Under his guidance some 1100 patients completed five years of endurance training, which included activities such as walking, jogging and cycling.[21] Over a five year period the average annual fatal recurrence rate was 0.88% compared with 4.8% per year for non-exercised patients. These results attested to the safety of supervised physical training for patients recovering from an uncomplicated myocardial infarction.

In North America, Hellerstein of Cleveland was one of the early supporters of exercise in post – coronary rehabilitation. In 1968 he described the results of a three year exercise programme involving 254 patients. In Canada, Rechinitzer and his associates from London & Ontario, first reported in 1967 on the short term benefits of a six month training programme and in 1972 published a five year follow up that compared data with results from patients treated at other hospitals in the London area[22].

A report on seven post coronary patients from the Toronto programme in the 1973, Boston Marathan [23] demonstrated that high level of fitness can be achieved by supervised training. Immediate studies in few years also focused on considerable attention on cardiac rehabilitation and did much to convince patients and public alike that most heart attack survivors could lead a full and active life[24, 25].

By the ‘80s, the demonstrable benefits of exercise rehabilitation training were sufficiently convincing that the various national and international heart associations were urging acceptance. In 1981 the council on Scientific Affairs of the American Heart Association recommended that “Cardiac rehabilitation should be considered one of the treatments for coronary heart disease complementary to drug therapy or surgery[26]. The following year the World Health Organization concurred, recommending “regular dynamic exercise as a rehabilitative measure is accepted as the major component of a cardiac rehabilitation programme[27]. The beneficial effects of aerobic training include improved efficiency of oxygen transport system, allowing an increase in maximal work capacity as well as greater tolerance for prolonged sub maximal physical tasks. Exercises brings about Structural and functional changes in working muscles, with enhanced ability to store and utilize carbohydrate and fat, as well as extract more oxygen from circulating blood[27].

The rate pressure product is decreased at the same sub maximal levels of effort, thereby reducing the workload on the heart muscle by doing regular exercises. For the angina sufferer, this means that a higher level of effort is possible

before the onset of symptoms. The stroke volume is increased as a result of augmentation in end diastolic volume, and enhanced myocardial contractility. In the presence of coronary artery disease, possible stabilization of atherosclerotic plaque, and/or improvement in blood supply to heart muscle by collateralization and / or regression in plaque size, may be possible.

The following changes also occurs due to exercise. They are, restoration of self confidence, improvement in mood, and alleviation of depression, reduction in CAD risk factors, decreased body fat, lowered serum triglycerides, increased HDL-cholesterol and decreased total cholesterol / HDL- Cholesterol ratio, increased insulin sensitivity and glucose tolerance (important in Type II diabetes), enhanced fibrinolytic activity, decreased resting and exercise plasma catecholamine levels (with increased resistance to stress and increase in threshold for ventricular fibrillation[27].

The Current Trend

During 1980 to 2005, there were more studies showing beneficial outcomes from CR. Possible outcomes include improvement in lifestyle, reduction of CVD risk factors, cost of care, reduction in disease progression, morbidity, and mortality. CVD is also a major cause of physical disability, particularly in the rapidly growing population of elderly persons [28, 29].

The prevention of subsequent coronary events and the maintenance of physical functioning in such patients are major challenges in preventive care. Cardiac-rehabilitation programs were first developed in the 1960s, [30,31] once the benefits of ambulation during prolonged hospitalization for coronary events had been recognized. [33]. After discharge from the hospital, the process of physical reconditioning was continued at home. The focus of these programs was almost exclusively on exercise. The hospital stay for acute coronary syndromes has now been shortened to three to five days so that deconditioning is minimal. [34]

With shorter stays, however, the opportunity to train patients about risk reduction and exercise is less. There is convincing evidence that regular exercise and modification of risk factors favorably alter the clinical course of coronary heart disease. [35,36]

The benefits of cardiac rehabilitation and secondary prevention are broad and compelling. Controlled trials of exercise after myocardial infarction, reported in the 1980s, have demonstrated reductions in overall mortality and in mortality from cardiovascular causes. [35,36]

Trials of exercise combined with nutritional counseling have demonstrated a slowing of the atherosclerotic process [37,38] and decreased rates of subsequent coronary events and hospitalization.

Home-base rehabilitation programs that are directed by physicians and coordinated by nurses have been developed as a way of expanding the delivery of secondary-prevention services [35,36].

Over the past 30 years, exercise therapy has evolved as one of the important component of CR. These programs also include nutrition counseling, smoking cessation, weight management, psychosocial counseling and metabolic risk-factor management, can be found in many hospitals and communities. The target population for CR has expanded, and includes men and women of all ages and those presenting with nonischemic CVD. Several national organizations have published extensive recommendations and guidelines for CR [39].

Challenges of CR

Even though there are numerous benefits of CR, several challenges exist that are common to most programs. These challenges include low participation rates, gender-biased referral and participation; problems with adherence, dropouts and resource management. Participation rates in CR programs by those eligible patients are less. Possible reasons may be lack of referral, distance to CR facility, lack of motivation, and patient's unwillingness to attend [40,41].

It is noted that referral rates are lower for women than men; however, it has also been reported that women are more likely to drop out of a CR program once referred [40].

The benefits of CVD risk reduction are only realized through long-term lifestyle and risk-factor management. CR dropouts and adherence continue to pose a challenge to the success of CR. Even after CR participation, adherence rates to favorable lifestyle behaviors have been reported to decline. However, good comprehensive data on adherence following completion of CR and its influence on risk-factor modification are not available [40,41].

Future directions of CR

As CR has evolved in the past 30 years, it has proven its value in the treatment of patients following MI and post CABG. In the coming years, the challenges will be no less demanding. As new target patient populations are recruited into CR programs and new models as well should be developed. Strategies for improving participation rates need to be developed, focusing on education of patients and health care providers. Extra efforts should be directed towards reducing the gender inequity. The possibility of disease regression needs to be explored in larger populations using clinically relevant practices [41].

The new CR programs will need to address issues of promoting long-term adherence, improving accessibility, particularly for patients in suburban and rural communities, and addressing the growing need for CR in an ageing population. Future CR programs will also need to be resource sparing, as current health care organizations cannot meet the demand of all eligible patients. ***For those patients living in rural areas, new communication technologies may be useful in delivering CR through telemedicine initiatives. Appropriate risk stratification will aid in health care resource management, restricting outpatient CR programs to those patients at high risk and utilizing less frequent contact for low and moderate risk patients.***

Integration with patients' family physicians and other health care providers is a potential strategy to improve adherence, as are behavior strategies aimed at patient empowerment. Other forms of contact than the traditional face-to-face session can be incorporated into CR as a method of continued follow-up and reaching those patients in nonurban areas. The growth of **telemedicine** can play a vital role from the simple use of the telephone, to the Internet and the use of personal digital assistants. Integration of these and other tools can address a number of the issues of CR [41].

CONCLUSION

Research and Clinical activities have reached the point where evidence, clinical practice, and professional experience have recommended exercise training in CR.

Cardiac rehabilitation has exclusively not been used and studied in patients at relatively low socioeconomic levels. However, the prevalence of coronary heart disease among persons at lower socioeconomic levels is increasing. Non compliance in continuation of the programme should be focused and measures to make patients to continue as home based programme in India.

An individualized program should be developed in close collaboration with the patient's primary care and needs [40].

To end, complete medical and physical fitness evaluation for middle age group targeting preventive rehabilitation should be on focus. Change in life style will prevent or slow down the development of heart disease. Regular medical check up, proper diet, supervised regular exercise, weight reduction, control of diabetes, control of high cholesterol and high blood pressure, stopping smoking and reduction in the stress may keep the heart more fit for the rest the life.

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EDIBLE VACCINES

V. Krishna Chaitanya*, Jonnala Ujwal Kumar

ABSTRACT

A new approach for delivering vaccine antigens is the use of inexpensive, oral vaccines. Edible oral vaccines offer exciting possibilities for significantly reducing the burden of diseases like hepatitis and diarrhea particularly in the developing world where storing and administering vaccines are often major problems. Even though they have some disadvantages like control of the "dosage" of the antigen that is present

in the recombinant fruit or vegetable, they have many advantages as they trigger the immunity at the mucosal surfaces which is the body's first line of defense. To overcome the disadvantage of adequate dosage, stable plant lines that produce fruits and vegetables with relatively constant amounts of the antigen need to be developed. The hope is that edible vaccines could be grown in many of the developing countries where their need is more.

Key words : Vaccines, edible, Immunity

INTRODUCTION

In the last decade, advancements in the field of medicine and healthcare have been possible because of the development of newer, safer and highly effective vaccines; recombinant vaccines, subunit vaccines, peptide vaccines and DNA vaccines to name a few. Although these vaccines have an undue advantage over traditional conventional vaccines, there is a flip side to them. Not only are these vaccines expensive, but their storage and transportation pose a problem as many of them require refrigeration. This is a disadvantage in many of the developing countries.

So, as alternatives had to be thought of, it was envisaged that plants could be used as a cheap, safe and efficient production system for vaccines and thus the concept of edible vaccines was born.

DEFINITION

Edible vaccines are nothing but transgenic plant and animal based production of or those that contain agents that trigger an animal's immune response. In simple terms, edible vaccines are plant or animal made pharmaceuticals. This essay highlights the importance of edible vaccines produced in plants.

INITIAL DEVELOPMENTS IN DESIGNING THE EDIBLE VACCINES

The concept of edible vaccines was developed by Arntzen (www.genomenetwork.org) in the 1990s. He currently heads the department of plant biology at the Arizona State University. He fell upon the idea after he attended a conference in New York, organized by the WHO. Although the idea seemed quite simple in the beginning, making it into a reality has required sophisticated science.

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The earliest demonstration of an edible vaccine was the expression of a surface antigen from the bacterium *Streptococcus mutans* in tobacco. As this bacterium causes dental caries, it was envisaged that the stimulation of a mucosal immune response would prevent the bacteria from colonizing the teeth and therefore protect against tooth decay.

CURRENT STATUS

Several plant derived vaccines for human use are approaching the market but it is likely that the first commercial Plant derived vaccine will be a veterinary vaccine.

At least 30 such products have been expressed in plants, some providing protection against challenges with disease causing agents.^[1]

The trial carried out by prodiGene Inc. showed for the first time that an oral vaccine produced in plants could protect live stock against virulence challenge.^[2]

The first product to reach market could be a poultry vaccine developed by Dow AgroSciences^[3], has been proposed for market release sometime in 2006.

HOW DO EDIBLE VACCINES WORK?

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response.

SOME EXAMPLES OF EDIBLE VACCINES

- **Transgenic Potatoes For Diarrhea**

The first human trial for an edible vaccine took place in 1997. Volunteers ate transgenic potatoes that contained the b-subunit of the *E. coli* heat-labile toxin, which causes diarrhea. Ten of the 11 volunteers showed a 4-fold increase in serum antibodies.^[4, 5]

Researchers at the Boyce Thompson Institute at Cornell University conducted another clinical trial of an edible vaccine in 1999. Potatoes containing the Norwalk

virus (which causes vomiting and diarrhea) fed to volunteers elicited an immune response in 19 out of 20 volunteers.^[6]

The disadvantage of using potato-based edible vaccine is that it has to be consumed raw; when cooked the protein may get denatured or in some cases less effective. Research has shown that by partial boiling at least half the vaccine remained alive.

- **Transgenic Tomatoes Against Diarrhea**

In the US at the Cornell University, researchers have developed transgenic tomatoes against the Norwalk virus, which causes severe diarrhea. The tomatoes produced a surface protein specific to the virus. Mice that ate these tomatoes developed an immune response to the virus.^[7]

Recently, banana has been explored as an alternative source because not only does it eliminate the need for cooking but also it's a locally grown plant. The expression of a protein in banana will depend on the identification of a tissue specific promoter. Other examples include rabies glycoprotein being expressed in viral vectors in spinach^[8] and hepatitis B surface antigen in lettuce and potato.^[9, 10]

ADVANTAGES OF EDIBLE VACCINES

1. They are cheap; therefore they can be mass-produced.
2. They can be ingested by eating the plant/part of the plant. So, the need to process and purify does not arise.
3. Extensive storage facilities like cold storage are not required.
4. If the local/native crop of a particular area is engineered to produce the vaccine, then the need for transportation and distribution can be eliminated.
5. Most importantly, they trigger the immunity at the mucosal surfaces such as those that line the mouth (mucosal immunity) which is the body's first line of defense.

DISADVANTAGES OF EDIBLE VACCINES

1. Will the antigens be able to survive the hostile, acidic conditions of the stomach and even if they did, will they be able to trigger the immune system in the right way? Although initial trials have shown promising results in human subjects, it is not clear what will happen when the person comes in contact with the actual virus.
2. How can the vaccine dose be controlled? This remains the most difficult task. There seems to be a danger that too high a dose could provoke oral tolerance of an invading bacteria or virus, instead of an immune response. Also, the dosage requirements for children and adults will be different. So, research is on its way to find a solution to these problems.
3. Plants are living organisms that change, so the continuity of the vaccine production might not be guaranteed.

4. Glycosylation patterns in plants differ from those in humans and could affect the functionality of the vaccines.
5. People may develop an allergy to the fruit or vegetable expressing the foreign antigen

CONCLUSION

The first trial on humans in 1997 (using the heat labile B- toxin from *E. coli*) is a milestone on the road to creating inexpensive vaccines that might be particularly useful in immunizing people in developing countries, where high cost and logistical issues, such as transportation and the need for certain vaccines to be refrigerated, can thwart effective vaccination programs.

The hope is that edible vaccines could be grown in many of the developing countries where they would actually be used.

Whatever may be the current situation, a day is not far off when we will be able to pluck a fruit from the garden, eat it and be protected from diseases...making needles needless...

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ORBITAL APEX SYNDROME IN A CHILD

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ABSTRACT

8 yr old male presented with visual loss, diplopia, ptosis, pain behind the left eye, facial numbness and vomiting of one week duration. The ophthalmological, neurological and radiological examination showed a lesion of the left orbital apex with extension into the cavernous sinus. Examination of the nose and paranasal sinuses did not reveal any abnormality. Transnasal Endoscopic orbital

decompression was performed and inflamed granulation tissue found in the orbital apex was removed. Microbiology showed fungal elements which on culture grew *Aspergillus flavus*. Antifungal therapy with new generation oral drug (voriconazole) resulted in complete resolution of symptoms. Relevant literature is reviewed and discussed.

Key words: *Aspergillosis, Orbital Apex Syndrome, Mycoses, Cavernous sinus thrombosis, c + casd*

INTRODUCTION:

Orbital Apex Syndrome consists of paralysis of all the three nerves (3, 4 & 6) supplying the extra ocular muscles and a sensory deficit in the distribution of the first division of the trigeminal nerve, combined with an optic nerve lesion [1]. Fungal lesions involving the orbital apex are the least common cause of this syndrome. At least 4 forms of fungal infection of the sinonasal tract have been recognized: Allergic, Non-invasive, Invasive and Fulminating. Infection usually spreads from the paranasal sinuses to the orbital Apex. *Aspergillus* is a fungus found in soil and organic debris. It usually presents as a localized disease of the lungs and the paranasal sinuses and mainly affects immunocompromised individuals. But the invasive form can also affect normal or mildly immunocompromised individuals. Orbital *Aspergillosis* is an uncommon condition, and is reported for its rarity and dramatic response to therapy.

CASE:

An 8 yr old boy presented to the neurosurgery department of Sri Ramachandra Medical College in Nov 2004 with complaints of blurring of vision in left eye following a head injury sustained 2 weeks earlier. He was evaluated and diagnosed to have a left carotico-cavernous fistula for which he underwent embolization with stenting. Patient improved symptomatically with no blurring of vision and was discharged from the hospital.

In April 2005, he was readmitted in neurosurgery with history of progressive loss of vision with diplopia of 1 week duration. At that time his vision was restricted to perception of light alone. MRI Brain and Paranasal sinuses revealed an enhancing lesion involving the left orbital apex in T2 weighted images. However all the paranasal sinuses were found to be

normal. In consultation with the ophthalmologist a diagnosis of ? granulomatous lesion was made and the patient treated with corticosteroids and antibiotics for a period of one month. Following this, his vision partially improved and he was discharged from the hospital.

In August, 2005 he was admitted with complaints of progressive worsening of vision in the left eye with diplopia, ptosis, retro-ocular pain, facial numbness and vomiting of 1 week duration. ENT opinion was sought for the same. On clinical examination the patient was conscious and oriented. He had proptosis of left eye and it was pushed outwards and downwards. There was left 3, 4 and 6 cranial nerve palsies with loss of sensation over the left forehead region [Fig. 1].



There was only perception of light in the superior quadrant in the nasal line in the left eye. MRI brain and paranasal sinuses revealed an enhancing lesion in T2 weighted images occupying the left orbital apex with extension to the cavernous sinus [Fig.2,3]. All the paranasal sinuses were however found to be normal and free of disease. The size of the lesion was found to have increased when compared to the earlier scan done in April, 2005. A doppler scan of the left eye revealed superior ophthalmic vein



thrombosis. A diagnostic nasal endoscopy could not be done due to severe pain. All the haematological investigations were within normal limits except ESR which was found to be elevated. Endoscopic biopsy with orbital decompression was done under general anaesthesia and an anterior to posterior approach was used. Uncinectomy with infundibulotomy and middle meatal antrostomy was done on the left side. The

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anterior and posterior ethmoid air cells were excised, sphenoidotomy was done. A bulge was visualized in the posterior aspect of the lamina papyracea at level of posterior ethmoids. The lamina was opened all the way from the region posterior to the maxillary ostium to the orbital apex. Bulging medial rectus muscle was identified and soft friable tissue was visualized lateral to the medial rectus extending posteriorly. This tissue was removed and sent for histopathological and fungal studies. A complete medial and inferior orbital decompression was done.

The nasal pack was removed on the first post operative day. The proptosis had subsided with partial recovery of the eye movements. The pain had completely subsided, however there was no improvement in vision. The child was on antibiotic coverage in the post operative period. Microbiological studies from biopsy specimen grew *Aspergillus flavus* on Sabouraud's dextrose agar and Lactophenol cotton blue mount revealed septate hyphae with spores and vesicles typical of *Aspergillus flavus* slide culture technique. The patient was started on oral voriconazole (150 mg BD) for a period of three months.

The patient was reviewed at the end of three months. He had full range of eye movements without any ptosis or proptosis [Fig.4]. Visual acuity had improved from perception of light to finger counting at a distance of two feet. Nasal endoscopy showed well



healed mucosa with no evidence of recurrence. A repeat MRI brain and paranasal sinuses showed no evidence of lesion in the region of the left orbital apex. However there was some evidence of disease persisting in the region of the cavernous sinus [Fig.5].



He was advised to continue voriconazole for a further period of 3 months. Patient was reviewed at the end of 6 months and he is totally asymptomatic.

Visual acuity is still confined to finger counting at a distance of two feet. A repeat MRI showed normal contrast uptake in the cavernous sinus and the orbital apex lesion had completely subsided. Patient has been advised to continue voriconazole for a further period of 3 – 6 months.

This case highlights the importance of endoscopic sinus surgery in lesions of the orbital apex.

DISCUSSION:

The orbital apex is defined as the region between the posterior ethmoidal foramen and openings of the optic canal and the superior orbital fissure [2]. The apex of the orbit is the entry portal for all the nerves and vessels of the eye and

the site of origin of all extraocular muscles except the inferior oblique. Orbital Apex syndrome can be due to a variety of causes like optic nerve glioma, Intraclinoid aneurysm of the internal carotid artery, trauma, orbital tumors, Paget's disease and fungal infections. Fungal infections are the least common. Only rarely is *Aspergillus* the causative pathology. In humans, almost all the invasive *Aspergillus*; also confirmed by infections are caused by *Aspergillus fumigatus*. In invasive human infections *A.flavus*, *A.glaucus*, *A.niger*, *A.restrictus*, *A.terreus* and *A.versicolor* have been found as causative agents. *Aspergillus fumigatus* is the most common species isolated in human infections, followed by *A.flavus* [3]. Invasive fungal infection is an opportunistic infection. Predisposing causes include alcoholism, low dose prednisolone therapy and diabetes mellitus. The route of infection is frequently by inhalation of *Aspergillus* spores and or airborne metabolites of *aspergillus*, causing at first allergic aspergillosis.

The main routes of central nervous system contamination are hematogenous dissemination from a distant primary source, mainly lung, and contiguous spread from an adjacent focus such as orbit or paranasal sinus [4]. It may manifest as single solid granuloma, multiple abscesses, necrotic lesions or meningitis. CNS aspergillosis is a dramatic disease with a mortality rate of over 90% in immunocompromised patients. Orbital aspergillosis is a rare condition that may mimic non-specific orbital inflammatory disease and hence it goes undiagnosed. Once the infection extends intracranially the morbidity is high in spite of aggressive management.

Systemic aspergillosis is a life threatening opportunistic infection that requires specific antimycotic therapy. Amphotericin B has been the first line drug for systemic therapy in spite of nephrotoxicity. In case of aspergillomata treatment includes aggressive surgical debridement and antifungal therapy with Amphotericin B, Itraconazole or Voriconazole [5]. Voriconazole is a second generation triazole antifungal drug which is very effective against *Aspergillus* species. It has been reported to be superior to Amphotericin B in treatment of *Aspergillus* infections of Paranasal sinuses.

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MANAGEMENT OF OBSTRUCTIVE SLEEP APNEA THROUGH DISTRACTION OSTEOGENESIS OF THE MANDIBLE: A CASE REPORT.

C. Ravindran*, S. Ramkumar, N.Nandakumar

ABSTRACT

The underlying pathophysiology of obstructive sleep apnea is beginning to be unraveled better in recent years. Mandibular hypoplasia is now considered to be a significant reason for this disorder. Mandibular hypoplasia can result from a variety of causes including congenital like, mandibulofacial dysostosis, and acquired conditions like TMJ ankylosis. The use of

distraction osteogenesis for the maxillofacial skeleton is a relatively new concept. We present a case of obstructive sleep apnea syndrome due to mandibular hypoplasia resulting from a TMJ ankylosis, treated with distraction osteogenesis of the mandible.

Key words: Distraction Osteogenesis, Sleep Apnea, Hypoplasia of Mandible, TMJ Ankylosis, case report

INTRODUCTION

Obstructive sleep apnea [OSA] has been identified as a significant health risk in recent times. Obstructive apnea is the absence of airflow despite respiratory effort. C.S.Burwell first used the term Pickwickian syndrome describing an obese patient with respiratory acidosis, heart failure and sleepiness [1].

Airway obstruction has been noted to occur in seven different sites in the upper airway. Retrognathism or repositioning of the jaw is beginning to be appreciated as a significant risk factor in the development of OSA. Maxillomandibular advancement to increase the airway space was the logical treatment for these conditions. Large advancements of the jaws through traditional orthognathic surgery were accompanied with a high rate of relapse. Distraction osteogenesis is a technique that offsets these problems.

Distraction osteogenesis was first developed by the Russian surgeon Ilizarov for correction of various extremity deformities. It was adapted to the maxillofacial region in the early 1990's to treat congenital and developmental hypoplasias of the maxilla and mandible [2]. It involves gradual separation of the osteotomised bone edges resulting in the formation of new bone.

CASE REPORT:

A 19-year-old male patient reported to the oral and maxillofacial surgical department with a primary complaint of an unaesthetic retruded lower jaw. His additional complaints included excessive daytime somnolence, loud and obnoxious snoring and decreased cognitive function during waking hours. History revealed that the patient was operated for bilateral temporomandibular joint ankylosis

10 years back. On examination patient had a short stature, was obese with a stout neck and had a very small chin



Fig 1

throat angle, exhibiting all the features of the bird face deformity. [Fig 1]. Lateral cephalogram revealed a severely



Fig 4

retruded mandible, decreased pharyngeal airway shadow and evidence of previous surgery [arthroplasty] in the temporomandibular joint [Fig 4]. The diagnosis of obstructive sleep apnea syndrome due to upper airway redundancy secondary to a hypoplastic mandible was confirmed through a overnight four channel polysomnogram which revealed apnea of over hundred with significant oxygen desaturation and resting tachycardia. To increase the upper airway space it was decided to advance the hypoplastic mandible by 20 mm. through osteodistraction. Under general anesthesia an osteotomy was performed in the bilateral angle region of the mandible transorally.

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Fig 2

Two indigenously sourced extra oral unifocal distractors were placed percutaneously in both the osteotomy sites [Fig 3]. After a latency period of one week, the mandible was advanced daily by incremental distraction of 1 mm a day



Fig 3

for 20 days. After 2 months of consolidation of the distracted segment the distractors were removed under local anesthesia.



Fig 5

Complete elimination of the defect was evidenced both clinically and radiographically in a lateral cephalogram [Fig 5].

A repeat polysomnogram revealed an improvement in the respiratory distress index. The surgical results were stable at the last follow up of 9 months [Fig 2].

DISCUSSION

Several modalities of treatment for OSA have evolved over the years. Non-surgical management includes weight loss, oral devices, Continuous Positive Airway Pressure [CPAP]. Medical management is plagued by poor patient compliance and can be employed only in moderate cases of OSA. A protocol developed by the university of Alabama recommends Maxillomandibular advancement and uvulopalatopharyngoplasty for respiratory distress index greater than 40³. In our case the respiratory distress was very high and hence the only treatment option was surgical. Since the etiology of respiratory obstruction was retrognathic and hypoplastic mandible, advancement was planned and achieved through distraction osteogenesis. The results were evidenced by the decrease in respiratory distress index in the polysomnogram, as well as an increase in the pharyngeal airway shadow in the lateral cephalometric radiographs [Fig 5].

Distraction osteogenesis is an emerging treatment modality used for correction of severe hypoplasia of the jaws. It carries with it the advantages of minimal relapse and added stability. In our case the mandible had to be advanced by 20 mm. Conventional osteotomies cannot achieve this magnitude of movement without bone grafting which has additional donor site morbidity. Though distraction osteogenesis of the jaws has been used to treat congenital hypoplasias in children, very few reports in the literature describe this as a definite modality in compromised airway following ankylosis of the temporomandibular joint. Further case studies in this regard will help in expanding our understanding of this complex deformity.

CONCLUSION

The use of distraction osteogenesis for the treatment of obstructive sleep apnea is a novel and groundbreaking concept. It simultaneously advances both the soft and hard tissues, in the process corrects the functional as well as the esthetic aspects of the deformity. In our case it has proved to be a successful alternative to other surgical options.

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GASTROINTESTINAL STROMAL TUMOUR

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ABSTRACT

'GIST' Gastro intestinal stromal tumours is one of the most common abdominal mesenchymal tumours. GISTs are thought to originate from the intestinal cell of Cajal, an intestinal pacemaker cell. We discuss a series of four cases of GIST with interesting

presentations for which immunohistochemistry confirmation was done. The primary therapy for GIST is surgery. Other treatment modalities are also discussed in detail.

Key words : gastrointestinal mesenchymal tumours, CD 117, c-KIT gene, imatinib mesylate, case report

INTRODUCTION:

Gastrointestinal stromal tumours (GISTs) are defined as gastrointestinal mesenchymal tumours expressing a protooncogene protein called CD117 detected by immunohistochemistry. GIST is the most common abdominal mesenchymal tumour reported in 16 -20 per million population in western literature [1] Majority of GIST occur in adults, rarely in neonates and children.[2]

CASE DETAILS :

Four different presentations of GIST in various parts of the G.I system are reported in this article. cases 1 & 2

presented as epigastric mass for which surgical resection was done. Case 3 presented as recurrence following surgical excision in rectum which was done elsewhere. Anterior resection was done for the same. Case 4 presented as a mass lesion in umbilicus with liver secondaries , biopsy proved to be GIST. Patient was subjected to imatinib mesylate chemotherapy regimen which noted a significant regression. Surgery is planned on a later date. All cases were subjected to upper gastrointestinal endoscopy, ultrasound , abdomen, CT scan and immunohistochemistry confirmation. All case details are discussed in tab col 1.1

Tabular column 1.1—Four cases of GIST are reported here

	CASE 1	CASE 2	CASE 3	CASE 4
Clinical presentation	40/F, Epigastric mass	45/F Mass in epigastrium & lt hypochondrium	70/M Recurrent GIST of rectum	30/F mass in umbilical region
Investigations <i>USG abd</i> <i>Ct scan</i>	Mass lesion in stomach with no lymph spread	Nodular mass from stomach, adherent to spleen & tail of pancreas	Mixed echogenic mass from rectum	Mul SOL in Rt lobe of liver Bx-positive
<i>UGI scopy/</i> <i>sigmoidoscopy</i>	Extraluminal mass in body of stomach	Normal	Normal	Periampullary growth
Treatment given	Total gastrectomy / esophagojejunostomy/Rou-en-y Jejunojunostomy Fig 2.3	Distal pancreatectomy / splenectomy / wedge resection of stomach Fig 2.4	Anterior Resection of rectum	Imatinib mesylate 400mg oral*6 mons
HPE	GIST	GIST	Reccurent GIST	Advanced GIST
IHC	CD 117 +	CD 117 +	CD 117 +	CD 117 +
Follow up	No recurrence	No recurrence	No recurrence & chemotherapy	Regression + after 6 mons

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DISCUSSION:

True nature of GIST is uncovered by immunohistochemistry and electron microscopy. It is now postulated that GISTs originate from the Intestinal cell of Cajal (Kaa- hal)[3]

Fig 2.1, 2.2 described by Ramon y Cajal which exhibit incomplete myogenic and neural differentiation. CD 117,

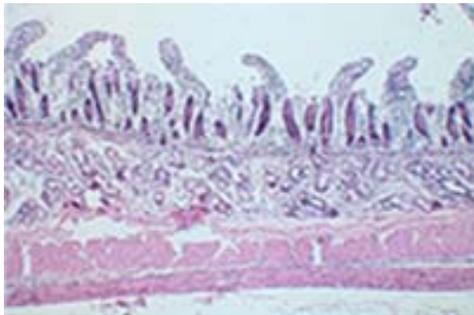


Fig. 2.1 Histopathology of GIST

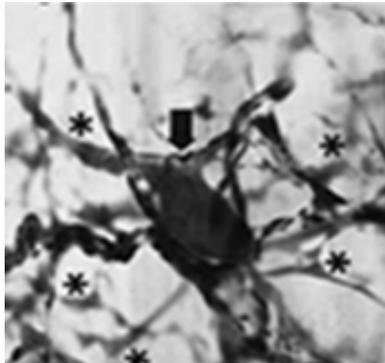


Fig. 2.2 Electron microscopic picture of interstitial cell of Cajal

the c- KIT[4] proto oncogene protein is a transmembrane receptor for the growth factor known as stem cell factor (SCF). It is encoded by the c-KIT proto oncogene located on chromosome 4q 11-21. Confirmation of diagnosis requires a biopsy with demonstration of immunohistochemistry staining with CD 117 positivity in tumour cells. Other markers[5] like CD 34, S 100, Desmin, are of limited value in distinguishing GISTs from other gastrointestinal mesenchymal tumours. CT and MRI scan will help in determining the extent & spread of disease.



Fig. 2.3 Specimen showing Stomach GIST



Fig 2.4 Operative picture of distal Pancreatectomy with Splenectomy

Surgical segmental resection with adequate marginal clearance is followed by surveillance for metastasis & recurrence. (Fig 2.3,2.4) There is no role for lymph node dissection. Preoperative tissue diagnosis is available only in ¼ of cases. Role of FNAC is controversial. Chemotherapy is doxorubicin based. There is minimal role for radiotherapy.

Imatinib mesylate is a synthetic analogue of tyrosine kinase inhibitor and is considered as the drug of choice for metastatic & inoperable GISTs and the response is found to be 40-69 %.[6]

Future of GIST:

Gene therapy would be the answer to the millions of questions unsolved! It would offer the ultimate treatment in future and render our future generations a syndrome free 23 pairs of chromosomes to live with...!

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ANTENATALLY DIAGNOSED, VEIN OF GALEN MALFORMATION: A CASE REPORT WITH REVIEW OF LITERATURE

Moni Pankhuri Singh ^a, Santosh Joseph ^b, Binu Ninan ^a, Prakash Aggrawal^c

ABSTRACT

Published reports are reviewed relating to pediatric patients diagnosed with a vein of Galen malformation (VGM). We report a case of antenatally

diagnosed VGM, including five patients from this institution; we reviewed the literature for antenatally diagnosed VGM.

Key words : Vein of Galen, Pre-natal, case report

INTRODUCTION:

Vein of Galen aneurysmal malformations (VGAM) are rare congenital vascular malformations characterised by shunting of the arterial flow into an enlarged cerebral vein dorsal to the tectum. Occurring during embryonic development, VGAM's are abnormal connections between arteries and the deep draining veins of the brain. VGAM's do not have capillaries, thus the blood flow can be extremely fast increasing the work of the heart. Incidence is <1/25000 deliveries, male: female ratio 3:1.

Most of these malformations present in early childhood, often causing intractable CCF in the neonate, which is the most common cause of death (1, 2, 3). In less severe cases, a child may develop hydrocephalus because the enlarged malformation blocks the normal flow or absorption of cerebrospinal fluid. Seizures and other neurological signs are unusual. Tranarterial embolisation with liquid adhesive agents or microcoils is the treatment of choice. Surgery has very little role.

CASE REPORT:

CASE 1 : Antenatal ultrasound of a 26 year old lady at 36 wks of gestation detected VGAM. Baby was delivered by elective LSCS at 39 weeks. Baby did not require any resuscitative measures at birth, but within half an hour baby went into congestive cardiac failure. Hence baby was electively intubated, connected to ventilator and treatment of cardiac failure was started. Echocardiography done showed severe right heart and PA dilatation. Cranial angiogram was performed which showed VGAM – choroidal type with multiple choroidal feeders. In view of innumerable shunt and tortuosity of feeders embolisation through arterial route was considered unsuitable and occluding the VGAM through venous route was considered. Treatment modality and prognosis was explained to the

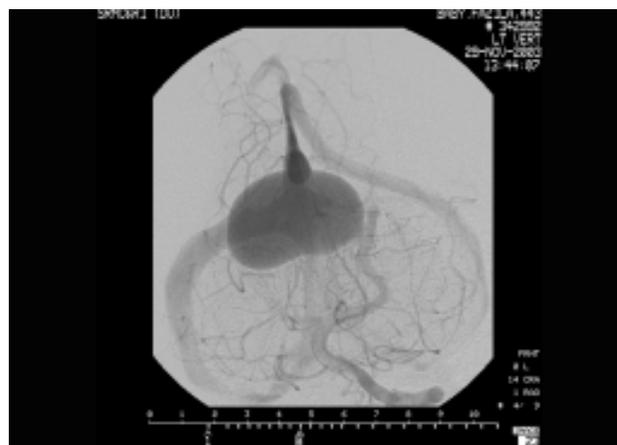


Fig.1. Cranial angiogram showing vein of Galen malformation parents who declined further management. Baby died of intractable CCF within 24 hours of life.

CASE 2: Antenatally diagnosed VGAM was referred to our institution for further management. After birth baby was clinically stable hence, no intervention was required. Later at the age of 6 months, child came with rapidly increasing head circumference. Child was conscious with no focal neurological deficit, hence was taken up for AV fistula embolisation. Four vessel angiogram revealed VGAM with arterial feeders from numerous posterior choroidal arteries. Arterial feeders were embolised but child died on 2nd post-op day due to intracranial bleed.

CASE 3: Another child with antenatally diagnosed VGAM; no intervention was required at birth as child was clinically stable. Later embolisation was done electively at 11 months. Angiogram done showed mural type of VGAM with simple fistula between left posterior choroidal artery and aneurysmal sac. The child underwent successful embolisation. At follow-up, the child has no developmental delay or any neurological deficit.

DISCUSSION:

There are two basic types of vein of Galen malformation. In the first, single or multiple arteries drain directly into enlarged venous structures of the Galenic system. The most common anomaly is the singular multiple direct arterio-venous fistula between the choroidal and the quadrigeminal arteries and a median venous sac. In

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the second type, a parenchymal AVM is present usually in the thalamus or midbrain and its nidus has deep Galenic drainage. Diagnosis is made by transcranial ultrasound, cerebral angiography, MR and MR angiography (4). Before the era of sophisticated imaging technologies and endovascular treatment, VGAM was fatal in 90% of patients under 1 month of age and half of those between 1 month and 1 year (2). With transcatheter embolisation there is 70-80% survival among neonates and young infants and cure rates of approximately 50% (5, 6).

Neonatal Rating Score (7)

Lasjaunias and ter Brugge score is derived from measures of cardiac, cerebral, hepatic, renal, and respiratory function.

- < 8 results in a decision not to treat.
- 8-12 prompts emergency endovascular intervention.
- 12 recommends medical treatment alone and delayed embolization at 5 months.

NEONATAL RATING SCORE

Score	Cardiac	Cerebral	Respiratory	Renal	Hepatic
5	Normal	Normal	Normal	_____	_____
4	Non-treated overload	Infraclinical EEG anomalies	Tachypnoea Bottle finished	_____	_____
3	CCF stable with treatment	Non-convulsive CNS signs	Tachypnoea Bottle not finished	Normal	Normal
2	CCF unstable with treatment	Isolated convulsion	Assisted ventilation	Transient Anuria	Hepatomegaly normal function
1	Need ventilation	Seizure permanen CNS signs	Assisted ventilation FiO ₂ > 25%	Unstable diuresis with treatment	Moderate hepatic impairment
0	Resistant to Rx.	Coma	Assisted ventilation	Anuria	Coagulation disorder

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FOREIGN BODY IN THE COMMON BILE DUCT - DEFYING NATURE - A CASE REPORT

K.Balaji Singh*, B.Premkumar, M.Sasikumar, Sukhbir Singh, Jameel Akhter, Gowrishankar

ABSTRACT

A 59-year-old male presented with the complaint of pain in the right upper abdomen of ten days duration. It was associated with passage of high colored urine and pale colored stools. There was minor elevation of liver enzymes but his bilirubin levels and alkaline phosphatase were grossly elevated (T.Bilirubin- 26.1mgs%, D.Bilirubin- 23.1mgs%, Alkaline phosphatase- 562). Viral markers were negative. The patient was clinically

diagnosed to have obstructive jaundice secondary to stricture, stone or sludge. During surgery, the gall bladder was distended and there were severe inflammatory adhesions in the lower CBD. The CBD and cystic duct were dilated. The CBD exploration was done. A coriander twig with surrounding sludge was found and was removed in toto.

Key words : Foreign bodies, commonbile duct, case reports

CASE HISTORY:

A 59-year-old male presented with a complaint of pain in the right upper abdomen of ten days duration. It was associated with passage of high colored urine and pale colored stools. He developed generalized itching three days later and also complained of non-bilious vomiting for four days. Appetite was lost with a weight loss of 7 kilograms.

EXAMINATION FINDINGS:

Patient was moderately built and nourished. Pallor and icterus were present. Systemic examination was otherwise unremarkable. His abdomen was soft and the gall bladder was palpable. Rectal examination was normal.

GENERAL INVESTIGATIONS:

The routine blood values were within normal limits. There was minor elevation of liver enzymes but his bilirubin levels and alkaline phosphatase were grossly elevated (T.Bilirubin- 26.1mgs%, D.Bilirubin- 23.1mgs%, Alkaline phosphatase- 562). Viral markers were negative.

SPECIFIC INVESTIGATIONS:

Upper gastro-intestinal endoscopy done was normal. Ultrasound of the abdomen showed an isoechoic lesion with few specks in the distal CBD, there was dilatation of the intra hepatic biliary radicles, Magnetic resonance cholangio pancreatogram (MRCP) showed a filling defect in the lower CBD suggestive of a stricture, stone or sludge. (Fig. 1)

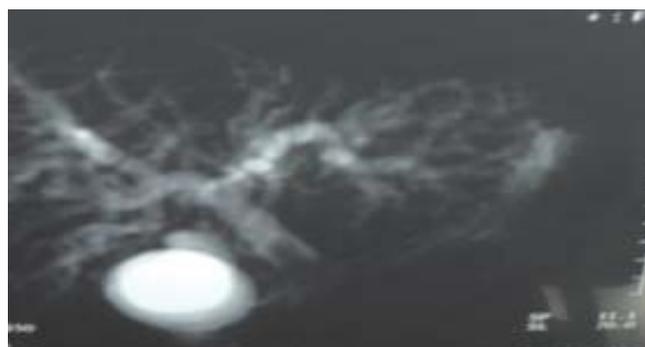


Fig 1 : MRCP showing filling defect in lower CBD:

SURGERY:

Under general anaesthesia the peritoneal cavity was entered. The gall bladder was distended and there were severe inflammatory adhesions in the lower CBD. The CBD and cystic duct were dilated. CBD exploration was done. A coriander twig with surrounding sludge was found and was removed in toto (Fig 2). On table T-tube cholangiogram showed free flow of dye into the duodenum.



Fig. 2 : THE FOREIGN BODY: Coriander twig with surrounding biliary sludge.

POST OPERATIVE PERIOD:

The patient had a smooth postoperative phase. On the ninth postoperative day a T- Tube cholangiogram was done which showed no residual foreign body and free flow of dye into the duodenum. The T tube was removed on 10th postoperative day.

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Follow up liver function test showed a T.Bilirubin of 7.2mgs%, D.Bilirubin of 5.9mgs% and alkaline phosphatase of 293.

DISCUSSION:

Very few cases of foreign body in CBD have been mentioned in literature. Most reported cases however have a history of intervention in the biliary tract previously either by surgery or endoscopic intervention[1].The presence of a foreign body in an unexplored or virgin CBD has not yet been reported. The occurrence of foreign bodies with the ligaclips as a nidus for stone formation has been reported following Laparoscopic cholecystectomy [2]. The occurrence of animal food matter i.e. chicken [3]and fish bones[4] have also been mentioned. Vegetable matter as in our case has been found in the form of cherry stalks [5].

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LISTERIA MONOCYTOGENES - A CASE REPORT

M. Kalyani*, P.K. Rajesh, Padma Srikanth, M. Mallika

ABSTRACT

Human Listeriosis is a zoonotic disease and usually presents as bacteremia or meningitis. Infants and immunosuppressed people are the most affected. We report a case of meningitis caused by *Listeria monocytogenes* in a 17-year-old immunocompetent

girl. The isolation of the organism from the cerebrospinal fluid (CSF) and subsequent treatment with ampicillin to which it was susceptible resulted in dramatic recovery. The case is reported for its rarity in an immunocompetent adolescent.

Key words: CSF, *Listeria*, meningitis, case report

INTRODUCTION

Listeria monocytogenes is a Gram positive, non-sporing, aerobic bacillus that is motile at room temperature and non motile at 37°C. Murray and associates first described it morphologically in 1926⁽¹⁾. Only *L.monocytogenes* and *L. ivanovii* are associated with diseases in humans. The organism may resemble *Corynebacteria*, *Streptococci* or *Pneumococci* on direct smears and hence the problem is identification and also the chance of regarding as a contaminant. Clinically *L.monocytogenes* causes meningitis and sepsis in immunocompromised individual. 25% of the cases of invasive listeriosis occur in pregnant women.

The annual incidence of listeriosis in Europe ranges from 0.1 to 11.3 cases per million. In the United States, the incidence reported in 1992 was 7.4 cases per million.⁽²⁾ In India there were no case reports on *Listeria* till 1973⁽³⁾. In 1981, a prospective study of 1300 births documented 2.2% as the prevalence rate of *Listeria* in meconium stained babies and 0.2% of live births.

Very few reports are available from India⁽¹⁾ especially in immunocompetent individuals and hence the present case is reported.

CASE REPORT

The CSF of a 17-year-old, unmarried girl who presented with signs and symptoms of meningitis was sent for biochemical, pathological and microbiological analysis. There was no history of steroid intake. Macroscopically CSF was turbid and the WBC count was 1250 cells/cubic millimeter. CSF protein was raised (182mg/dl) and sugar was low (15mg/dl). CT scan of brain was normal.

Gram staining of the CSF showed mononuclear cells with moderate Gram-positive, short, nonsporing bacilli with diptheroid like arrangement (Fig. 1). The CSF was inoculated onto Blood Agar (BA), Chocolate Agar (CA), and Mac conkey Agar (MAC). After 24 hours of incubation, the BA plate showed moderate growth of small, round, smooth, translucent colonies with minimal beta hemolysis. CA also showed moderate growth of tiny colonies. No growth was seen on MAC. Two blood cultures were done and were reported negative after 14 days of incubation. CSF culture for tuberculosis and smear for acid-fast bacilli were negative.

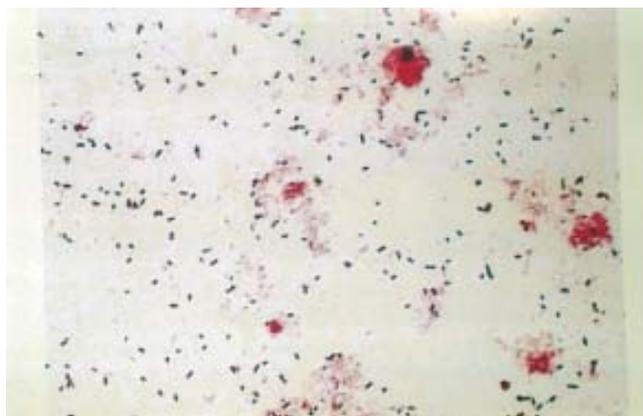


Fig. 1 Gram Staining of CSF

Gram staining of the colonies also showed Gram-positive, short, nonsporing bacilli with diptheroid like arrangement (Fig. 2). Tumbling motility was demonstrated in nutrient broth incubated at 25° C and the organism was non motile in nutrient broth incubated at 37° C. It was catalase, esculin and hippurate hydrolysis positive. Umbrella shaped motility pattern was observed in semisolid nutrient butt. CAMP test (Christie, Atkins and Munch-Peterson) using plazen strain of *Staphylococcus aureus* was positive. The isolate was identified as *Listeria monocytogenes* based on Gram stain, tumbling motility at 25°C, catalase positive and ability to grow at 4°C. The isolate was sensitive to penicillin, ampicillin, gentamicin, cefotaxime, and ciprofloxacin and resistant to cotrimoxazole.

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Fig. 2 - Gram Staining - Culture Smear

The patient had been empirically treated with cefotaxime and antituberculous drugs (rifampicin, pyrazinamide, etambutol) but was not responding. However after the culture and sensitivity report, she was switched over to intravenous ampicillin which was continued for ten days. There was dramatic improvement clinically within 24 hours and after a week all parameters returned to normal. There was no neurological deficit at the time of discharge and the patient was advised to continue oral ampicillin for two more weeks.

DISCUSSION

Listeria monocytogenes usually presents as bacteremia or meningitis. It is mostly common in neonates but children and adults may also be affected. The predisposing factors are pregnancy, diabetes mellitus, malignancy, collagen disorders and immuno-suppressed patients. The incubation period of listeriosis averages about 3-4 weeks with a range of 3-90 days⁽²⁾. In cases of early onset neonatal listeriosis, the infant is infected in utero presumably by transplacental infection from mother who is bacteremic.

Though listeriosis is common in neonates, children more than one year and adults may also be affected and about one third of the patients with meningitis have no predisposing conditions and occur even in healthy children and adults⁽³⁾. In this case it was isolated from a 17-year-old immunocompetent girl.

Regarding the treatment of listeria meningitis combination therapy of ampicillin (dose 25- 100mg/kg body weight) and gentamicin (dose 3-5 mg/kg body weight) is

recommended for 21 days. It is always resistant to cephalosporins, which are used in the empirical treatment of bacterial meningitis of unknown etiology. In this case though there was in vitro susceptibility to cefotaxime, the patient responded well only after switching over to ampicillin.

Due to its morphological resemblance with diphtheroid species, *L.monocytogenes* could be regarded as a contaminant in the laboratory. It is difficult to isolate this organism from certain clinical specimens particularly from tissues removed at surgery or at autopsy.

The important points for identification of *L.monocytogenes* in laboratory diagnosis are listed in Table I⁽²⁾

Table - I – Key Points for Identification of *L.monocytogenes*

- Narrow zone of beta hemolysis around the colonies on blood agar.
- Growth at 4° C
- Tumbling motility at 25° C and non motile at 37° C
- Catalase positive
- Hydrolysis of esculin
- Fermentation of glucose, trehalose and salicin
- CAMP test positive
- Negative reaction for hydrogen sulphide

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A RARE TYPE OF RIGHT SIDE BOCHDALEKS DIAPHRAGMATIC HERNIA PRESENTING IN AN ADULT : CASE REPORT

M Subramanian*, Prasad A Sasnur

ABSTRACT

Literature describes less than a dozen cases of right-sided diaphragmatic hernia. Here we present an unusual case of this kind in an adult patient. The hernial contents were the Liver, stomach, large bowel. Diaphragmatic hernias, in which peritoneal contents are

found in the thoracic cavity, are well documented in medical literature. Hernias of this type may be congenital in origin, such as Morgagni's or Bochdalek's hernia, or traumatic in origin.

Key words : Diaphragmatic hernia, Right diaphragmatic hernia, adult diaphragmatic hernia, case report

INTRODUCTION

This case is reported for its rarity because it is seen in an elderly patient on the right side of the diaphragm with liver being one of the contents of hernia.

CASE REPORT

A 55-year-old male patient presented with dyspnoea, vomiting and abdominal pain since one month. CT Scan revealed herniation of right diaphragm, raised liver in to the right lower thorax along with hepatic flexure [Fig1]. Dilated stomach and duodenum with herniation of the hepatic flexure were seen on Barium meal study [Fig2]. Lung functions were normal.

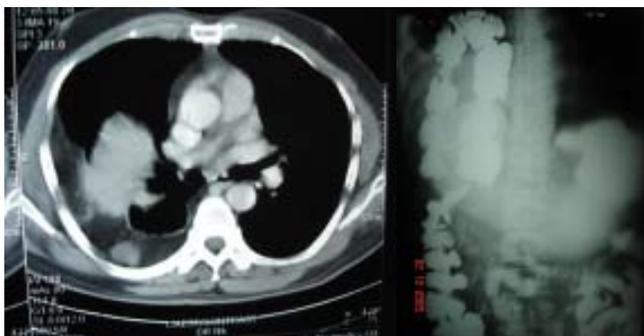


Fig. 1 & Fig 2.

Operative findings— Diaphragm was thinned out and distal stomach, duodenum, omentum, right lobe of the liver, hepatic flexure herniated into the right side of chest through large space of Bochdalek was observed on thoracoabdominal incision[Fig3]. Diaphragm was repaired through abdomen using prolene interrupted sutures and Marlex mesh reinforcement. Postoperative period was uneventful and he is doing well after nine months of surgery.

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Fig. 3

DISCUSSION

The true incidence of Congenital diaphragmatic hernia is 1 in 7000 live births[1] while right side diaphragmatic hernia (15%) is rare comparing to left side diaphragmatic hernia (85%) [2] because liver plugs the opening. Congenital diaphragmatic hernia usually develop in the small areas of weakness in the diaphragm (space of Bochdalek and Morgagni) observed during fetal development where diaphragm has to sustain pressure developed by growing lungs above and intestines returning in to the abdomen below. These canals get normally closed by the pleuroperitoneal membranes at 8th week of gestation. Failure to close due to various reasons leads to Bochdalek hernia [3]. Neonates are born with respiratory distress, tachypnea, cyanosis. Infants with well developed lungs with no symptoms immediately (24hrs) after birth will do well after surgery[4]. In adults, chest or abdominal pain (75%), dyspnea (75%), and vomiting (25%) are common symptoms, whereas bowel obstruction and strangulation are not[5]. Diaphragmatic hernia rarely presents during adult life and patient do well after surgery [6].

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