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Volume 3 Number 1 July - December 2011 ISSN 0976 - 402X

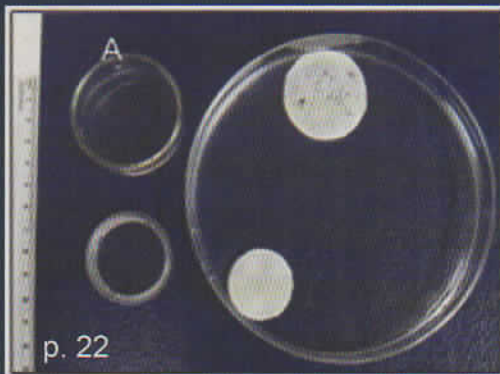
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An International Journal

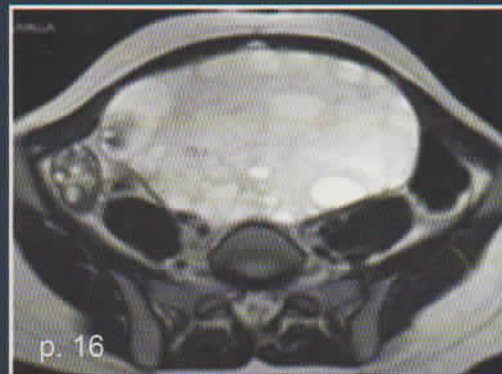


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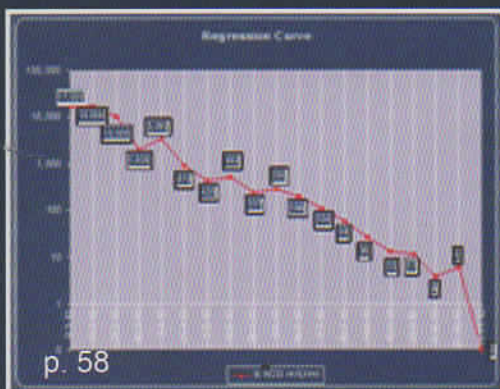
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'Pushpagiri Medical Journal', an International Journal, is the official publication of Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla. It is a peer reviewed multi-disciplinary journal providing health professionals with a forum to discuss current challenges in healthcare, sharing innovative evaluation and treatment techniques, learning about and assimilating advanced methodologies being developed in various disciplines in modern medicine as well as related professions, and communicating information regarding newer developments and research programmes. The journal serves as a valuable tool for helping therapists deal effectively with the emerging problems, stumbling blocks and challenges in the field, and emphasizing articles and reports that are directly relevant to medical practice and public health. It will publish original research articles, concise evidence based review articles, unusual and interesting case reports and technical reports. We offer an online submission facility, and a fast reviewing process and editorial decisions so as to avoid delay and inconvenience to the authors. The journal is being included and indexed with many international databases, and it will be published half-yearly, in March (January-June) and September (July-December).

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PMJ

Pushpagiri Medical Journal

An International Journal

Volume 03, Number 01

July - December 2011

Official Publication of
Pushpagiri Institute of Medical Sciences and Research Centre



Editorial Office:

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PIMS & RC, Tiruvalla
Pathanamthitta

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Printed, Published and Owned by:

Rev Fr Dr Abraham Mulamoottil

in his official capacity as Chairman and

CE of Pushpagiri Group of Institutions

Phone: 0469-2603833, 2700755 (Ext. 401)

E-mail: ceopushpagiri@gmail.com

Printed at:

Furore Digital Printing, Pathanamthitta

Published at:

Tiruvalla - 689 101

Pathanamthitta, Kerala, India by the
Pushpagiri Institute of Medical Sciences
& Research Centre

ISSN 0976-402X

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PUSHPAGIRI MEDICAL JOURNAL

Volume 3, No. 1

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Impact of Electronic publishing in Biomedical Research

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Scientific publications are meant for dissemination of information, scientific validation and also for specific objectives set by the author. Published papers generally archive scientific knowledge, documenting all successful and unsuccessful results of research¹. Validation is done by peer review and editing whereby scientific knowledge is presented in a sound form, ensuring that the data is complete and clearly presented. British Medical Journal was the first journal to be published electronically². Most biomedical journals at present produce an electronic version along with their print version. In view of the fact that Pushpagiri Medical Journal is also being published now in digital format, it will be worthwhile knowing some relevant facts about electronic publishing.

What is electronic publishing? It is the process of creating and disseminating information by electronic means including the web, and the search engines help to identify and make available to all those who seek, the vast data collected from sources worldwide. The materials to be published may originate as traditional paper publishing or may be specifically created for electronic publishing. This method of accessing information is gradually becoming more and more popular since the number of subscriptions to scientific journals has been substantially reduced by many libraries. Publishers respond to this cancellation of subscription by raising the rates, forcing the libraries to pay more for practically less information. Institutions and individuals with good academic interests are commonly being exploited in this way.

Electronic method of publishing journals has many advantages for the authors, scientists and academics:

- ❖ Lower cost: compared with the print cost 70% saving can be made
- ❖ Speed: worldwide interconnection of the latest status in the field of interest is possible at amazing speed
- ❖ Inclusion of graphics like video, animation etc. are possible
- ❖ Search is direct and easy - for author, title, year of publication or full text search
- ❖ Publications can be catalogued in international databases to facilitate search
- ❖ Information can be archived and made available to future generations
- ❖ Papers can be easily linked to those that are cited

Some disadvantages have also been pointed out with regard to electronic journals:

- Difficulty in reading computer screen
- Some journals are often not included in indexing and abstracting services
- Problems in archiving (whose responsibility is it?)
- Perishable citations - web sites may change or disappear

It is not very clear at present whether electronic publishing will improve or worsen the quality of scientific publications. Most professionals who use this method in research, patient care and teaching are attracted to the potential benefits of digital technology. International Committee of Medical Journal Editors (ICMJE) has given uniform requirements for manuscripts submitted to biomedical journals for electronic publishing. (Ref: ICMJE.ORG © 2009).

New digital technologies are radically changing the relationship between producers, middlemen and consumers of information on all aspects of life. Biomedicine is no different. PMJ is hopeful of seeing a new era of scientific collaboration, and access to and interaction with health information sources, which will be of overall benefit to the medical profession and the society.

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✦ MEDICAL ETHICS

Why IVF should be considered illicit?

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The Sunday times, January 31, 2009, reported local news titled, **“Octuplets' mother had multiple IVF although she has six children”**. A thirty-three-year-old single mother Nadya Suleman-Guiterrez gave birth to the eight babies and it took a team of 46 doctors and nurses at the Kaiser Permanente Medical Center in the Los Angeles suburb of Bellflower to deliver the octuplets. Aside from the octuplets, the elder children were aged seven, six, five, three and two. The youngest were twins, suggesting that the mother had undergone IVF treatment before. Madam Suleman is said to be a divorcee studying for a master's degree in Psychology.



Fig.1: Octuplets with mother, grandmother

The other side of the same coin is that in vitro fertilization (IVF) treatment guidelines produced by the American Society for Reproductive Medicine say that a woman under 35 should have no more than two embryos implanted at a time.

Who is more at fault? The woman who chose to IVF and embryo transfer (in spite of six elder children), but refused termination of some of them when she came to know the number of foetuses, or the *“expert medical professionals”* who transferred not less than eight embryos to such an *“infertile patient”*?

Procedure of IVF and Embryo transfer (ET)

Initially fertility drugs are

administered to the woman to stimulate her ovarian follicles to produce as many mature ova as possible in an ovarian cycle. Maximum numbers of ova are retrieved by ultrasonographically guided aspiration or laparoscopy, and are inseminated by a sample of semen containing sperms of good quality. Embryos are graded by the embryologist and transferred to the uterus, depending on the number available, the age of the woman and other health and diagnostic factors.

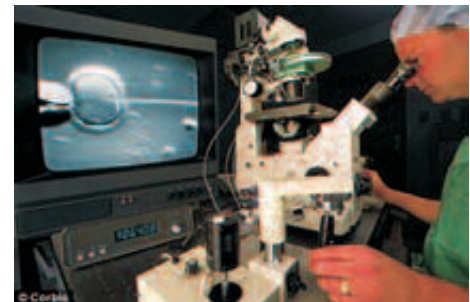


Fig.2: Sperms injected into ovum before ET

In countries such as Canada, the UK, Australia and New Zealand, a maximum of two embryos are transferred except in unusual circumstances. In the USA, younger women may have many embryos transferred based on individual fertility diagnosis. The embryos judged to be the *“best”* are transferred to the patient's uterus; several embryos may be passed to improve chances of implantation. Most clinics and country regulatory bodies seek to minimize the risk of pregnancies carrying multiples. As it is not uncommon for more implantations to take than desired, the next choice for the expectant mother is selective abortion.

Success rates

IVF success rates are the percentage of all IVF procedures which result in a favorable outcome. This outcome may represent the pregnancy rate or the live birth rate. Due to the advancements in reproductive

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technology, the IVF success rates are substantially better today than they were just a few years ago.

Table. 1 Success rates of IVF in various age groups

Success rate	Age < 35	35 - 37	38 - 40	41 - 42
Pregnancy rate	47.6	38.9	30.1	20.5
Live birth rate	41.4	31.7	22.3	12.6

The most current data available in the United States, 2009, compiled by the Society for Reproductive Medicine (SART) reports the average national IVF success rates in various age groups (Table. 1).

Are all technological advances ethical?

God created man in his own image and likeness: "male and female he created them", entrusting to them the task of "having dominion over the earth" (*Gen 1:27, 28*). Scientific research constitutes a significant expression of this dominion of man over creation. Science and technology are valuable resources for man when placed at his service and when they promote his integral development for the benefit of all.

God, who is love and life, has inscribed in man and woman the vocation to share in a special way in his mystery of personal communion and in his work as Creator and Father. For this reason marriage possesses specific goods and values in its union and in procreation. Such values and meanings are of the personal order and determine the meaning and limits of artificial interventions on procreation and on the origin of human life. These interventions are not to be rejected just on the grounds that they are artificial. As such, they bear witness to the possibilities of the art of medicine. But they must be given a moral evaluation in reference to the dignity of the human person. What is technically possible does not for that very reason, become morally admissible.

The current rapid development of technological discoveries gives greater urgency to respect the fact that science without conscience can only lead to man's ruin. **No biologist or doctor can ethically claim, by virtue of his scientific competence, to be able to decide on an individual's origin and destiny.** This norm must be applied in a particular way in the field of sexuality and procreation, in which man and woman actualize the fundamental values of love and life. The two basic issues which need to be addressed in IVF are: *the value of life of embryos and the ethical aspects of procreation.*

❖ **Respect for the life of human embryos**

Human life is sacred because from its beginning it involves "the creative action of God" and it remains forever in a special relationship with the Creator, who is its sole end. God alone is the Lord of life from its

beginning until its end: no one can, in any circumstance, claim for himself the right to destroy directly an innocent little human being. Careful reflection on this teaching enables us to respond to the numerous moral problems posed by technical interventions upon the human being in the first phases of his life and upon the processes of his conception.

The implementation of procedures of artificial fertilization has made possible various interventions upon embryos and fetuses. At the Second Vatican Council, the Church presented to modern man her constant and certain doctrine according to which: *"life once conceived, must be protected with the utmost care; abortion and infanticide are abominable crimes"*.

Modern genetic science brings valuable confirmation to this view. It has demonstrated that, from the first instant, the programme is fixed as to what this living being will be: a man, this individual-man, with his characteristic aspects, already well determined.

The human being is to be respected and treated as a person from the moment of conception; and therefore from that same moment his rights as a person must be recognized, among which the first is the inviolable right of every human being to life. The embryo must also be defended in its integrity, tended and cared for, to the maximum extent possible, in the same way as any other human being.

Is prenatal diagnosis morally licit?

Prenatal diagnosis makes it possible to know the condition of the embryo and of the foetus when still in the mother's womb. It help to anticipate earlier and carry out, certain therapeutic, medical or surgical procedures. Such diagnosis is permissible, *if the methods employed safeguard the life and integrity of the embryo and the mother, without subjecting them to disproportionate risks.*

But this diagnosis is gravely opposed to the moral law *when it is done with the thought of possibly inducing an abortion depending upon the results.*

Thus a woman would be committing a gravely illicit act if she were to request such a diagnosis with the deliberate intention of having an abortion should the results confirm the existence of a malformation or abnormality. So too the specialist would be guilty of illicit collaboration if he were deliberately to contribute to establishing or favouring a link between prenatal diagnosis and abortion. This is to be condemned as a violation of the unborn child's right to life and as an abuse of the rights and duties of the spouses.

Are therapeutic procedures on embryos licit?

As with all medical interventions on patients, one must uphold as licit, procedures carried out on the human embryo respecting its life and integrity, and do not involve disproportionate risks for it but are directed towards improvement of its condition of health, or survival. Whatever the type of medical, surgical or other

therapy, the free and informed consent of the parents is required, according to the deontological rules followed in the case of children. The application of this moral principle calls for delicate and particular precautions in the case of embryonic or foetal life.

Is research and experimentation on human embryos and fetuses licit ?

Medical research must refrain from operations on live embryos, unless there is a moral certainty of not causing harm to the life or integrity of the unborn child and the mother. It follows that all research, even when limited to the simple observation of the embryo, would become illicit, if it involves risk to the embryo's physical integrity or life, by reason of the methods used or the effects induced. *If the embryos are living, whether viable or not, they must be respected just like any other human person; experimentation on embryos which is not directly therapeutic is illicit.*

No objective, even though noble in itself, such as a foreseeable advantage to science, to other human beings or to society, can in any way justify experimentation on living human embryos or fetuses, whether viable or not, either inside or outside the mother's womb. To use human embryos or fetuses as the object or instrument of experimentation constitutes a crime against their dignity as human beings, having a right to the same respect that is due to the child already born and to every adult human person.

The *Charter of the Rights of the Family* published by Pope John Paul II affirms: "Respect for the dignity of the human being excludes all experimental manipulation or exploitation of the human embryo". The practice of keeping live human embryos *in vivo* or *in vitro* for experimental or commercial purposes is totally opposed to human dignity. *The corpses of human embryos and fetuses, whether they have been deliberately aborted or not, must be respected just as the remains of other human beings. Also, It is immoral to produce human embryos destined to be exploited as disposable "biological material".*

In the usual practice of *in vitro* fertilization, not all of the embryos are transferred to the woman's body and some are destroyed. Just as the Church condemns induced abortion, she also forbids acts against the life of these little human beings. *It is a duty to condemn the particular gravity of the voluntary destruction of human embryos obtained 'in vitro' for the sole purpose of research, either by means of artificial insemination or by means of "twin fission".* By acting in this way the researcher usurps the place of God; and sets himself up as the master of the destiny of others, inasmuch as he arbitrarily chooses whom he will allow to live and whom he will send to death.

The freezing of embryos, even when carried out to preserve the life of an embryo - cryopreservation - constitutes an offence against the respect due to human beings by exposing them to extremely grave

risks of death or harm to their physical integrity and depriving them of maternal shelter and gestation, thus placing them in a situation in which further offences and manipulation are possible.

❖ **Interventions upon human procreation**

By "artificial procreation "or" artificial fertilization" are understood the many different technical procedures directed towards obtaining a human conception in a manner other than the sexual union of man and woman. It may be heterologous or homologous.

The term *heterologous artificial fertilization or procreation* implies techniques used to obtain a human conception artificially by the use of gametes coming from at least one donor other than the spouses who are joined in marriage. Such techniques can be of two types: *Heterologous IVF and ET which occurs* through the meeting *in vitro* of gametes taken from at least one donor other than the two spouses joined in marriage, and *Heterologous artificial insemination which occurs* through the transfer into the genital tracts of the woman of the sperm previously collected from a donor other than the husband. *Homologous artificial fertilization* implies fertilization taking place artificially *in vitro*, using gametes from legally bound spouses.

a. Heterologous artificial fertilization

Why must human procreation take place in marriage?

Every human being is always to be accepted as a gift and blessing of God. The procreation of a new person, whereby the man and the woman collaborate with the power of the Creator, must be the fruit and the sign of the mutual self-giving of the spouses, of their love and of their fidelity. The child has the right to be conceived, carried in the womb, brought into the world and brought up within marriage: it is through the secure and recognized relationship to his own parents that the child can achieve his own proper human development.

Through IVF and ET, and *heterologous artificial insemination*, human conception is achieved through the fusion of gametes of at least one donor other than the spouses who are united in marriage. *It is contrary to the unity of marriage, to the dignity of the spouses, to the vocation proper to parents, and to the child's right to be conceived and brought into the world in marriage and from marriage.* Respect for the unity of marriage and for conjugal fidelity demands that the child be conceived in marriage, with the exclusive right to become father and mother solely through each other.

Recourse to the gametes of a third person, constitutes a violation of the reciprocal commitment of the spouses and a grave lack in regard to that essential property of marriage which is its unity. Heterologous artificial fertilization violates the rights of the child; it deprives him of his filial relationship with his parental origins and can hinder the maturing of his personal identity. *Furthermore, the artificial fertilization of a woman who is unmarried or a widow, whoever the donor may be, cannot be morally justified.*

"Surrogate" motherhood

The term surrogate mother denotes a woman who carries an embryo implanted in her uterus and who is genetically a stranger to the embryo because it has been obtained through the union of the gametes of "donors". It could also be a woman who carries an embryo to whose procreation she has contributed the donation of her own ovum, fertilized through insemination with the sperm of a man other than her husband. In either case she carries the pregnancy with a pledge to surrender the child once it is born, to the party who commissioned or made the agreement for the pregnancy.

Surrogate motherhood represents an objective failure to meet the obligations of maternal love, of conjugal fidelity and of responsible motherhood; it offends the dignity and the right of the child to be conceived, carried in the womb, brought into the world and brought up by his own parents.

b. Homologous artificial insemination

Since heterologous artificial fertilization has been declared unacceptable, the question arises of how to evaluate morally the process of homologous artificial fertilization, IVF and ET and artificial insemination between husband and wife. For this, first the question of principles of procreation needs to be addressed.

Connection between procreation and conjugal act

The conjugal act, while most closely uniting husband and wife, capacitates them for the generation of new lives. "By safeguarding both the unitive and the procreative aspects, the conjugal act preserves in its fullness the sense of true mutual love and its ordination towards man's exalted vocation to parenthood". The origin of the human being follows from a procreation that is "linked to the union, not only biological but also spiritual, of the parents, made one by the bond of marriage". It should never be permitted to separate these different aspects to such a degree, as to exclude either the procreative intention or the conjugal relation. The one conceived must be the fruit of his parents' love. He cannot be desired or conceived as the product of an intervention of medical or biological techniques; that would be equivalent to reducing him to an object of scientific technology.

If the technical means facilitates the conjugal act or helps it to reach its natural objectives, it can be morally acceptable. If on the other hand, the procedure were to replace the conjugal act, it is morally illicit.

Conclusion

The suffering of spouses who cannot have children or who are afraid of bringing a handicapped child into the world is a suffering that everyone must understand and properly evaluate. On the part of the spouses, the desire for a child is natural: it expresses the vocation to fatherhood and motherhood inscribed in conjugal love.

Nevertheless, marriage does not confer upon the spouses the right to have a child, but only the right to perform those natural acts which are *per se* ordered to procreation. *The child is not an object to which one has a right, nor can he be considered as an object of ownership: rather, a child is a gift, "the supreme gift" and the most gratuitous gift of marriage, and is a living testimony of the mutual giving of his parents.* The normal protocol of IVF involving intentional killing or immoral risking of the lives of a great many embryos in order to achieve a live birth is contemptible.

Whatever its cause or prognosis, sterility is certainly a difficult trial. Many researchers are engaged in the fight against sterility. Scientists are to be encouraged to continue their research with the aim of preventing the causes of sterility and of being able to remedy them so that sterile couples will be able to procreate in full respect for their own personal dignity and that of the child to be born. *The doctor is at the service of persons and of human procreation. He does not have the authority to dispose them off, or to decide their fate.*

Although the manner in which human conception is achieved with IVF and ET cannot be approved, every child which comes into the world must in any case be accepted as a living gift of the Divine Goodness, and must be brought up with love.

Reference

Instruction on respect for human life in its origin and on the dignity of procreation reflects to certain questions of the day: Congregation for the Doctrine of the Faith, given at Rome, February 22, 1987.



✦ ORIGINAL ARTICLE IN SERIES

Magnetic Resonance Imaging of Female Pelvis: A systematic evaluation

Part 2: Imaging of ovarian and adnexal lesions

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Abstract

Background: Since magnetic resonance imaging (MRI) offers high contrast resolution, provides good tissue characterization, and is capable of multiplanar imaging capabilities, it is becoming a useful tool for evaluation of female pelvic pathology. Since MRI is more expensive and potentially less readily available than ultrasound, it is important to know when patients should undergo MRI. **Objectives:** To evaluate the advantages of MRI in evaluation of female pelvic pathologies. **Materials and methods:** The usefulness of MRI in the female pelvis has been studied in detail. The MRI study was done on 1.5 Tesla MRI scanner. The first part of this series article included the significance of MR Imaging in evaluating uterine pathologies. This second part would signify evaluation of ovarian and adnexal masses. The third part would consider the various congenital anomalies of female genital tract (Mullerian duct anomalies). **Subjects:** Selected patients referred to the Department of Imaging, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala. **Results:** The situations in which MRI should be considered strongly to evaluate the ovarian and adnexal lesions have been delineated in depth in this part of the series article. **Conclusion:** Certain radiologic findings predominate in each type of ovarian tumour. Knowledge of these key imaging features of ovarian tumours may allow a specific diagnosis or substantial narrowing of the differential diagnosis. Characterization of ovarian tumours can aid in surgical planning, whether exploration or laparoscopic excision, and may help distinguish benign from malignant tumours and thus avoid inappropriate management. Exquisite display of pelvic anatomy and tissue characterisation on different sequences of MR imaging may help to differentiate ovarian and adnexal non-neoplastic pathologies as well.

Key words: Functional cysts, Haemorrhagic cysts, Endometrioma, Epithelial serous and mucinous tumours, Germ cell tumours, Metastatic tumours, Collision tumours, Paraovarian cyst, Pelvic abscess, Retrorectal developmental cyst, Giant cell tumour of sacrum.

Introduction

Ovarian cancer is the second commonest gynaecologic malignancy; however, it remains the leading cause of death from such diseases. Ovarian and non-ovarian adnexal masses present a special diagnostic challenge, in part because benign adnexal masses greatly outnumber the malignant ones. A high degree of clinical suspicion is necessary, and the diagnosis is based largely on the imaging appearance. Endovaginal ultrasonography (US) is the most practical modality for assessment of ovarian tumours because of its ready

availability and the high negative predictive value. Morphologic analysis of adnexal masses enables their accurate identification as either low risk or high risk. MRI is better reserved for problem solving when US findings are non-diagnostic or equivocal, because, though MRI has better diagnostic accuracy, it is also more expensive.

The accuracy of MR imaging in diagnosing mature cystic teratomas, endometriomas and leiomyomas is well established, and is derived from its superb contrast resolution and its usefulness in tissue characterization. Several types of tissue and fluid can be

* Part I of this Original Article appeared in PMJ Vol.2, No.2 (January-June 2011) - Pg. 90-96

distinguished at MR imaging based on their signal intensity characteristics. In general benign epithelial ovarian neoplasms are predominantly cystic, and malignant epithelial neoplasms have both cystic and solid components. Cystic lesions containing simple fluid have prolonged T1 and T2 relaxation times with low signal intensity on T1WI and very high signal intensity on T2WI. Although solid lesions contain large amounts of both intracellular and extracellular fluid, resulting in increased T1 and T2 relaxation times, they have relatively intermediate signal intensity on T2WI that is considerably lower than that of fluid. Fat, haemorrhage, and some high-viscosity, mucin-containing lesions have high signal intensity on T1WI. Fibrosis or smooth muscle has low or intermediate signal intensity on T1WI and low signal intensity on T2WI. A typical fat-containing lesion is a mature cystic teratoma. Haemorrhagic lesions include endometriosis, haemorrhagic cysts, haemorrhagic foci of adenomyosis, haematosalpinx etc. Fat-saturated T1WI help distinguish between haemorrhage and fat. Identifying the signal intensity of a mass can help narrow the differential diagnosis. Injection of gadolinium-enhanced contrast agent is recommended for the accurate characterization of some adnexal lesions, especially for delineation of necrosis, papillary projections, solid components, septations, peritoneal implants, and omental disease.

Materials and methods

The usefulness of MRI in the ovarian and non-ovarian adnexal pathologies was studied in selected patients referred to Department of Imaging, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala.

Observations

In this part of the series we describe various ovarian and adnexal pathologies evaluated by MRI. Studies were done on 1.5 Tesla MR scanner with 8-channel body coil. Different sequences performed were the same as already described in the previous article.

A) Non-neoplastic ovarian masses

(a) Haemorrhagic corpus luteum cysts

Most ovarian cysts are functional cysts (follicular cysts or corpus luteum cysts). Corpus luteum cysts may enlarge by internal haemorrhage and cystic transformation. Cysts larger than about one cm often represent corpus luteum cysts. They exhibit relatively



Fig. 1: Haemorrhagic corpus luteum cysts. (a) T1W SAG (b) T2W SAG (c) T2W AX: a complex right ovarian cyst with fluid-fluid level, exhibiting high signal in non-dependent portion, and intermediate signal in dependent portion on T1/T2W.

high signal intensity on T1WI and intermediate to high signal intensity on T2WI (Fig. 1). Corpus luteum cysts do not demonstrate the profound T2 shortening that is seen with many endometriomas.

(b) Endometriomas

The most specific MR imaging findings here include multiple cystic masses with high signal intensity on T1WI and low signal intensity on T2WI (Fig. 2,3). Endometriomas acquire an iron concentration in their cyst contents many times higher than even whole blood. This gives them the characteristic appearance of very high signal intensity on T1WI (similar to fat) and low signal intensity on T2WI, a combination not seen in pelvic haematomas at any stage of evolution.

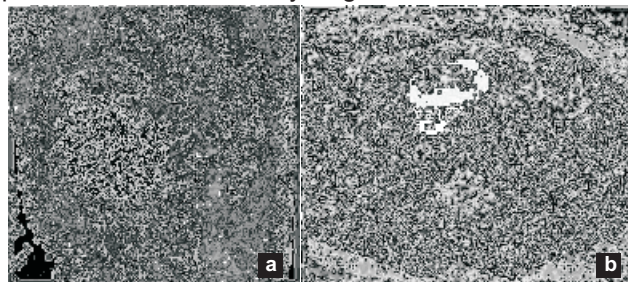


Fig. 2 Endometrioma: (a) T1W SAG (b) T2W AX: Bulky left ovary with multiple follicles; largest follicle exhibits high signal on T1WI/T2WI. Peripheral hypointensity was noted on GRE images (image not shown). Multiple septae noted within.



Fig.3 Endometrioma: (a) T1W SAG (b) T2W SAG (c) GRE SAG: a well defined complex cyst, exhibiting high signal with shading on T2W. On T1W anterior component is hyperintense while posterior component exhibits low to intermediate signal. GRE image shows blooming along dependent part of posterior component, suggestive of blood products.

Other possible MR imaging findings in endometriomas are high signal intensity on both T1- and T2WI, adhesion to surrounding organs, and a thickened, low-signal-intensity wall. Noncystic endometrial implants may be especially difficult to define at MR imaging due to their small size, potential obscuration by artifact from bowel peristalsis, and occasional lack of differentiation from adjacent fat on contrast-enhanced images.

B) Neoplastic ovarian masses

1. Benign Neoplasms

a. Epithelial ovarian tumours

In many instances, epithelial tumours tend to be cystic and solid at gross morphologic examination, and their cell types cannot be differentiated on the basis of their appearance at MR imaging, CT, or US. However,

some features can aid in differentiating mucinous from serous tumours.

Benign serous cystadenoma: occurs as unilocular or multilocular cystic mass with homogeneous MR imaging signal intensity of the locules, a thin regular wall or septum, and no endocystic or exocystic vegetation (Fig. 4-8).

About 60% of all the serous ovarian neoplasms are smooth-walled benign cystadenomas; 15% are of low malignant potential, and 25% are malignant.



Fig. 4: (a) T2W COR (b) T2 FAT SAT COR (c) T2W AX: large multiseptated lesion with thick septum superiorly; no solid mass. **Papillary serous cystadenoma** of right ovary confirmed surgically and on Histopathological examination.

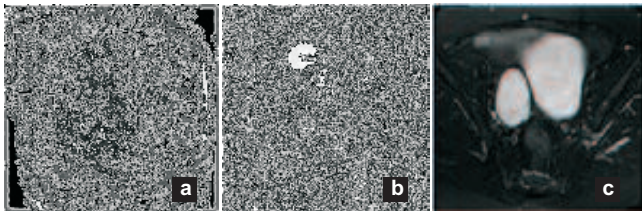


Fig. 5 **Benign serous cystadenoma:** (a) T1W SAG (b) T2W SAG (c) T2 FAT SAT AX: shows multiseptated cystic lesion on both sides. Asymmetrical septal wall thickening seen; but no solid nodules or papillary projections.

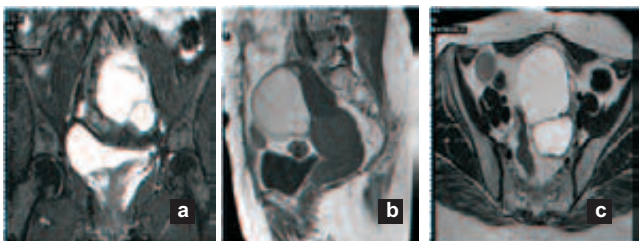


Fig. 6 **Benign serous cystadenoma:** (a) T2W FS COR (b) T1W SAG (c) T2W SAG: large multi-septate complex cystic lesion with thick walled septae; cyst content exhibits high signal on both T1W and T2W images.

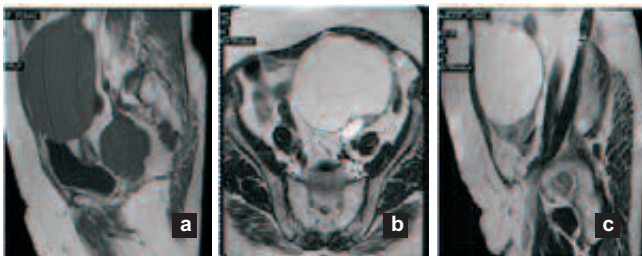


Fig. 7 **Benign serous cystadenoma:** (a) T1W SAG (b) T2W AX (c) T2W SAG: large unilocular cystic lesion in lower abdomen and pelvis; incomplete septae and solid nodule noted within.

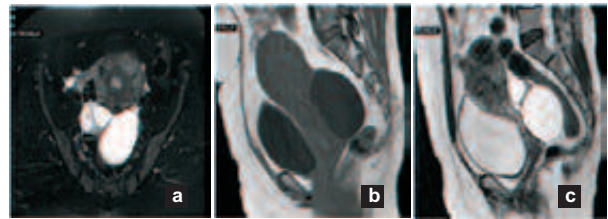


Fig. 8 **Benign serous cystadenoma:** (a) T2W FS AX (b) T1W SAG (c) T2W SAG: Multiloculated cystic lesion in pouch of Douglas (POD) with thickened septae and solid portion. Abnormal location of ovary in POD suggests torsion of cyst.

Benign mucinous cystadenoma: manifests as a multilocular cystic mass that has a thin regular wall and septa, or that contains liquids of different attenuation or signal intensity but has no endocystic or exocystic vegetation (Fig. 9,10). Mucinous cystadenomas tend to be larger than serous cystadenomas.

Features more suggestive of benign epithelial tumours include a diameter less than 4 cm, entirely cystic components, a wall thickness less than 3 mm, lack of internal structure, and the absence of both ascites and invasive characteristics such as peritoneal disease or adenopathy.

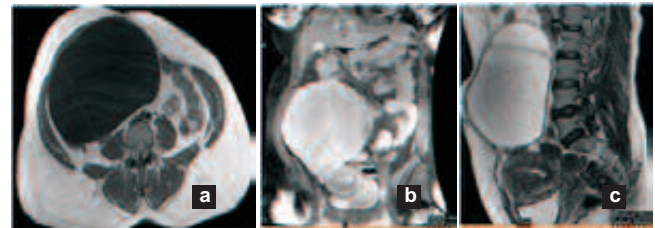


Fig. 9: (a) T1W AX (b) PD FS COR (c) T2W SAG: large multiseptated cystic mass in lower abdomen & pelvis with septae of variable thickness; **Benign mucinous tumour** found at surgery.

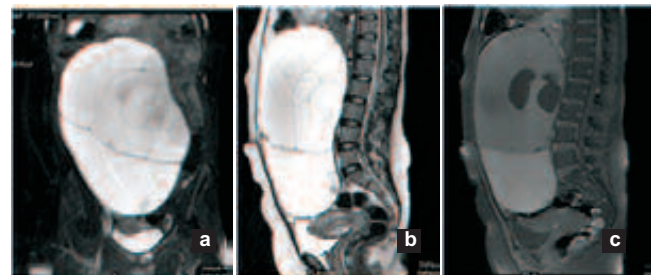


Fig. 10: (a) T2W FS COR (b) T2W SAG @ T1W FS SAG: Large multiseptated predominantly cystic lesion of heterogeneous signal intensity - cysts within cyst appearance - note high signal intensity content on T1W images. **Mucinous cystadenoma of ovary** was proved by histopathology.

b. Germ cell tumours

They include mature and immature teratomas, dysgerminoma, endodermal sinus tumour, embryonal carcinoma and choriocarcinoma. Of all the germ cell tumours, only mature teratoma is benign.

Mature teratoma

Mature teratoma is the most common benign ovarian tumour in women below 45 years of age. At any imaging modality, mature teratomas demonstrate a

broad spectrum of findings, ranging from purely cystic, to a mixed mass with all the components of the three germ cell layers, to a non-cystic mass composed predominantly of fat. At MRI, the sebaceous component of dermoid cysts has very high signal intensity on T1WI similar to retroperitoneal fat (Fig. 11,12). The signal intensity of the sebaceous component on T2WI is variable, usually approaches that of fat. Internal patterns of mature cystic teratomas such as palm tree-like protrusions or dermoid nipples are typical findings.

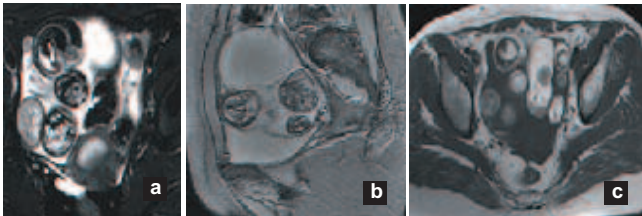


Fig. 11: (a) T2W FS AX (b) T2*W SAG (c) T1W AX: Heterogeneous signal intensity bilateral adnexal masses with high signal on T1W & suppression of signal on T2W fat sat sequences, suggesting fat. Areas of peripheral and central blooming on T2*W images suggest calcification. High signal intensity within cyst on T2W images represents fluid. These signal intensities are highly specific for **mature cystic teratoma**, which was subsequently confirmed by surgery.

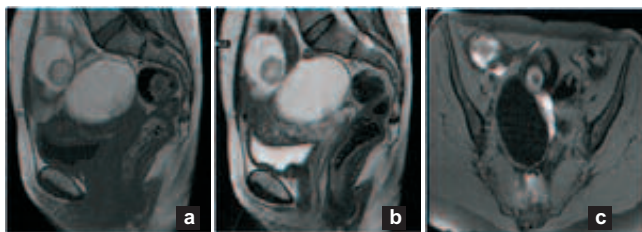


Fig. 12: (a) T1W SAG (b) T2W SAG (c) T1W FS AX: Bilateral adnexal fat containing (high signal on T1W & T2W images with suppression of signal on fat sat sequences) masses with solid soft tissue nodules - Rokitansky protuberance (dermoid plug). Surgery confirmed **mature cystic teratoma**.

c. Fibroma

They account for approximately 4% of all ovarian neoplasms, typically detected in middle-aged women during routine gynaecologic examination. They are important from an imaging standpoint because they appear as solid masses, thereby mimicking malignant neoplasms. Fibromas demonstrate homogeneous, relatively low signal intensity on T1WI and appear as well-circumscribed masses with low signal intensity

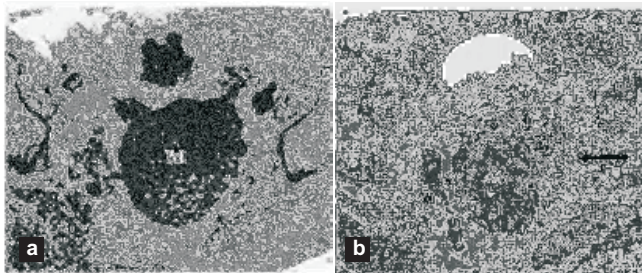


Fig. 13: (a) T1W AX (b) T2W AX: mass (M) with nonspecific intermediate signal intensity on T1WI, and very low signal intensity similar to that of muscle on T2WI, a finding characteristic of an **ovarian fibroma**. Small ovarian cysts at the margin of the mass (arrow) help identify the mass as ovarian.

containing scattered high-signal intensity areas representing oedema or cystic degeneration on T2WI (Fig. 13). This low signal intensity results from the abundant collagen content of these tumours and is relatively diagnostic of fibroma.

2. Malignant neoplasms

Imaging findings suggestive of malignancy include a thick, irregular wall, thick septae, papillary projections and a large soft-tissue component with necrosis. Ancillary findings of pelvic organ invasion, implants (peritoneal, omental, mesenteric), ascites, and adenopathy increase diagnostic confidence for

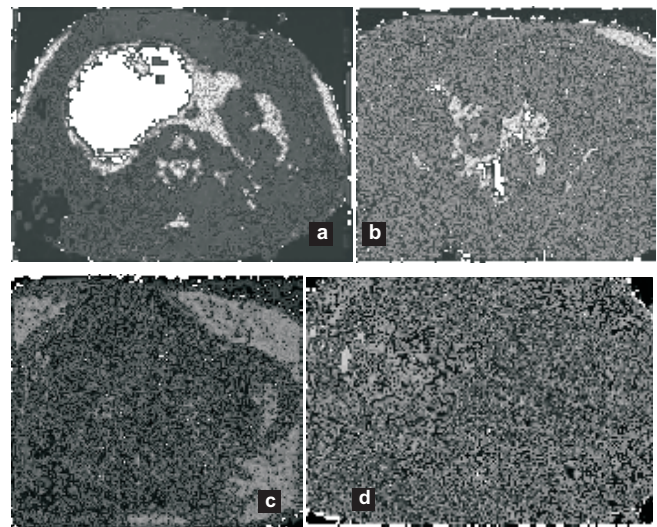


Fig. 14 **Malignant serous cystadenocarcinoma**: (a) T2W FS AX (b) T2W FS AX (c) T1W AX (d) T2W AX: Large multiseptated predominantly cystic lesion with thick septae, papillary excrescences and solid nodules. Marked ascites noted. Large size of the cyst and ascites may point towards malignancy; however difficult to differentiate between the benign and malignant neoplasm on MR imaging.

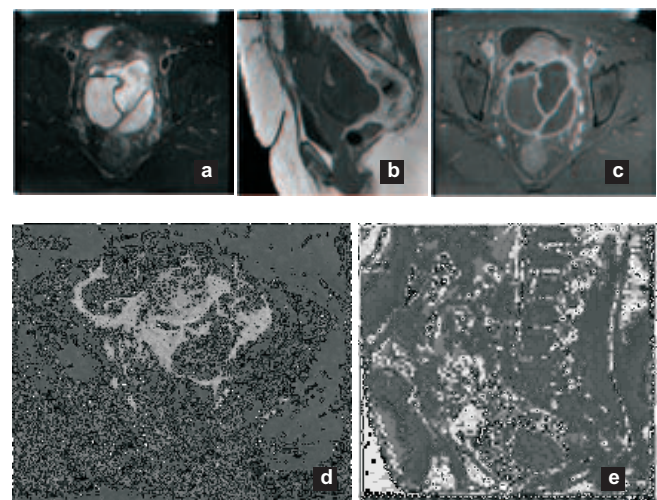


Fig. 15 **Malignant cystadenocarcinoma** : (a) T2W FS AX (b) T1W SAG (c) & (d) T1W post-contrast FS AX (e) T1W post-contrast FS SAG: Multiseptated mixed signal intensity solid and cystic mass lesion in lower abdomen and pelvis. Post-contrast study shows intense enhancement of thick septae, solid nodules and peritoneal deposits; ascites also noted; peritoneal deposits favour malignant neoplasm.

malignancy (Fig. 14,15,16). Bilaterality and peritoneal carcinomatosis are seen more frequently in serous cystadenocarcinomas. The Psammoma bodies are calcifications seen at histologic analysis in up to 30% of malignant serous tumours.

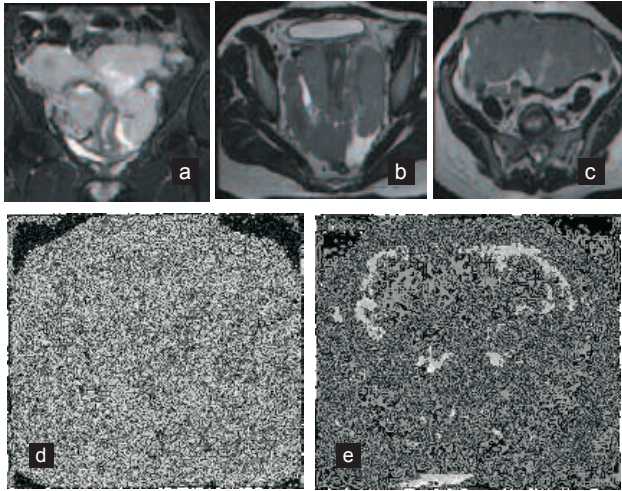


Fig. 16 Pelvic lymphoma: (a) T2W FS COR (b) & (c) T2W FS AX (d) & (e) post contrast T1W FS AX & SAG: Large solid multilobulated mass lesions in both adnexae with peritoneal implants. These masses are of intermediate signal on T1W and homogeneously hyperintense on T2W with intense uniform enhancement on contrast study. Predominantly solid masses with lobulations, bilaterality and peritoneal implants likely to represent malignancy.

Features such as wall thickening, septae and multilocularity are less reliable indicators of malignancy as they are frequently seen in benign neoplasms as well, particularly cystadenofibromas, mucinous cystadenomas, and endometriomas.

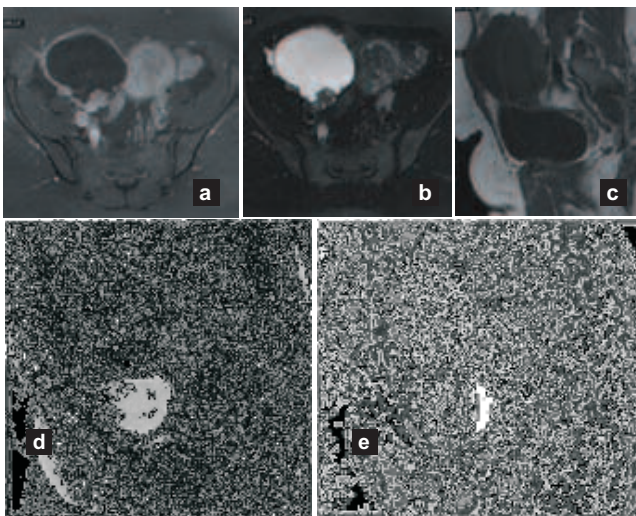


Fig. 17: (a) T2W FS AX (b) T1W SAG (c) T2W SAG (d) Post-contrast T1W FS AX (e) Post-contrast T1W FS SAG: Complex predominantly cystic mass with solid portion in right adnexa; fundic fibroid. Focal well defined mass exhibits high signal on T2W in lower endometrial cavity. Intense enhancement of cyst wall and solid portion in right adnexa as well as endometrial lesion. Most likely imaging diagnosis is **Endometrioid right ovarian carcinoma with synchronous endometrial carcinoma or hyperplasia**. Patient lost to follow up.

a. Endometrioid carcinoma

Almost always malignant, about 15%-30% are associated with synchronous endometrial carcinoma or endometrial hyperplasia, with bilateral involvement in 30%-50% cases. Imaging findings are nonspecific and include a large, complex cystic mass with solid components (Fig. 17). Endometrial thickening can also be seen on imaging studies.

b. Immature teratoma

The Benign mature teratomas must be differentiated from malignant, immature teratomas, which have prominent solid components and may demonstrate internal necrosis or haemorrhage. Mature tissue elements similar to those seen in mature cystic teratoma are invariably present. Radiological examination reveals a large, complex mass with cystic and solid components and scattered calcifications; in contrast, calcification in mature teratomas is localized to mural nodules. Small foci of fat are also seen in immature teratomas. These tumours grow rapidly and frequently demonstrate perforation of the capsule. The tumour capsule is not always well defined.

c. Collision tumours

It represents the coexistence of two adjacent but histologically distinct tumours with no histologic admixture at the interface. They are rare and are most commonly composed of teratoma and cystadenoma or cystadenocarcinoma. The mechanism of its development is uncertain. When an ovarian tumour demonstrates imaging findings that cannot be subsumed under one histologic type, especially in cases of ovarian teratoma, a collision tumour should be considered.

d. Metastatic ovarian tumours

Colon and stomach are the most common primary tumour sites, followed by breast, lung, and contralateral ovary. Differentiation between primary and metastatic tumours is of great importance in the treatment and prognosis. Imaging findings in metastatic lesions are nonspecific, consisting of predominantly solid components or a mixture of cystic and solid areas.



Fig. 18 Pelvic metastasis: (a) T1W SAG (b) T2W SAG (c) Post-contrast T1W FS AX: Irregularly marginated solid masses in peritoneal cavity and bilateral adnexae. Intense enhancement of these masses seen on contrast study; mild ascites. These findings are non-specific. Bilaterality, irregularly marginated solid masses, ascites and peritoneal involvement suggest metastasis.

Krukenberg tumours are metastatic tumours that contain mucin-secreting “signet ring” cells and

usually originate in alimentary tract. They demonstrate some distinctive findings, including bilateral complex ovarian masses with hypointense solid components (dense stromal reaction) and internal hyperintensity (mucin) on T1- and T2WI respectively (Fig. 18).

Key imaging features in differential diagnosis of ovarian tumours

Although ovarian tumours have similar clinical and radiologic findings, predominant or specific key features are present in each type of ovarian tumour.

1. Although there is considerable overlap in morphologic characteristics and corresponding imaging features that in many cases prevents definitive preoperative characterization as benign or malignant, features that are suggestive of malignant epithelial tumours include a thick irregular wall, thick septae, papillary projections and a large soft-tissue component with necrosis.
2. The ovarian tumours associated with endometrial hyperplasia or carcinoma include endometrioid carcinoma, granulosa cell tumour and occasionally, thecoma or fibrothecoma.
3. Solid ovarian tumours that have very low signal intensity on T2-weighted MR images include fibroma, Brenner tumour and occasionally, fibrothecoma.
4. Although rare, endometrioid carcinoma is the most common malignant neoplasm that arises from endometriosis, followed by clear cell carcinoma.
5. The presence of fat opacity or fat signal intensity in an ovarian lesion is highly specific for a teratoma. Mature cystic teratomas are predominantly cystic with dense calcifications, whereas immature teratomas are predominantly solid with small foci of lipid material and scattered calcifications.
6. Ovarian tumours that are frequently associated with calcifications include serous epithelial tumor, fibrothecoma, mature or immature teratoma, and Brenner tumor.
7. When bilateral complex ovarian masses are seen, metastatic ovarian tumors and serous epithelial tumors of the ovary should be considered.
8. When an ovarian tumour demonstrates imaging findings that cannot be subsumed under one histologic type (especially in cases of ovarian teratoma), a collision tumor should be considered.

C) Adnexal masses

Mimics of ovarian cystic masses include peritoneal inclusion cyst, paraovarian cyst, mucocoele of the appendix, obstructed fallopian tube (eg. hydro-, pyo-, and haematosalpinx), uterine leiomyoma, adenomyosis, spinal meningeal cyst, unicornuate uterus, lymphocoele, lymphangioliomyomatosis, cystic degeneration of lymph nodes, haematoma and abscess. It is important to understand the relationship of a mass with its anatomic location, identify normal ovaries at imaging, and relate imaging findings to the patient's clinical history to avoid misdiagnosis.

(a) *Paraovarian cyst*: arises from the broad ligament or fallopian tube, so the ipsilateral ovary is not affected and maintains its normal configuration. MR imaging also clearly depicts the independent relationship of the ovary to an adjacent paraovarian cyst, which usually appears as a well-defined homogeneous structure, with high signal intensity on T2-wieghted images, and low signal intensity on T1-weighted images (Fig. 19). If the cyst is complicated by torsion or haemorrhage, it may demonstrate high signal intensity on T1-weighted images and have thick walls. The presence of soft-tissue component is indicative of a neoplasm.

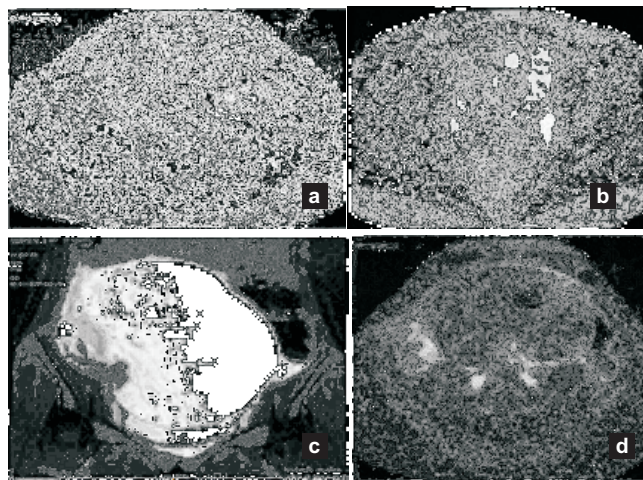


Fig. 19: (a) & (b) T2W AX (c) T2W FS COR (d) T1W Post-contrast FS AX: Large multiloculated cystic lesion in lower abdomen and pelvis with high signal intensity fluid and low signal septae seen. The lesion is in close proximity to right ovary which shows multiple small follicles. Left ovary is in Pouch of Douglas. Marked ascites noted; peripheral and septal enhancement of lesion; **right paraovarian cyst** found at surgery.

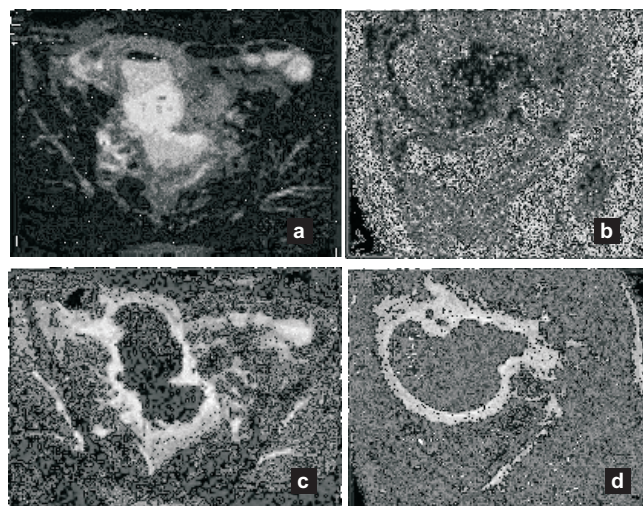


Fig. 20 **Pelvic abscess**: (a) T2W FS AX (b) T1W SAG (c) Post-contrast T1W FS AX (d) Post-contrast T1W SAG: Irregular thick walled collection in pelvic cavity with air-fluid level within. Post-gadolinium images show marked peripheral enhancement with central non-enhancing collection, suggesting abscess formation. Surgical drainage and antimicrobial therapy shows marked reduction in the abscess size on follow up study.

(b) *Pelvic abscess*: may be intra- or extra-peritoneal. Imaging features of pelvic abscess vary, with thick or thin walls, simple or complex fluid collections, adjacent inflammatory fat stranding, free fluid and inflammation of surrounding organs seen at all modalities (Fig. 20, 21). Air from gas-forming organisms or fistulization with adjacent bowel may be seen.

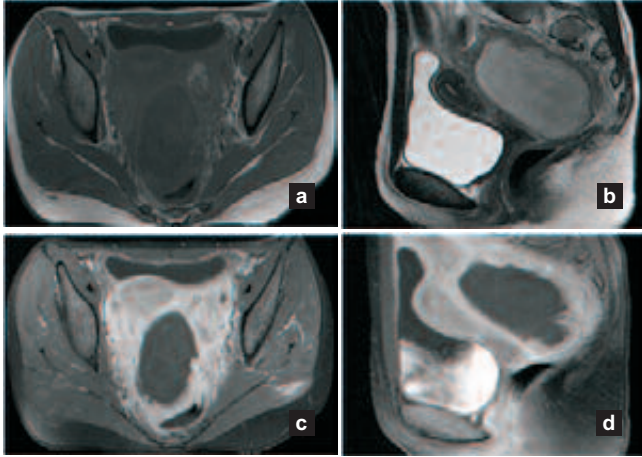


Fig. 21 **Pelvic abscess**: (a) T1W AX (b) T2W SAG (c) Post-contrast T1W FS AX (d) Post-contrast T1W SAG: Well defined thick walled collection in Pouch of Douglas. Post-gadolinium images show marked peripheral enhancement with central non-enhancing collection suggesting abscess formation. Surrounding fat shows enhancement, suggestive of inflammation. Medical therapy shows marked reduction in the abscess size on follow up USG study.

(c) *Mesenteric/ omental cyst*: is a descriptive term for any cystic lesion within the mesentery, usually a lymphangioma, a benign lesion of vascular origin (only 5% are abdominal). They show enhancing septae, better depicted by MR or US. MR imaging helps to determine the mesenteric origin of lymphangioma, specifically with the use of multiple planes, and allows

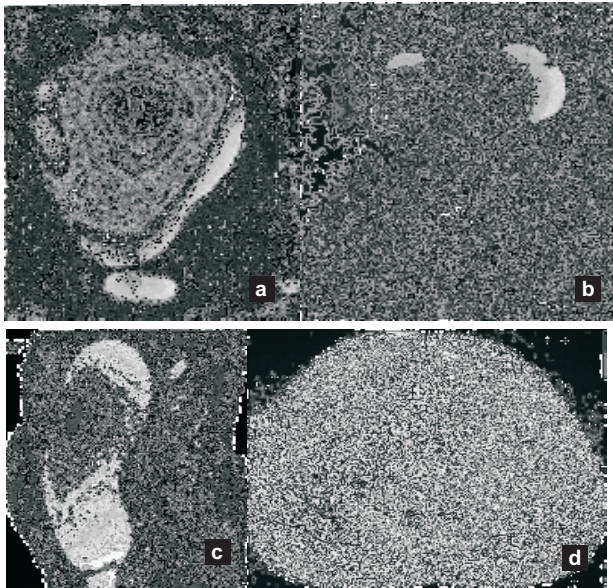


Fig. 22: (a) & (b) T2W FS COR (c) T2W FS SAG (d) T1W AX: Large unilocular cystic lesion of uniform signal intensity with low signal on T1W and high signal on T2W images. Both ovaries are seen separately. **Benign mesenteric cyst** was the most likely diagnosis.

differentiation of cyst contents. Serous contents appear hypointense on T1WI and hyperintense on T2WI (Fig. 22). Cysts with haemorrhagic or fatty contents appear hyperintense on T1WI and T2WI.

(d) *Retro-rectal developmental cyst*: a rare congenital cystic lesion that originates from the vestiges of embryonic tissue. It encompasses dermoid, epidermoid and enteric (eg. tailgut and duplication) cysts. They may be multicystic, and it may be difficult to differentiate them from an adnexal mass at US. MR imaging is the best to determine the relationship of the mass to the rectum, ovaries, and spine (Fig. 23). Because these lesions are located in the retroperitoneal space, anterior displacement of the ureter, uterus, and rectum may occur, indicative of a presacral location.

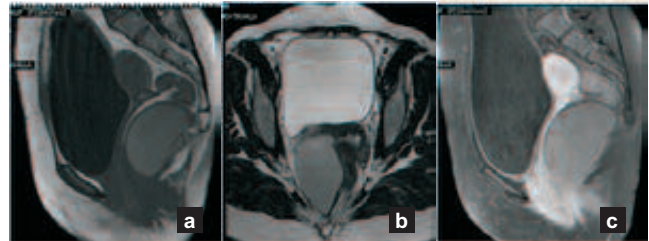


Fig. 23: (a) T1W SAG (b) T2W AX (c) T1W post-contrast T1W FS SAG: Cystic mass lesion in pelvis posterior and to the right of rectum and cervix & upper part of vagina. Predominantly high signal on T1W images, suggestive of long standing proteinaceous collection with no enhancement on post-contrast examination. Cysto-cervical canal communication well seen on T2W Axial sequence. **Rectal duplication cyst** was the most likely possibility.

(e) *Giant cell tumour of sacrum*: They are most common in the 2nd to 4th decade of life, with a female predominance. These neoplasms manifest a lytic, expansile, and destructive process that is often eccentrically located. These very vascular neoplasms show intermediate signal intensity on both T1- and T2-weighted MR images, (Fig. 24) demonstrate significant enhancement at CT and MR imaging, and

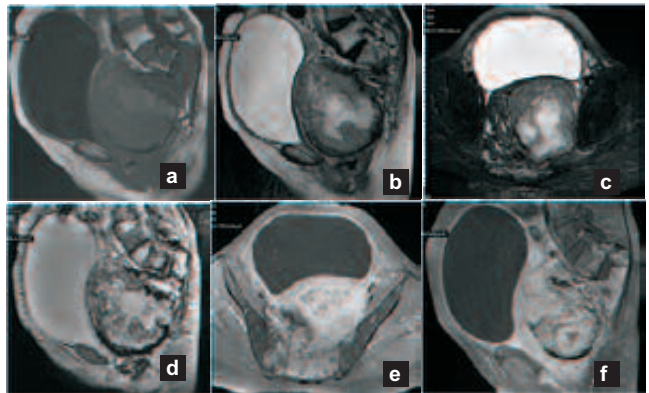


Fig. 24: (a) T1W SAG (b) T2W SAG (c) T2W FS AX (d) T2*W SAG (e) Post-contrast T1W FS AX (f) Post-contrast T1W FS SAG: Known and operated case of **Giant cell tumor of sacrum**. Now has bladder outlet obstruction and feeling of solid mass per vaginum. Large extra-osseous soft tissue mass in pelvis with lower lumbar and sacral destruction. Post-operatively non-visualization of sacro-coccygeal vertebrae. Internal heterogeneity with areas of necrosis, haemorrhage and calcification with intense enhancement of the mass.

may contain areas of haemorrhage or necrosis, aggressive appearance with ill-defined borders, extension to the soft tissues and destruction of subchondral bone plate.

MR as well as skeletal scintigraphy play a crucial role in identifying, localizing, and characterizing lesions of the sacrum, thereby contributing to the correct diagnosis and facilitating treatment planning. MRI findings of a giant cell tumour in the spine are often characteristic, allowing an accurate diagnosis and preoperative evaluation of the extent of the mass.

Conclusions

Despite the development of effective surgical and chemotherapeutic approaches, ovarian carcinoma remains a leading cause of death from gynaecologic malignancies. The treatment of patients with ovarian masses requires initial stratification of risk based on the imaging appearance of the mass, clinical presentation and findings, and serum CA-125 level. Laparoscopic management of masses is largely restricted to those having a mostly benign imaging appearance. Recommendations based on the US imaging appearance include no further evaluation (eg. simple cysts), follow-up US (eg. haemorrhagic cyst), MR imaging (eg. suspected endometrioma, mature cystic teratoma, fibroma, leiomyoma), or staging laparotomy (eg. cystic and solid masses).

Certain radiologic findings predominate in each type of tumour. Knowledge of these key imaging features of ovarian tumours will allow a specific diagnosis or substantial narrowing of the differential diagnosis. Characterization of ovarian tumours can aid in surgical planning, whether exploration or laparoscopic excision, and may help distinguish benign from malignant tumours, thus avoiding inappropriate management.

Exquisite display of pelvic anatomy and tissue characterisation on the various sequences help differentiate ovarian and adnexal lesions and narrow down the differential diagnosis in adnexal masses on the basis of signal intensity, location and involvement of other structures.

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✦ ORIGINAL ARTICLE

Preliminary biocompatibility assessment of Polylactide membrane intended for wound healing

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Abstract

Background: Artificial skin substitutes aim to provide a biocompatible cover for wounds to fasten up the process of epithelialization and wound closure. Membranes based on both animal-derived materials like collagen, and synthetic biodegradable polymers have been used for wound treatment. A major attraction for using biodegradable and biocompatible polymers for fabricating membranes for wound treatment is their safety profile in comparison with xenograft materials like lyophilized collagen. **Materials and methods:** Biocompatible polylactide membranes were fabricated by fusing porous polylactide particles. Preliminary biocompatibility assessment of these membranes was carried out by cytotoxicity tests, and evaluation of cutaneous sensitivity by subcutaneous implantation in guinea pigs. **Results:** Preliminary results show that these polylactide membranes are not toxic to cells and their surface is suitable for cell attachment and growth. These membranes did not provoke any severe inflammatory reaction on subcutaneous implantation in guinea pigs. This was confirmed by histological studies and protein estimation of extracellular matrix content of the excised skin. **Conclusion:** Preliminary results of the study indicate that polylactide membranes are biocompatible, and have the potential to promote faster epithelialization and wound closure. Synthetic biodegradable polymers also exhibit a controlled degradation rate to achieve complete resorption within the intended time.

Key words: Polylactide membrane, Wound healing, Biocompatibility, ISO 10993.

Introduction

Treatment of chronic wounds continues to be a challenge to the clinician, given its refractory nature to the various modes of treatment. Conventional wound care, including debridement, saline washes, sterile cotton gauze dressing, good nursing care, proper nutrition and treatment of any underlying systemic disease, prove effective in the majority of cases of difficult-to-heal wounds. But a minor subset of chronic wounds and ulcers seem to be refractory to the conventional treatment methods, and warrants exploration with more advanced wound care methodologies to provide relief to the patient. Various approaches and technologies have been explored by research groups across the world to hasten the process of wound healing in diabetic ulcers, pressure sores and burn injuries.

Recombinant DNA technology has been used to produce growth

factors like epidermal growth factor (EGF) and fibroblast growth factor (FGF) to aid in wound healing, but their high costs prevents their wide use. Some groups have explored controlled delivery of these growth factors and drugs from local delivery devices like hydrogels and polymeric membranes to the wound bed to fasten healing process^{1,2}. This was partly due to the realization that beyond a certain critical size of the wound, it would be advantageous to have a biocompatible scaffold like material to aid and guide the proliferating cells to achieve wound closure.

Scaffold based dressings were preceded by the development and use of dressings like hydrocolloid and hydrogel based dressings, which aimed to provide a moist wound environment for the proliferating cells in the wound bed³. A wide variety of biocompatible synthetic and natural materials have been used to fabricate

membranes for wound healing, with or without cells grown on them. An example of acellular skin substitute is '*Integra*', which consists of collagen and glycosaminoglycan composite scaffold, which acts as a dermal regeneration template. It has a superficial layer of silicone, which can be removed once the collagen layer is infiltrated by the proliferating fibroblasts and gets integrated into the wound bed⁴. '*Integra*' has been widely used to treat second degree burns in US with good results⁵.

An example of a template with cells is '*Apligrath*', which consists of a collagen membrane with neonatal fibroblasts grown on them. Though expensive, it has been used in the treatment of chronic ulcers⁶. A recent study suggests that the neonatal cells may be indirectly helping in the healing process by secreting a wide variety of growth factors which help in wound healing, and these alloplastic cells are gradually replaced by the native fibroblasts of the body.

Various research groups in India are working towards developing advanced wound dressings based on different types of biocompatible and biodegradable materials. A case in point is the development of collagen membranes for wound healing by the Central Leather Research Institute (CLRI) in Chennai. A research group in the Sree Chitra Thirunal Institute of Medical Sciences and Technology, Trivandrum is actively involved in the development of *chitosan-based* wound dressings.

Our focus at Pushpagiri Research Centre is to develop polylactide (PLA) based biocompatible and biodegradable membranes for wound treatment.

Any biomaterial based device before entering clinical trials has to undergo the requisite tests prescribed under ISO 10993, which describes the biological evaluation of medical devices. The present paper describes some of the preliminary biocompatibility results obtained during the evaluation of the polylactide membranes intended for wound healing.

Materials and methods

Materials:

Poly-D,L-Lactic acid (PDLLA, 45 kDa) was purchased from Durect Corporation, Pelham, USA, and the surfactant Cetyltrimethyl ammonium bromide (CTAB) was procured from Amresco chemicals, USA. Bovine serum albumin (BSA), sodium carbonate, sodium hydroxide, copper sulphate, sodium potassium tartarate, Folin's reagent and other chemicals were purchased from Himedia, Mumbai, India and MP Biomedicals, India. Guinea pigs used for the experiments were maintained in cages kept in the animal house of the Institute. They were fed with chow and water. Anaesthetic agents used for the animal experiments were ketamine for general anaesthesia and lignocaine for local anaesthesia. Sterile surgical instruments were used for the animal experiments.

Methods:

a. Design and fabrication of polylactide membranes: The polylactide membranes were fabricated in a two step process. Initially, polylactide porous particles were formulated and they were later fused to form membranes. Polylactide particles were prepared by double emulsion solvent evaporation method⁷ (Fig. 1).

A primary emulsion was made between an internal aqueous phase (IAP) consisting of two ml sterile water, and an organic phase (OP) consisting of the polymer (200 mg) dissolved in four ml dichloromethane, by sonication (Bandelin, Germany). The resultant *water in oil* (w/o) primary emulsion was dripped slowly into 300 ml of an external aqueous phase (EAP) containing the surfactant (at a concentration of 1% w/v). This resulted in the formation of *water in oil in water* (w/o/w) secondary emulsion, which was continuously stirred, slowly overnight, using a magnetic stirrer, for the dichloromethane to evaporate.

As the solvent evaporates, polylactic acid polymer hardens to form particles within the external aqueous phase. The resulting particles were collected by centrifugation (Hettich, Germany) and lyophilized (Labconco, USA) to obtain free-flowing polymer particles.

To fabricate polylactide membranes, the particles were spread on plastic petri dishes and wetted with ethanol. This resulted in fusion of particles into a membrane form. Ethanol effects partial solubilization of the polylactide particles, resulting in fusion of the particles at their points of contact.

After the membrane is formed, it was washed repeatedly with sterile water to remove any residual ethanol. Additional sterility was achieved by exposing them to UV light in a laminar flow hood. To check the sterility of the polylactide membranes, they were kept on nutrient agar plates and observed over days for any sign of growth indicating bacterial contamination.

b. Evaluation of cytocompatibility of the polylactide membranes: The morphology of the membranes composed of the fused particles was visualized by optical microscope (Magnus) and scanning electron microscope (SEM), (Zeiss, Germany).

The cytocompatibility of the polylactide membranes was assessed using cell lines (B-16 melanoma cells). Polylactide membranes were transferred to six-well culture plates and incubated with RPMI complete media containing high concentration of B-16 cells (for 50 mts), for initial cell attachment to the polylactide membranes to take place. The scaffolds were then cultured in two ml of RPMI complete medium in a CO₂ incubator, with the culture medium changed on alternate days. Optical microscope was used to observe the growth of cells on the polylactide membranes over days.

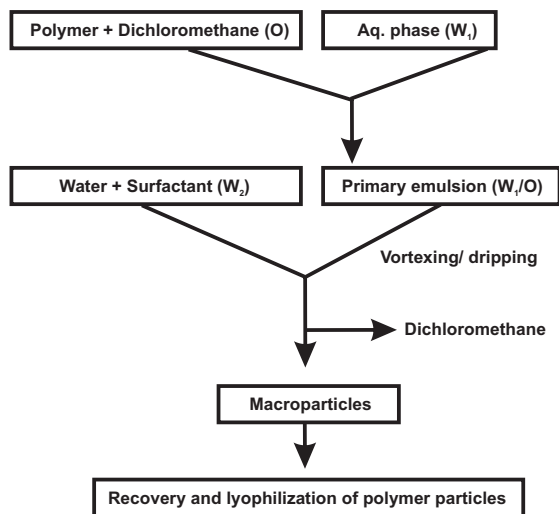


Fig. 1: Scheme of preparation of PLA particles (double emulsion solvent evaporation method)

Preliminary assessment of cutaneous sensitivity in animal models

Animals were maintained according to the guidelines established by the Institutional Animal Ethics Committee (IAEC) of Pushpagiri Institute of Medical Sciences and Research Centre. IAEC clearance was obtained for conducting the animal experiments. Subcutaneous evaluation of the polymeric membrane was done on guinea pigs surgically. All the surgical procedures were carried out under anesthesia.

A small area on the dorsum of the guinea pigs was shaved, wiped with surgical spirit and anaesthetized with lignocaine injection. This was in addition to the ketamine injection given to achieve painless surgical procedures. Two sided full thickness skin incision was made and the flap was reflected to expose the subcutaneous tissue. A two sq cm poly lactide membrane was implanted subcutaneously, and the flap was sutured to retain the membrane in a subcutaneous pouch.

Re-entry of the pouch was carried out at different time points (days 5,12) to assess the effect. Visual inspection of the wound bed was carried out to identify any signs of inflammation caused by the membrane. The overlying skin was excised for histological examination and for estimating the protein and extracellular matrix (ECM) content. The wound was then allowed to close naturally.

The excised skin sample was weighed, minced and treated with a lysis buffer to lyse the cells, releasing the cellular protein, and then centrifuged at 4500 rpm for ten minutes to pellet down the skin pieces. The supernatant (1 ml) was again centrifuged at 12,000 rpm (15 mts) for any debris to settle down. This was used for protein estimation of the released cellular proteins. The de-cellularized skin pellet which mostly consisted of the ECM protein was washed with distilled water; ten ml

10N HCl was added to it, and then kept in boiling water bath for hydrolyzation of the pellet. It was then neutralized with sodium hydroxide solution (1N), and the filtered solution was then used for estimation of ECM content. Folin's test was used to carry out the protein estimation of skin samples. Standard protein solutions were used to plot the standard curve for finding out the protein concentrations of the test samples (Fig. 2). One ml of each test sample was taken, five ml of alkaline copper reagent was added, mixed well and allowed to stand at room temperature for ten minutes. Folin's reagent (0.5 ml) was added to all the tubes and kept at the room temperature for 30 minutes. Blank sample was used to set the colourimeter at zero, and readings of test samples were taken at 540 nm, and protein concentrations calculated using the standard curve.

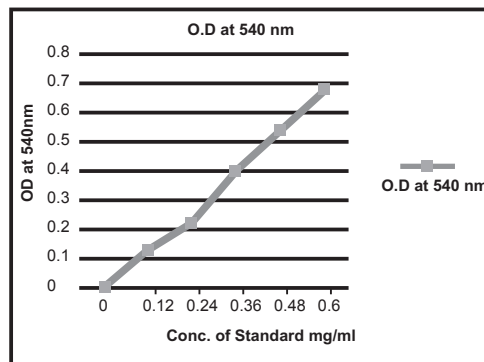


Fig. 2: Standard curve of protein concentrations using Folin's test

Results

Formulation and characterization of poly lactide particles

Poly lactide particles were prepared using double emulsion solvent evaporation method as described earlier. Clinical grade PLA pellets were used for the particle preparations. The lyophilized macroparticles were found to be of spherical morphology and had an average particle size of 250 μm (Fig. 3A). These particles had large internal pores as confirmed in earlier studies using scanning electron microscopy (Fig. 3B). These pores are formed by the removal of the internal aqueous phase during the process of lyophilization. The porous nature of the particles would be beneficial during drug loading of the membrane by diffusion of drug molecules into them.

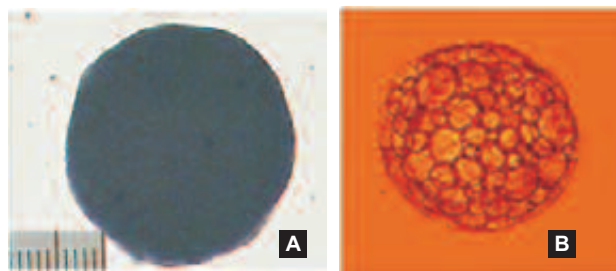


Fig. 3: Images of Poly lactide particles (A) Optical image of a spherical poly lactide particle (Scale bar denotes 100 μm. (B) Image showing the internal pores of a poly lactide particle

Fabrication of polylactide membranes

Fusion of polylactide macroparticles into membranes was carried out at room temperature in suitable plastic moulds like sterile petri dishes in the presence of ethanol (Fig. 4A, B). PLA is sparingly soluble in ethanol, with the result that exposure of PLA particles filled in moulds results in their fusion at the points of their contact in the presence of ethanol, while still retaining the structural integrity of the particles.

The fusion regions between the particles after exposure to ethanol was visualized using optical and scanning electron microscope, denoting the formation of a real membrane as opposed to a simple aggregation of polymer particles, with the **fusion regions between the particles clearly visible** (Fig. 5A, B). The membrane after its formation with ethanol is fragile, but with repeated washes with sterile water it stabilizes into a stable structure.

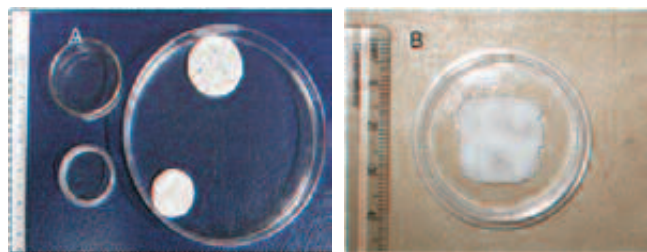


Fig. 4A, B: Fabrication of polymeric membranes of different sizes by fusion of polylactide particles in the presence of ethanol at room temperature

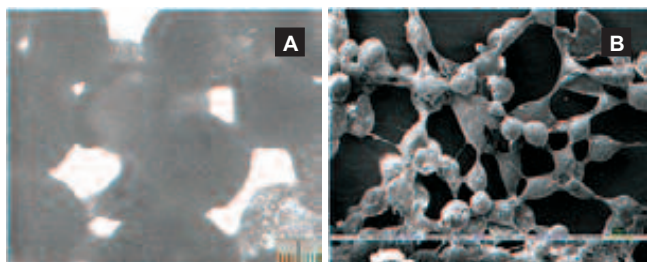


Fig. 5: Polylactide membrane observed using optical microscope (A) and scanning electron microscope (B)

Evaluation of cytocompatibility of polylactide membrane

Fabrication of the membranes was carried out in a clean room enclosure fitted with HEPA filters for maintaining sterility. Sterility checks were done for all the batches of polylactide membranes used for the experiments and were shown to be sterile.

Evaluation of the polylactide membrane with B16 melanoma cell lines showed that they were non toxic to the cells and that they readily adhered to the particles of the membrane and proliferated over days to form organized cellular masses on the membranes (Fig 6A,B). The polylactide membrane thus has the requisite characteristics necessary for cell attachment and growth.

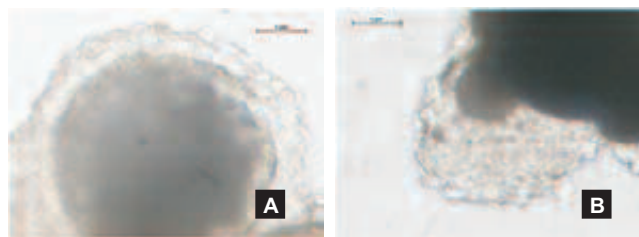


Fig. 6 A, B: Three dimensional growth of B16 melanoma cells on PLA scaffold (day 6)

Subcutaneous evaluation of polylactide membranes

Evaluation of the polylactide membrane with cells showed that they were cytocompatible. Preliminary evaluation of cutaneous sensitivity of the polymeric membranes was done by implanting them subcutaneously in guinea pigs. The animals were anaesthetized and the sterile membranes of approximate size two sqcm were placed in a subcutaneous pouch created on the dorsum of the animals. The edges of the pouch were sutured to secure the membrane in the subcutaneous pouch. The animals were observed daily for any signs of infection, inflammation or oedema at the site of implant. Re-entry of the pouch was done on days five and twelve to assess the impact of polylactide membrane on subcutaneous tissue. There was no sign of any inflammation or oedema at the site of implant during the period of experiments.

When re-entry of the subcutaneous pouch was done on day five, it was seen that the polylactide membrane had stuck well to the underlying subcutaneous tissue (Fig. 7A). A visual inspection showed no signs of inflammation surrounding the membrane, indicating a non-irritant effect of the membrane. The skin overlying the membrane was excised, and the open wound was seen to heal normally (Fig. 7B).

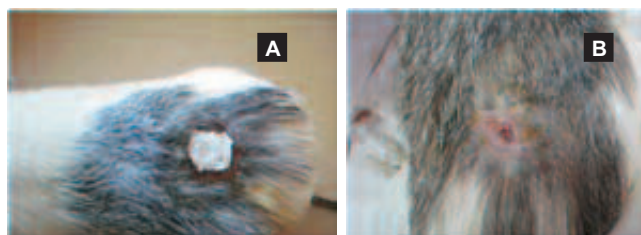


Fig. 7A,B: Subcutaneous evaluation of polylactide membranes
(A) PLA membrane on wound bed (re-entry of subcutaneous pouch by skin excision) on day five
(B) Same wound healed by day twenty one

Histology of the excised skin was done and compared with control guinea pig skins taken from the dorsal area (Fig. 8A, B). The sections did not show any inflammatory cells, which could be induced by the underlying polylactide membrane. Estimation of the protein concentration from the lysed cells of the excised skin,

and its ECM content were done using Folin's test, and compared with that of control guinea pig skins samples. The cellular protein levels and ECM content of the excised skins overlying the membrane were similar to that of the normal skin of the guinea pigs excised from the dorsal area (Fig. 9A, B). The results indicate that there was no significant inflammatory cell infiltration or ECM destruction in the presence of polylactide membranes.

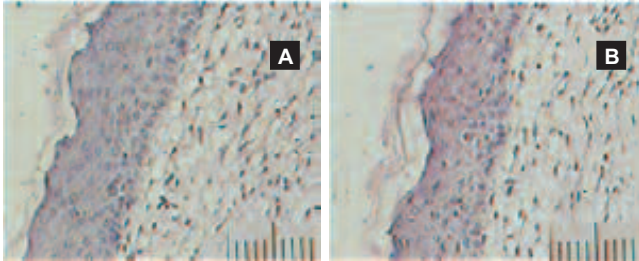


Fig. 6: Histology of guinea pig skin
 (A) Membrane treated skin - no inflammatory cells seen
 (B) Normal dorsal skin of the guinea pig

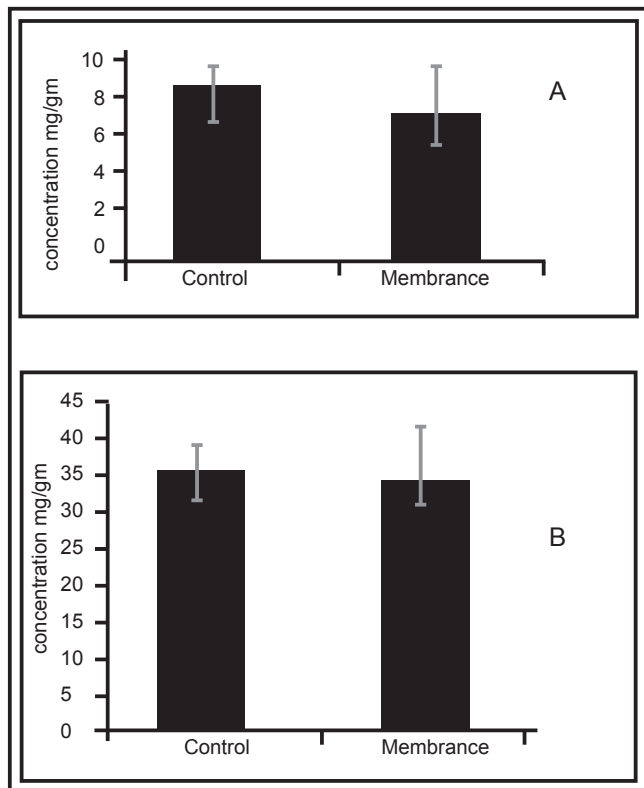


Fig. 7: Cellular protein and ECM content of guinea pig skin samples

- (A) Cellular protein concentration of membrane treated skin and normal skin
- (B) ECM concentration of membrane treated skin and normal guinea pig skin

Discussion

The objective of the present work was to conduct a preliminary biocompatibility assessment of

polylactide membranes intended for wound healing. Preliminary results showed that they did not cause any inflammatory reaction or irritation during subcutaneous implantation in guinea pigs. PLA is a thermoplastic aliphatic polyester, derived from renewable resources such as corn starch (in the USA), tapioca products (roots, chips or starch mostly in Asia) or sugarcane (in the rest of world). The biodegradability and biocompatibility of PLA has been proven in numerous studies⁸. PLA is approved by Federal Drug Agency (FDA) of USA, to be used for a wide variety of clinical applications and has been in clinical use for about 60 years, especially as absorbable sutures.

Burn injuries form a major health problem in the developing world, resulting in a high mortality and morbidity as compared to the west⁹. But in the developed world, increased life expectancy and affluence have increased the incidence of chronic wounds associated with aging and diabetes. In burn wound management, early tangential excision and skin graft is still considered the gold standard. Autologous skin grafts however have limited availability depending on the available area of normal skin on the patient. Also, skin grafting creates additional donor site wounds, thus increasing the total body surface area affected. As an alternative, and to augment existing approaches of burn and wound management, artificial skin substitutes have been widely explored. Attempts have been made to use membranes and scaffolds based on biocompatible polymers and materials to treat cases of burns, and chronic wounds like diabetic ulcers.

'Integra', one of the first acellular regeneration templates introduced in the wounds' market has now been widely adopted for the treatment of second degree burns. It consists of a porous dermal component made of bovine type I collagen and shark chondroitin-6-sulphate glycosaminoglycan, bonded to a silicone layer. The matrix becomes populated with fibroblasts in the wound and contributes towards neo-dermis formation; at the same time the scaffold degrades in a controlled way. The silicone layer protects the wound from fluid loss and bacterial infection. One disadvantage of using materials derived from animal origin like collagen is the risk of transmitting infections like bovine spongiform encephalopathy, if proper sterilization is not ensured. This has led to the investigation of synthetic biocompatible polymers to fabricate porous membranes.

'Suprathel' is a polylactide-based composite membrane for wound treatment, developed in Germany. It rapidly adhered to the wound bed, reduced the pain, protected against infections and promoted wound healing. The relative safety profile and its already proven use in clinics have led us to choose polylactic acid polymer as the substrate to fabricate biocompatible membranes to be used for wound treatment. Polylactide membranes need to comply with three major requirements before they can enter clinics, namely, they should be safe for the patient, be clinically effective and

be convenient in handling and application. Since PLA is already approved for human use, only minimal testing is required to ascertain its safety profile for human use. The tests are enumerated in ISO 10993, Biological evaluation of medical devices. The polylactide membrane used for wound cover comes under the classification of surface devices used in breached or compromised surfaces. The tests recommended for such devices are cytotoxicity testing, and irritation and intracutaneous reactivity testing.

Evaluation of the membrane proved that it was non-toxic to cells, and that the cells were able to attach to and proliferate on these membranes. The irritation and inflammatory potential of these membranes were tested in subcutaneous implants in guinea pigs. Re-entry of the implant site was done at different time points, and visual inspection showed that it did not invoke any adverse inflammatory reaction at the site of implant, which was confirmed by histological studies. Cellular protein estimations and ECM content estimations of the excised skin from the site of implant showed a profile similar to that of normal skin of guinea pigs.

Conclusion

Preliminary biocompatibility evaluation of porous polylactide membranes showed that they are non-toxic to cells and do not cause any adverse inflammatory reactions in the dorsal subcutaneous pouches of guinea pigs. PLA has been well characterized for its biocompatibility, but since fabrication of the membranes is carried out using a novel process, it is necessary to carry out the minimal requisite tests before using them for clinical trials. Detailed and in depth tests are underway for these membranes. The main advantage of these membranes is that it is not necessary to change the polylactide

membrane dressings frequently, since they are biodegradable and biocompatible; traditional wound dressings require frequent changes, causing injury to the underlying healing skin. The porous nature of the polymeric membrane maintains a moist environment needed for proper wound healing, at the same time preventing excessive loss of fluids. Since infections of open wound with microbes and subsequent delay in healing are major problems faced in burn cases, it would be advantageous if the membrane also releases drugs in a controlled manner to combat wound infections.

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✦ ORIGINAL ARTICLE

Evaluation of Oxidative stress and Antioxidant status in Coronary artery disease patients with smoking and/or alcoholism

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Abstract

Background: Coronary artery disease (CAD) is a multi-factorial, possibly fatal disease - highly predictable, preventable, and treatable. Many studies have linked excess generation of reactive oxygen species (ROS) with cellular damage and CAD. No systematic studies have been conducted to evaluate the role of ROS and antioxidant status in correlation with the physical habits (smoking and alcoholism) in the development of CAD. Hence an attempt is made here to correlate the role of oxidative stress and antioxidant status in the presence of various risk factors. **Materials and methods:** One hundred and twenty four angiographically proved CAD patients and ninety one age- and sex-matched controls were included in this study. Detailed clinical and anthropometric data were collected using proforma. Levels of the lipid peroxidation product, Malondialdehyde (MDA), antioxidants such as Super oxide dismutase (SOD), Glutathione (GSH) and Ascorbic acid were estimated in the serum of patients. These values were compared with that of the control group. **Results:** A significantly high level of MDA was observed in CAD patients compared to the normal counterparts ($p < 0.05$), whereas significantly decreased levels of SOD, GSH and ascorbic acid were observed in the study subjects. This study could not observe a significant elevation in oxidative stress and decrease in antioxidant status among smokers and/or alcoholics. **Conclusion:** CAD is a multifactorial disease caused by interaction of many external or internal risk factors. It is associated with greater than normal lipid peroxidation, and an imbalance in the antioxidant status.

Key words: Oxidative stress, Reactive oxygen species, Lipid peroxidation, Anti-oxidant status, Coronary artery disease.

Introduction

Coronary artery disease (CAD) is the single most important disease entity in terms of both the mortality and the morbidity in the entire world population¹. The huge burden of CAD in the Indian subcontinent is the consequence of the large population and the high prevalence of risk factors.

Kerala has the highest prevalence of coronary artery disease among all Indian states with a rural prevalence of 7.5% and urban prevalence of 12%. Despite wide spread efforts in the prevention and management of this disease, it still remains a major challenge to the health managers and scientists².

A constant supply of oxygen is indispensable for cardiac viability and

function. However, the roles of oxygen and oxygen associated processes in the heart are complex; they can be either beneficial or can contribute to cardiac dysfunction and death. As oxygen is a major determinant of cardiac gene expression, and a critical participant in the formation of reactive oxygen species (ROS) consideration of its role in the heart function is essential in understanding the pathogenesis of cardiac dysfunction³.

Lipid peroxidation is a free radical mediated process, which is potentially harmful to membranes, lipoproteins and polyunsaturated fatty acids leading to the formation of malondialdehyde (MDA). The main physical habits contributing to lipid peroxidation are cigarette smoking and alcohol consumption⁴.

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Cigarette smoking is the main risk factor for various chronic diseases, including cardiovascular disease, pulmonary disease and cancer⁵.

The effect of alcohol consumption has been linked to alcohol-related toxicity and diseases are considered relevant to alcohol-atherosclerosis interrelationship⁶.

A good antioxidant status may be important for human health and especially for the prevention of chronic diseases such as cancer and CAD. Low plasma levels of antioxidant as well as low intake of dietary antioxidants have been associated with an increased risk of atherosclerotic heart disease⁷.

Several studies have been conducted to evaluate the effect of smoking in CAD patients. But no detailed studies correlating the effect of smoking along with alcohol consumption, on oxidative stress and antioxidant status in coronary artery disease have been observed in the available literature. So we planned to conduct a systematic study to evaluate the oxidative stress and antioxidant status in CAD patients with smoking and/or alcohol consumption.

Materials and methods

One hundred and twenty four angiographically proven patients below the age of 65 years from the Pushpagiri Heart Institute were selected as cases, and ninety-one age and sex matched persons were included as controls in this study. Detailed clinical and anthropometric data were recorded from all subjects using proforma.

Blood samples were collected after getting proper consent; it was done along with blood collection for clinical diagnosis to avoid unnecessary repetitive venepuncture. Detailed personal data and clinical history was collected using proforma, from the patients as well as normal subjects. Three millilitres of peripheral blood was collected aseptically by venepuncture in an EDTA tube. The oxidative stress was assessed by estimating malondialdehyde (MDA)⁸. Antioxidant status was determined by estimating SOD, glutathione and ascorbic acid^{9,10,11}.

Table 1. Levels of MDA, SOD, GSH, and ascorbic acid of test and control subjects

Parameters	Test (n = 124)	Control (n = 91)	T	p
MDA	1.63 ± 0.633	0.74 ± 0.216	12.80	0.000
SOD	2.58 ± 1.883	10.99 ± 1.894	-32.217	0.000
GSH	8.545 ± 3.949	23.487 ± 5.177	-24.011	0.000
Ascorbic acid	0.93 ± 0.503	1.77 ± 0.313	-32.991	0.000

Values are mean ± SD

Results

1. The levels of MDA, SOD, GSH, and ascorbic acid of the patients as compared to that of age and sex matched control subjects are given in the Table 1.

The MDA level of the patients was significantly higher than that of the normal subjects and the levels of SOD, GSH, and ascorbic acid were significantly lower than that of the normal controls.

2. The patients (n=124) of the study group were divided into four sets according to their life style (Table 2).

Table 2. Grouping of test subjects according to life style

Groups with habits	Frequency	%
Group I (Smoking only)	n = 13	6.0
Group II (Alcoholism only)	n = 19	8.3
Group III (Both)	n = 26	12.1
Group IV (None)	n = 66	30.7

3. The levels of MDA, SOD, GSH, and ascorbic acid were compared between the different groups of patients.

(a) MDA

The levels of MDA were compared among patients with smoking only (group-I), alcoholism only (group-II), having both alcoholism and smoking (group-III) and without both these habits (group-IV) [Fig. 1].

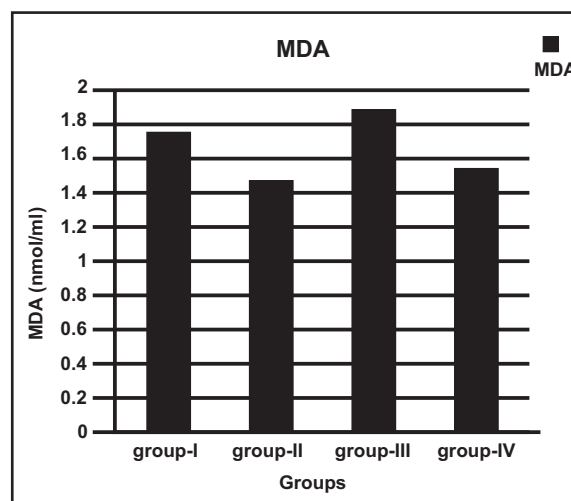


Fig. 1: Levels of MDA in the various groups

There was no significant difference in MDA level among the various groups.

(b) SOD

The level of SOD compared among patients of Groups I, II, III and IV are given in Fig. 2.

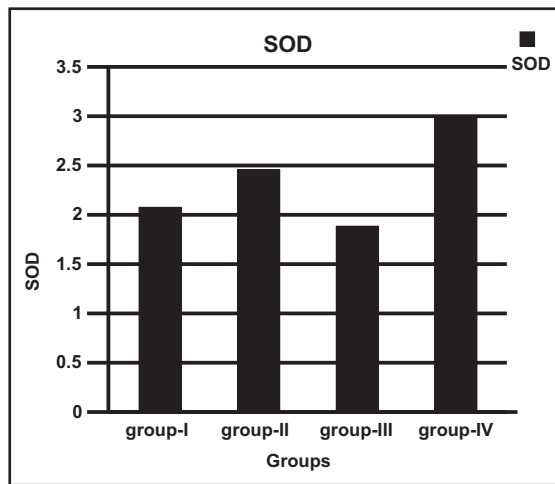


Fig. 2: Levels of SOD in the different groups

The SOD levels in patients with alcoholism and smoking (Group-III) were significantly lower than patients without any such habit (Group-IV). No significant variation was observed in other groups.

(c) GSH

The levels of GSH compared among patients of Groups I, II, III and IV are depicted in Fig. 3.

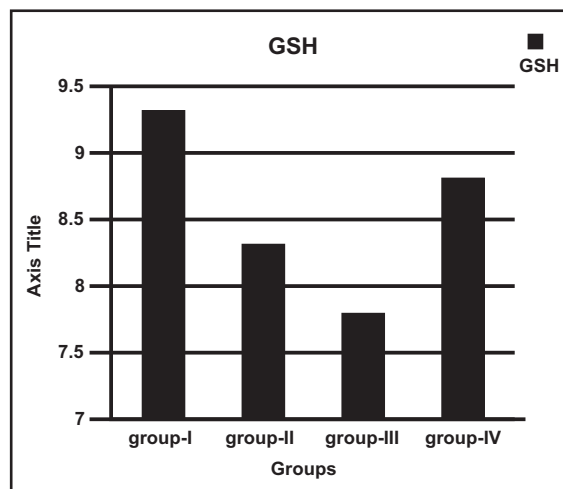


Fig. 3: Levels of GSH

There is no significant difference in GSH levels among the various groups.

(d) Ascorbic acid

The levels of ascorbic acid were compared among the patients of the different Groups, and are given in Fig. 4.

There is no significant difference in ascorbic acid level among the four groups.

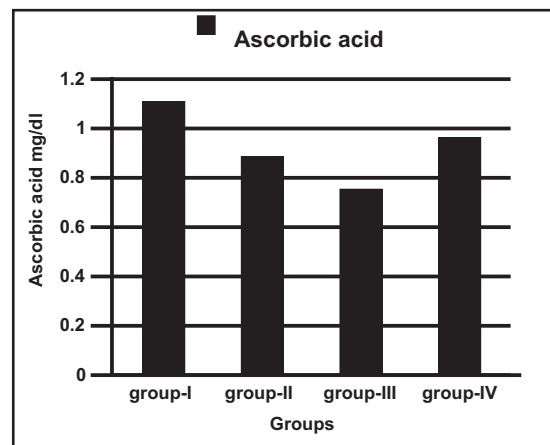


Fig. 4: Levels of Ascorbic acid

Discussion

Coronary artery disease is the major cause of mortality and morbidity worldwide. Increased lipid peroxidation is thought to be a consequence of oxidative stress, which occurs when the dynamic balance between pro-oxidant and anti-oxidant mechanisms is impaired. It has been suggested that there was increased lipid peroxides levels in the blood of patients with CAD.

MDA is a decomposition product of auto-oxidation of poly-unsaturated fatty acids, which is used as an index of oxidative damage¹². It was observed in the present study that increased concentration of MDA is present in the circulation of total CAD patients, indicating increased lipid peroxidation¹³. This rise in MDA could be due to increased generation of reactive oxygen species (ROS), resulting from the excessive oxidative damage that occurs in these patients. Similar reports of elevated MDA levels have been reported in patients with coronary artery disease^{14,15}.

We observed a significant decrease in the levels of glutathione (GSH), ascorbic acid (non-enzymatic antioxidants) and SOD in patients with coronary artery disease, when compared to controls, which is also in agreement with the previous studies^{13,16,17}. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to their increased turnover, which could prevent oxidative damage in these patients. This suggests an increased defence against oxidant damage in coronary artery disease. This study has shown a higher oxygen free radical production and decreased antioxidant activity in coronary artery disease, suggesting a higher incidence of oxidative stress.

The decreased activity of antioxidant enzymes may be a compensatory regulation in response to the increased oxidative stress. Free radical-scavenging enzymes such as SOD, CAT and GPx are the first

line of cellular defence against oxidative injury. In the present study we observed a decreased activity of serum SOD. These results are also in conformity with previous reports^{18,19}.

Cigarette smoking constitutes the single most important, independent and effective risk factor of CAD as per many previous studies²⁰. The extent of lipid peroxidation was found to be higher in smokers than in non-smokers, as shown by the significantly higher levels of erythrocyte MDA. Also the available data confirms that cigarette smoking is associated with decreased SOD and ascorbic acid concentration levels. This suggests that with an increase in oxidative stress, there is a corresponding proportionate decrease in the antioxidant defence system²¹. In our study also the MDA levels were high, whereas antioxidants such as GSH, ascorbic acid and SOD levels were low among smokers than nonsmokers; but the elevation was not significant.

Ethanol is known to deplete GSH levels by the generation of oxidants as well as by inhibiting the mitochondrial glutathione transporter²². Ethanol intoxication causes imbalance between pro-oxidants and anti-oxidants, and results in the oxidative damage of biomolecules such as fats, proteins, or DNA, finally leading to cell injury. In the present study also MDA level was elevated and ascorbic acid, GSH and SOD levels were decreased in alcoholic users²³. But the difference was not statistically significant

Conclusion

The present study clearly reveals significant increase in oxidative stress, and decrease in antioxidant status in CAD patients. But the study would not observe any significant elevation in oxidative stress and decrease antioxidant status among smokers and/ or alcoholics. CAD has for a long time been known as a multifactorial disease caused by interaction between many hereditary, biological and environmental risk factors. It is concluded that the condition is associated with greater than normal lipid peroxidation and with an imbalance in antioxidant status.

Acknowledgments

The financial support given by the Kerala State Council for Science, Technology and Environment is gratefully acknowledged. The authors should like to express their sincere thanks and gratitude to faculty and staff of Dept. of Cardiology and Dept. of Biochemistry, PIMS & RC, Tiruvalla for their advice and guidance.

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✦ ORIGINAL ARTICLE

Epidemiological factors and profile of hospitalized children in measles outbreak in Central Kerala, 2010-2011

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Abstract

Aim: To find out the epidemiological factors and profile of hospitalized children in the Measles outbreak in Central Kerala. **Subjects and methods:** Children admitted with measles in the recent outbreak were evaluated with immunization history, the presence of complications and the duration of hospital stay. **Results:** Of the 64 cases admitted from April 2010 till March 2011, 60.94% were boys and 39.06% were girls. The age of 40.62% cases was less than or equal to one year. Unvaccinated children constituted 48.44% cases. A contact history was available in 25% of them. Only 20.31% patients were malnourished, and the rest apparently had normal nutrition. Most common complication was bronchopneumonia (79.69%) followed by diarrhoea. Average duration of hospital stay was three and a half days for those with pneumonia, and five days for those with pneumonia and diarrhoea. **Conclusion:** Measles vaccination needs wider coverage. Infants may be vaccinated earlier than nine months, especially during epidemics. All preadolescents should be given an extra dose of measles vaccine. Strict cold chain maintenance also is important.

Key words: Measles, Vaccination, Epidemiological factors

Introduction

Measles forms one of the leading causes of death among young children, even though a safe and cost-effective vaccine is available¹. Measles is caused by a virus belonging to the paramyxovirus family. The measles virus, after transmission, grows in the cells that line the back of the throat and lungs and spread to other parts of body haematogenously. Measles is a human disease and is not known to occur in animals. As shown by previous studies, the highest age group affected is around two years². With good vaccination coverage this particular disease can be eliminated.

Back ground

Our Institution caters to urban and suburban population and is also a referral centre for hospitals 25 km around, we attending to approximately 100 outpatients per day. We observed an increase in the flow of measles cases, which started in January 2010 and continued till April 2011. This was significantly higher compared to the previous years (Table 1), and hence a

prospective observational study was planned from April 2010. It was aimed at finding out the epidemiological variables, vaccination status and the complications of children who presented with measles in this one year.

Table 1: No. of measles cases admitted in paediatric ward in last five years

Years	2006	2007	2008	2009	Jan 2010 - April 2011
No. of cases	5	16	4	2	64

Subjects and methods

This was a prospective observational study of all the cases admitted with a diagnosis of measles from April 2010 to April 2011. Age limit was one month to 15 years since it is the hospital policy to admit children up to this age group in Paediatric ward.

The cases were diagnosed as measles if they had the typical prodrome of fever, rhinorrhoea and prominent cough followed at least three days later by appearance of the typical rash. Measles IgM had been done in a few representative cases to confirm

that the clinical inclusion criteria were correct. Those exanthematous fevers which do not satisfy the clinical inclusion criteria were excluded from the study. Age and sex were noted. History was taken with regard to contact with measles. Vaccination status was recorded from the vaccination card and socio-economic categorization was done according to Modified Kuppuswamy's classification. Weight was recorded and assessed for presence of any protein energy malnutrition. PEM was defined as weight for age of less than the third percentile in WHO growth chart. Presence of complications like pneumonia and diarrhoea were recorded, and the duration of hospital stay was noted. The whole data was entered in Microsoft excel worksheet and analyzed with respect to different epidemiologic variables.

Results

A total of 64 cases of measles were admitted from April 2010 to April 2011. Of these 25 (39.06%) were girls and the rest, 39 boys (60.94%) [Table 2]. Among the 64 cases, 26 were aged not more than one year (40.62%), and four were below six months. Eleven children (17.19%) were one to five years old, five (7.81%) were of five to ten years age, and 22 (34.38%) were above ten years [Table 2]. Regarding measles vaccination status, 16 (25%) were vaccinated; vaccination status was not known (lack of availability of reliable vaccination records) in 17 cases (26.56%), and the rest 31 were unvaccinated (48.44%) [Table 2]. Among the sixteen vaccinated children, two (12.5%) aged nine months developed measles within two weeks of vaccination, four (25%) were between five and ten years and the rest ten cases above 10 years (62.5%). There was nobody of age one to five years. Among the 31 unvaccinated, parents of twelve children (38.70%) had just postponed or forgotten to give the vaccination, three (9.67%) did not give immunization because of fear of vaccine and 16 children (51.61%) were too early for measles vaccination. A definite contact history was available in 16 cases (25%) and no history of contact in the rest (75%).

Table 2: Distribution of patients and their vaccination status

Sex distribution	Number	Percentage
Girls	25	39.06%
Boys	39	60.94%
Age distribution		
≤ 1 year	26	40.62%
1-5 years	11	17.19%
5 - 10 years	5	7.81%
> 10 years	22	34.38%
Vaccination status		
Vaccination	16	25%
Unknown status	17	26.56%
Unvaccinated	31	48.44%

Thirteen children were malnourished (20.31%), but the majority (79.69%) did not have malnutrition. Thirty seven children (57.81%) were from Modified Kuppuswamy class 3, twenty one (32.81%) from class 2, and six (9.37%) from class 4. There was nobody from classes 1 and 5. Forty nine (76.6%) had bronchopneumonia as a complication, out of which 17 had diarrhoea also. Six (9.4%) patients had diarrhoea alone, without pneumonia. A total of 26 out of 64 had diarrhoea (40.63%). Eight children with pneumonia had earache as well. Only nine (9.4%) were without any complications and were admitted just for the high fever. All the 13 children who had malnutrition had pneumonia along with diarrhoea and had the longest hospital stay, averaging six and a half days. Average duration of hospital stay was four days with the shortest being one day and the longest, eight days. Average duration of hospital stay was three and a half days for those with bronchopneumonia, and five days for those with both pneumonia and diarrhoea.

Discussion

The major observation made in the present study was a shift in the age distribution of children affected with measles, with two peaks, first in the age group of twelve months or below, and another above ten years. Previous studies have shown the maximum incidence of measles to be from the age one to five years^{2,3}. Singh J and Datta K K, (1997) reviewed the Indian data on age distribution of measles prior to large scale immunization⁴. In metropolitan areas, the median age was about 24 months and virtually all the cases were recorded in children below five years of age, whereas the median age in most of the rural studies was between four to five years.

A measles survey by Satpathy S K *et al.*,⁵ conducted in ten villages of the Rural Health Unit and Training Centre, Singur, West Bengal involving 581 cases, observed the highest attack rate (43.5%) to be in the age group of one to two years, about 11.5% of the cases to be below the age of nine months and 10.4% above fifteen years. In the measles outbreak in Chandigarh reported by Sharma M K *et al.*,⁶ the maximum affected age group was one to five years. In contrast to these Indian studies, the present study showed the maximum number of patients in the two extremes of childhood, infancy and adolescence (40.62% and 34.38% respectively).

The changing trend of more young children affected in the present outbreak in middle Kerala could be a pointer towards waning immunity in the general population with lack of transferred antibodies from mothers, which should have otherwise protected the babies during infancy. Again the waning of immunity during adolescence could explain the second peak during adolescence.

Sinha N P⁷ carried out a study on 989 cases of measles attending the Communicable Disease Clinic,

University of Benin Teaching Hospital in 1977, and observed that multiparity and higher age group of mothers were probable factors in the increased susceptibility of children under six months of age. We had only four cases below six months, and these cases did not show such risk factors, and the number in this age group in the present study was too small. The former study had also shown that poor environmental hygiene and low socio-economic status had a definite role in the transmission of the disease, but malnutrition was not found to be a contributory factor. In our study also most of the children were not malnourished (79.69%) and were from the middle income group (modified Kuppuswamy's class 2 and 3). The limitation of our study to make firm conclusions in this aspect is the fact that normally, most of the people attending to our institution belong to the middle income group.

Regarding the vaccination status, most important observation made was that a significant number of children got measles before the age of vaccination (16 out of 64, forming 25%). Also, there was a group of children whose measles vaccination was postponed or forgotten (12 out of 64, 18.75%). Nobody was unaware of the existence of the vaccine. Similar observation of missed measles vaccine was seen in other studies also⁹. According to a study conducted by Ray *et al.*, in Kolkata⁹ amongst the measles cases in slum areas, 19.7% were immunized with measles vaccine. In our study, 16 out of 64 were vaccinated early (25%).

Respiratory tract complications are generally common in measles and have been previously reported as 39.3%¹⁰, 46.8%¹¹ and 50%¹² in different studies. Certain studies like the retrospective study of measles cases in the outbreak in Sudan¹⁰, showed a higher incidence of diarrhoea. In the present outbreak, respiratory complications were more (76.6%) whereas only 40.63% had diarrhoea. But the main limitation of our study was that, it was a hospital based study. The average length of hospital stay was 3.6 ± 4.2 in a study conducted in Pakistan¹³ which is very similar to our observation.

Conclusions

Measles vaccination coverage is far below the target. Measles occurs even below the age of vaccination which needs consideration of vaccination earlier than nine months, especially during an outbreak setting. Vaccination of preadolescents also should be made routine practice since there is chance of waning immunity by that age even if previously vaccinated. Strict cold chain maintenance also is important, since there are possibly some vaccine failures.

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✦ ORIGINAL ARTICLE

Marital adjustment in families of workers of a factory in Kerala and its social correlates

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Abstract

Background: A good family environment is essential for the efficient working of an employee in a factory. Marital happiness of the workers can be a subject matter of welfare department of an establishment as long as it does not infringe on the privacy of employees. **Objectives:** To determine the level of happiness of wives of employees in a manufacturing establishment. **Design:** A cross-sectional assessment of adjustment between the woman and her husband. **Setting:** A focal group meeting organized by a voluntary agency to educate the women on marital happiness. **Subjects:** The wives of 34 employees of a manufacturing unit who attended the focal group meeting. **Methods:** A translated version of Dyadic Adjustment Scale (DAS) was administered to assess the marital adjustment among the couples. **Results:** About one-third of the women were very much happy about their marital lives. Almost half of them were happy enough to continue with the way of life. About one-fifth were not happy with their married life. On the whole, major aspects of life were in general agreement except the finer aspects such as recreational matters and leisure activities. Lack of marital agreement due to physical violence was present in a minority of families, which was traced mostly to alcohol abuse. **Conclusions:** More leisure time interests and activities, shared household tasks and matters of recreation could improve family environment from within, and career guidance for the wives from external agencies.

Introduction

From a social point of view, any occupational health environment could take marital adjustment of the workers as a part of the day-to-day concern of family welfare division of companies without infringing on their private lives. Kerala has a mother/ woman centred view of family arrangements and women typically hold much larger role in the upkeep of the health of their partners. We deduced, hence, that a good family environment would make a man stick to his job in a factory in a much healthier fashion, provided the woman evinces a keen interest in the financial and social angles of family life in general. With a view to analyzing the relationship of physical, social and mental well being of the workers of a factory with marital adjustment as a starting point of health, we conducted a focal group meeting of the women who made up the families in question. Marital adjustment is something very vague and ambiguous¹ and hence difficult to measure according to many experts. However, Spanier and Cole²

had expanded the concept of a dyadic adjustment scale more objectively to gauge the overall dynamic satisfaction a couple might be feeling at any given point of time. We steered clear of attempts to define dyadic adjustment in terms of co-habitation and other such latter-day concepts which are alien to Indian culture.

Methods

The scale, used in the study was a modified version of the Spanier and Cole Dyadic Adjustment Scale (DAS) which was translated into Malayalam and used among the wives of an industrial manufacturing unit of Kottayam District. Family Health Association of India (FHA) organized the gathering of the women belonging to the families of the workers. A non-probability purposive sampling had to be used as the wives who arrived for the focal group meeting by choice comprised of the sample. The questionnaire was explained to them, even though it was self-administered. There were no divorced individuals

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among the sample of women who participated. Demographic and other facts which might have a bearing on satisfaction in life were elicited in detail.

The scale itself was made of four subscales which were consensus, satisfaction, cohesion and affectional expression. Some of the items did not fit in with the conceptual model which the original authors constructed into the factor structure. These items were singled out for explanation in the results, whenever relevant. Affectional expression is always a critical issue in a culture like Kerala where the open expression of emotion of females will not be a socially acceptable phenomenon. The demographic variables were summarized as frequencies. The scale items were tabulated according to the number of women choosing each option ignoring those who did not attempt the item. Mean scores were found for the subscale totals and are expressed along with standard deviations in the results. Some cross-tabulations of the variables and items were carried out against the wholesome level of happiness which the wives chose wherever necessary for describing associations.

Results

A majority of the women (61.6%) who answered the survey were educated till pre-university level. Only 20.6% had lower grades of education. Generally the women achieved higher education than men in the sample. Only one of the men was a graduate as compared to six of the women. Family income ranged from a lowly 1000 rupees per month to 24,000 rupees per month. Only eight women were employed and out of them two had higher salaries than their husbands. Out of the 34 women, 26 were Christians. There was no inter-religious marriage among any of the women.

Age at marriage of the women and men were 19-32 years and 21-35 years respectively. Most of the women came from two-child families while their husbands often had two siblings or more. The parents were mostly from agricultural or other skilled backgrounds and mothers often had tenth standard education or less. Women disclosed their own properties very little whereas the properties of husband were projected from as low as five cents in land ownership up to Rs. 80 lakhs in monetary terms. Most of the savings which the women had was in the form of bank deposits, post office savings, life insurance policies or chit funds. Except for 17.6% families, all of them owned vehicles. The two-child family norm was followed by 58.8% of the women, where as 26.5% had three children each. Only 11.8% had single child families.

There were no ailments which the women or their spouses had during their childhood which bore any significance on the present level of their health. Jaundice was the commonest childhood illness, either of the partners among the couples had. Chicken pox also was mentioned as one of the frequent childhood illnesses. Renal stones affected two husbands in the

study in addition to a chronic renal failure patient who had a renal transplant donated by his wife. Back ache affected two other couples. Hypercholesterolemia affected three of the females whereas high blood pressure was a common complaint of the male partners of the women. Myocardial infarction affected one husband. Four of the husbands had regular habitual intake of alcohol and four were smokers (one being both smoker and alcoholic). Visit to the hospital was a common event in the families covered under the study. Six families had not done this in the past two months, which did not mean they were free from health problems. Eleven of the females had undergone caesarian section during their fertility period. Two cases of hysterectomy and four sterilization procedures were also logged during the past for these women. Men had less number of operative procedures compared to women. X-ray and ECG investigations seemed to be a fairly common event among these women and their husbands as part of their general health checkup or in other eventualities such as accidents.

Marital adjustment is a dyadic process wherein both the woman and the man comes to terms with their individual qualities of life in terms of the major domains of consensus, satisfaction, cohesion and affectional expression (Tables 1-4). The consensus subscale among the women studied, reached a pooled mean of 25.1 (6.0) out of a worst possible score of 56. The components measured among the various marital adjustment areas which were not in general agreement were as follows; leisure time interests and activities 2.39 (0.88); household tasks 2.38 (0.91); matters of recreation 2.28 (0.96); amount of time spent together 1.88 (0.75); friends and relatives 1.88 (0.66); sex relations 1.79 (0.55); daily life or routines 1.76 (0.83); career decisions 1.75 (0.76). Agreement was visible, more or less all the time, in areas related to taking major decisions 1.45 (0.77); religious matters 1.48 (0.77); financial matters of family 1.52 (0.71); way of dealing with parents or in-laws 1.55 (0.62); demonstrations of

Table 1: The consensus sub-scale among the participants

Consensus	Always N (%)	Almost N (%)	Occasional N (%)	None N (%)
Handling family finance	20 (60.6)	9 (27.2)	4 (12.1)	0 (0)
Recreational matters	8 (25)	10 (31.2)	11 (34.3)	3 (9.37)
Religious matters	20 (64.5)	8 (25.8)	2 (6.45)	1 (3.22)
Matters of affection	16 (50)	13 (40.6)	3 (9.37)	0 (0)
Friends & relatives	9 (28.1)	18 (56.2)	5 (15.6)	0 (0)
Physical relationship	9 (27.2)	22 (66.6)	2 (6.06)	0 (0)
Behaviour in daily life	15 (45.4)	12 (36.3)	5 (15.1)	1 (3.03)
Dealing with in-laws	17 (51.5)	14 (42.4)	2 (6.06)	0 (0)
Aims & goals in life	18 (56.2)	9 (28.1)	4 (12.5)	1 (3.12)
Time spent together	11 (34.3)	14 (43.7)	7 (21.8)	0 (0)
Major decision making	21 (67.7)	7 (22.5)	2 (6.45)	1 (3.22)
House hold tasks	6 (18.7)	11 (34.3)	12 (37.5)	3 (9.37)
Leisure activities	5 (16.1)	12 (38.7)	11 (35.4)	3 (9.67)
Career decisions	14 (43.7)	12 (37.5)	6 (18.7)	0 (0)

affection 1.59 (0.67); philosophy of life 1.62 (0.83). On the whole, other major aspects of life were in general agreement (Table 1).

On the satisfaction subscale (Table 2) the following items showed relatively high frequency of occurrence. Common interests 2.22 (1.0); physical proximity 1.7 (0.85); compromise on issues 1.55 (0.79) and trust in the partner 1.45 (0.99). Low frequency was visible in dialogs about divorce 1.21 (0.49); physical violence 1.21 (0.42); leaving the house after a fight 1.37 (0.66) and harassing behaviour 1.41 (0.66). The overall score in satisfaction subscale was 13.41 (2.55) out a worst possible score of 36.

Table 2: The satisfaction sub-scale among the participants

Satisfaction	Most of the time N (%)	Occasionally N (%)	Rarely N (%)	None N (%)
Divorce/separation	0 (0)	1 (3.12)	5 (15.6)	26 (81.2)
Fighting/leaving	0 (0)	3 (9.37)	6 (18.7)	23 (71.8)
Confidence in spouse	25 (80.6)	1 (3.22)	2 (6.45)	3 (9.67)
General adjustment	20 (60.6)	9 (27.2)	3 (9.09)	1 (3.03)
Regret of being married	0 (0)	1 (3.22)	5 (16.1)	25 (80.6)
Violence	0 (0)	0 (0)	7 (21.8)	25 (78.1)
Irritating behavior	0 (0)	3 (9.37)	7 (21.8)	22 (68.7)
Physical intimacy	17 (51.5)	10 (30.3)	5 (15.1)	1 (3.03)
Common interests	9 (28.1)	11 (34.3)	8 (25)	4 (12.5)

Working together in a project, as per the women in the Indian context, seemed rarer in comparison to other activities with a score of 2.75 (1.29) on a worst score of four (Table 3). Sharing of ideas (2.24 ± 1.32) also was less frequent in the male dominant families of our setting. Calm discussions (2.19 ± 1.3) were also not the main agenda of family life. Laughing together and enjoying jokes may be the only activity which was happening on a weekly basis with an average score of 1.91 (1.28). Overall cohesion score was 8.94 (3.98) out of a maximum possible 16.

Table 3: The cohesion sub-scale among the participants

Cohesion	Daily N (%)	Weekly N (%)	Monthly N (%)	Rarely N (%)
Stimulating exchange of ideas	15 (45.4)	5 (15.1)	3 (9.09)	10 (30.3)
Laughing together	20 (60.6)	4 (12.1)	1 (3.03)	8 (24.2)
Calm discussions	15 (46.8)	6 (18.7)	1 (3.12)	10 (31.2)
Working on a project	9 (28.1)	4 (12.5)	5 (15.6)	14 (43.7)

Of the total 34, fourteen (46.6%) felt that tiredness was a deterrent factor to the physical relationship between the partners (Table 4). Thirteen (41.9%) of women felt that there was inadequate expression of love. Only five (16.6%) of the women felt that sexual relations end up just being a routine chore. In spite of all of this, 79.4% felt that the sexual aspect of their lives was satisfactory.

Table 4: Affectional expression sub-scale among the participants

Affectional expression	Yes N (%)	No N (%)
Always tired for physical relation	16 (53.3)	14 (46.6)
Never show love openly	13 (41.9)	18 (58.0)
Just a routine	5 (16.6)	25 (83.3)
Satisfied	27 (90)	3 (10)

From a stability point of view, none of the women felt their marriages were shaky. 41.2% of them felt ready to try as much as they could to make their family life a success. 35.3% were ready to go to any extent, to make the relationship succeed especially in the face of adversities. The minority opinion of 'doing my part' to make the marriage a success was recorded in the remaining cases.

The global degree of happiness centred mostly on being "happy" about the marital life among the 34 women interviewed (two refused to comment). Only three of the women were extremely/perfectly happy about their family life. There were six (17.6%) women who were having 'a little unhappiness' (the limit to which they were ready to accept any unhappiness) regarding the same. The unhappy women were mostly graduates and four of them were employed. The salary of these employed women did not reach that of their husbands, though they were more educated than their spouses. They were more likely to be closer in age to the husbands than the happier wives of the study. Four of the six also complained of physical violence from their husbands. Among the seven wives, who complained of physical violence, four regretted having been wedded at least on some occasions. Alcohol was associated with three of these physically abusive husbands.

Overall, the happiness was found to be dependant broadly on consensus on major domains of life as well as some of the social variables. From a cultural point of view, the analysis showed that happiness was the least dependent on matters of recreation, religious matters, and the demonstration of affection and overall philosophy of life.

Discussion

From a matrimonial point of view, a happy family can be built on some assumptions. The positive education gradient in a society like Kerala where the family is headed by a more educated man would ensure a higher satisfaction level among the females. Employed wives somehow derive significantly lower values in satisfaction compared to unemployed wives as per the present study. This could be due to a variety of confounding reasons as outlined below. One of the major reasons is the salary bias in favor of males. Four of the employed females who get lower salaries as compared to their husbands were rather unhappy about

their married lives. However, it also stands to argument the amount of work a woman can contribute to the job considering her dual conflicting role of an employee and a home-maker. An employer also may be biased towards a male employee in that he doesn't require any extra leaves such as maternity leaves once employed. Extremes of age at marriage for both men and women could be a factor in reduced satisfaction levels. Either they are unprepared for a planned married life or at the other extreme they land up in unhappy marriages because of advancing age and other factors.

Compatibility is a major issue among the couples which affect the mental status of a working person so much so that it may overwhelm his attitude to healthy working practices. The scales scores showed a low range of values which can be expected in a conservative society such as Kerala. The consensus subscale among the women studied, reached only a pooled mean of 25.1 (6.0) out of a worst possible score of 56. The overall score in satisfaction subscale was just 13.41 (2.55) out of a worst possible score of 36. Overall cohesion score is 8.94 (3.98) out of a maximum possible 16. Other studies in the field of matrimonial happiness, often show two-thirds to four-fifths showing happiness; one-fifth to one-third being pretty happy, and under one-tenth not too happy or unhappy in their marriages³. Our findings are not too far away from these estimates.

The incompatibilities which showed up between happier and not-so-happy couples were not so much in terms of material happiness (even though families without a vehicle were a bit more common among less happy families) but more in terms of perceptive differences. More leisure time interests and activities, shared household tasks and matters of recreation would result in the amount of time spent together or with friends and relatives. More attention

paid to the daily life or routines of the women and also their career decisions could foster better spousal relations. Common interests could be fostered. Working together for a gainful project for improving the financial status of the families could in turn increase sharing of ideas through calm discussions and this could become the main agenda of a happy family life. The present study was an attempt of a family health organization along with the welfare department of a major industrial manufacturing unit in Kerala to reach out to the women counterparts of the working men. The attempt is on to involve the spouses in the dyadic assessment such that the process could be completely conceptualized in line with modern concepts⁴.

Acknowledgement

We thank the authorities of the factory for agreeing for the organization of such a study. Members of Family Health Association of India (FHA) and the Family Welfare group of the factory who assisted in the conduct of the programme were extremely co-operative. We thank our Dean and the Head of our department for having permitted us to conduct the study.

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✦ ORIGINAL ARTICLE

Correlation of umbilical cord macroscopic structure with the foetal outcome in singleton pregnancies

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Abstract

Background: An altered structure of the umbilical cord can be associated with maternal and foetal complications like pre-eclampsia, diabetes, foetal growth retardation and demise. **Objectives:** To study the morphology of umbilical cord in normal, preterm and low birth weight babies. **Materials and methods:** This was a study of 308 placentae and umbilical cord specimens collected from the labour room of Pushpagiri Medical College Hospital over a period of one year. Of all the singleton deliveries considered, 232 were full term normal babies, 68 were of low birth weight and 08 were preterm babies. The type of insertion of the cord, amniotic web, knots and oedema, if any, and the number of umbilical vessels were examined. Samples from all three groups were subjected to macroscopic examination. **Results:** The umbilical cord thickness and the type and site of insertion of the cord showed wide variations. A significant number of cases showed amniotic webs, knots and nuchal cord. A sole case of single umbilical artery was also observed. **Conclusions:** Examination of umbilical cord would throw light into prenatal condition of the newborns, and at least in some cases provide information valuable for the future pregnancies.

Key words: Umbilical cord, Marginal insertion, Velamentous insertion, Amniotic web, Cord knots, Nuchal cord, Single umbilical artery.

Introduction

Umbilical cord complications from the most common cause of foetal demise in the third trimester¹. Traditionally, even in the most sophisticated hospital set-ups, the prenatal ultrasonographic assessment of the umbilical cord is limited to an observation of the number of vessels, and an evaluation of umbilical artery blood flow parameters. Morphological aspects of the cord like type of insertion, and the number of and the branching pattern of the umbilical vessels have been studied by the pathologists, specifically only in cases of unfavourable foetal outcome. Obviously thin or thick umbilical cords, anomalies in the number of blood vessels, a variation in the amount of Wharton's jelly, presence of knots, amniotic webs, etc. have been proved to result in unsatisfactory foetal growth and development. Hence an attempt is made here to correlate morphological features of umbilical cord with the pregnancy outcome in singleton pregnancies.

Objectives

To correlate the gross features of umbilical cords of normal, preterm and low birth weight babies (LBW).

Materials and Methods

The study was conducted in Pushpagiri Medical College hospital over a period of one year, after obtaining approval from the Institutional Ethics Committee (No. PIMS & RC/ Eth/ 809/ 007). Placentae and umbilical cords from 308 singleton deliveries were collected (both vaginal deliveries and caesarean sections included). The relevant antenatal history was noted, with maternal age, gravidity, gestational period (in days), and maternal hemoglobin levels. Post-natally the sex and weight of the foetuses, type of cord insertion, pattern of vessel arrangement as seen on the foetal surface, cord thickness, presence of abnormalities, if any, were looked for in all the 308 specimens. Intra uterine death, still birth and

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macerated babies were excluded. All the samples of umbilical cord were subjected to macroscopic examination in the Department of Anatomy, and the observations were analyzed.

Observation and Results

Out of 308 placentae and umbilical cords collected and examined, 232 were from full term normal babies (birth weight more than 2500 gm), 68 from full term LBW babies (less than 2500 gm), and 08 from premature babies (age below 224 days/ 32 weeks of gestation). In all the three groups the maternal age was between 18 to 42 years.

The thickness of the umbilical cord (measured using calipers) was correlated with the age and birth weight parameters, as shown in Table 1. The cord thickness appeared significantly higher in the preterm deliveries as compared to term babies, and lower in low birth weight babies as compared to normal babies.

Table 1: Cord thickness correlated with baby birth weight and gestational age

	Birth wt (gm)	Gestational age (days)	Cord thickness (cm)	Maternal age (yrs)
Normal (n=232)				
Mean	2.94	269.08	0.29	28.47
SD	0.33	8.26	0.13	4.54
LBW (n=68)				
Mean	2.12	258.60	0.25	27.71
SD	0.40	13.80	0.08	4.04
Preterm (n=8)				
Mean	1.17	207.13	0.32	30.38
SD	0.26	10.36	0.12	5.53

Insertion of umbilical cord was observed to be of various types (Table 2), the more common occurrence in our study being paramarginal and paracentral.

Table 2: Types of insertion of umbilical cord

	Central	Paracentral	Marginal	Paramaginal	Velamentous	Furcate
Normal (n=232)	9 (3.8%)	95 (41%)	6 (2.6%)	116 (50%)	5 (2.2%)	1 (0.4%)
LBW (n=68)	—	28 (41.2%)	3 (4.4%)	34 (50%)	2 (2.9%)	1 (1.5%)
Preterm (n=8)	—	2 (25%)	1 (12.5%)	5 (62.5%)	—	—

We observed battle dore insertion of unmbilical cord (Fig. 1) in six (2.6%) cases in normal group, three (4.4%) cases in the LBW group and one case (12.5%) in the preterm group.

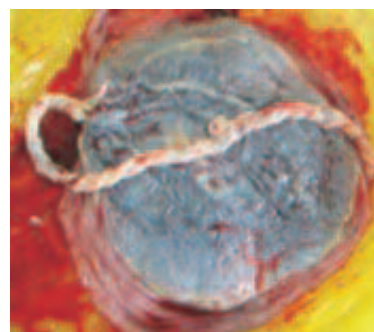


Fig. 1: Marginal insertion of cord, with a false knot

We found five cases (2.2%) of velamentous insertion of the cord in the normal group and two cases (2.9%) in the LBW group (Fig. 2). Here the umbilical cord gets inserted on the chorioamniotic membranes rather than on the placental mass. A variable segment of the umbilical vessels runs between the amnion and the chorion, losing the protection of the Wharton's jelly.

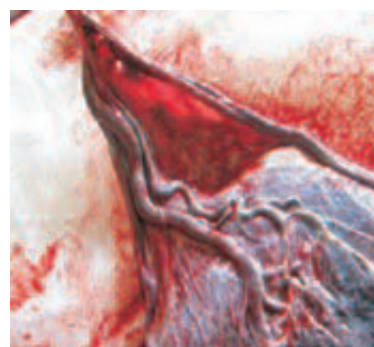


Fig. 2: Velamentous insertion of cord

Each umbilical artery divides into eight or more chorionic plate arteries, which perforate the placental tissue and form the basis for determining the number of the cotyledons in the placenta. The subdivisions of the umbilical vessels also appear variable in different specimens, and have been classified (Fig. 3) into disperse, magistral and mixed types.

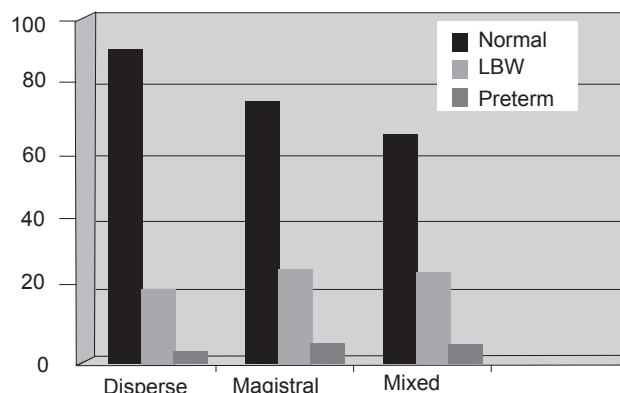


Fig. 2: Pattern of arrangement of umbilical vessels on foetal surface of placenta

A number of other cord anomalies were observed, such as amniotic webs, true knots, false knots and cord oedema (Table 3).

Table 3: Incidence of cord anomalies in the various study groups

	Amniotic web	False knots	Marginal	True knots	Cord oedema	Nuchal cord
Normal (n=232)	20 (8.6%)	30 (12.9%)	6 (2.6%)	1 (0.4%)	3 (1.4%)	5 (2.16%)
LBW (n=68)	11 (16.2%)	13 (19%)	3 (4.4%)	—	—	1 (1.5%)
Preterm (n=8)	1 (12.5%)	1 (12.5%)	1 (12.5%)	—	—	—

An amniotic web is a fold of amniotic membrane enclosing the yolk stalk and extending from the point of insertion of the umbilical cord to the yolk sac (Fig. 4a). In the present study webs were observed in all three groups.

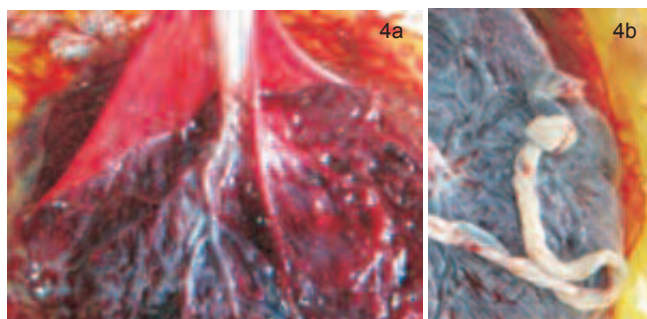


Fig. 4a: Amniotic web enclosing the cord 4b: a true knot

False knots in the umbilical cord are caused by the umbilical vein twisting around the artery, leading to localized thickening of Wharton's jelly (Fig. 1). True knots (Fig. 4b) are less frequent, and pose serious risk to the life of the baby.

In the present study, cord oedema (Fig. 5) was found only in three cases (1.2%) of the normal group.



Fig. 5: Umbilical cord oedema

Cord around neck was found in five cases (2.16%) of the normal group and one case (1.5%) in LBW group.

On seeing the cross section of the umbilical

cord, a single umbilical artery (Fig. 6) was observed in a case with normal pregnancy outcome. The umbilical vein was normally present, with a thinner wall and wider lumen.

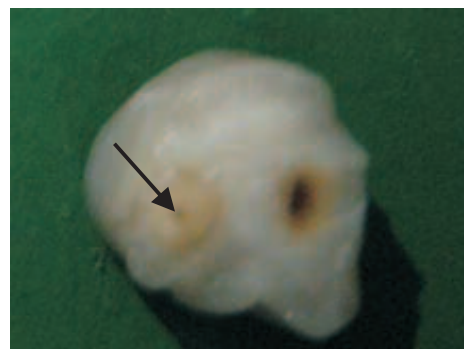


Fig. 6: Cord showing single umbilical artery (arrow)

Discussion

The embryology of the umbilical cord begins as early as the third week with the formation of the umbilical arteries as branches of the paired dorsal aortae. It is made up of the body stalk made of extraembryonic mesoderm, the omphalomesenteric duct, the secondary yolk sac, the extra-embryonic allantois and the umbilical arteries and veins.

During the second month of gestation the right umbilical vein regresses, resulting in two umbilical arteries and a single left umbilical vein. The paired arteries become branches of the internal iliac arteries after growth and differentiation. These vessels carry deoxygenated blood to the placenta. The persistent left umbilical vein carries oxygenated blood to the foetus, joining the left portal vein and ductus venosus, eventually entering the inferior vena cava and right atrium. Umbilical cord growth normally continues until the end of the second trimester. An average umbilical cord is roughly 55 cm long, with a diameter of 1-2 cm and 11 helices.

Many studies indicate that most cords are inserted centrally (3% to 28%) or nearly centrally (62% to 91%)². In marginal insertion of the cord (battledore placenta occurring in five to seven percent) the shortest distance between the cord insertion and placental edge is within two cm (including 0.0 cm)³. Such a cord may be more susceptible to vessel rupture or compression and have been associated with foetal growth retardation, still birth, and neonatal death. But this occurs only when placental maturation is unevenly accelerated because of low uteroplacental blood flow.

Stephen et al.,⁴ reported one to two percent incidence of velamentous insertion of umbilical cord. Preterm birth associated with the condition reportedly accounts for three per 1000 births and has statistically significant correlation with advanced maternal age⁴. But in the present study we never found any such variable insertion in the preterm group. It has been found in

one percent of stillbirths, usually in multifoetal pregnancies⁵. The most significant clinical problem arising from this variation is vasa previa, a dangerous condition in which the umbilical vessels traverse the fetal membranes in the lower uterine segment below the presenting part. Vasa previa occurs in six percent of singleton pregnancies with a velamentous insertion. The unprotected umbilical vessels may rupture at any time during pregnancy, causing foetal exsanguination and death.

Traditionally the chorionic vasculature is classified into disperse, magistral and mixed types⁶. dispersed type of chronic vasculature occurs with near centered insertion of the cord; the two arteries divide dichotomously several times into a number of smaller vessels, rapidly diminishing in calibre. The magistral type of chorionic vasculature is associated with fewer penetrating arteries, fewer cotyledons, and abnormal umbilical artery blood flow⁷. In the present study the mixed type of vessel arrangement pattern were more in the preterm and LBW groups.

Nordenvall and team⁸ correlated marginal cord insertion to magistral or mixed allanto-chorial vascular pattern. A paucity of lobes was associated with marginal cord insertion, pre-eclampsia, gestational age less than 38 weeks and small for gestational age babies⁹. They suggested that common denominator for abnormal configuration and marginal cord insertion is the paucity of lobes.

Amniotic webs may be loose or tight, binding the cord to the foetal surface. This would limit cord mobility and possibly predispose to subamniotic vessel haemorrhage⁴. Circulatory compromise and decreased in utero movement are thought to be associated findings.

Cord knots may have a transient stenosing effect on the umbilical vessels in utero, causing disturbances in blood flow and higher resistances seen on waveform Doppler studies⁹. True knots arise from foetal movements and are more likely to develop during early pregnancy, when relatively more amniotic fluid is present and greater foetal movement occurs. True knots are also associated with advanced maternal age, multiparity, and long umbilical cords. False knots have no known clinical significance. Various factors predisposing to cord knot formation have been described, including a long cord (more than 80 cm), polyhydramnios, and a small foetus^{10,11}.

In the presence of true knots foetal mortality is very high, with up to a fourfold increase in foetal loss, presumably because of compression of the cord vessels when the knot tightens. Modern examination techniques (Colour doppler sonography) are able to detect such cord knots and hence it is necessary in all multiple pregnancies to examine precisely for real knots of umbilical cord in regular intervals¹². If a knot is present, the pregnant woman had to be supervised by

cardiotocography (CTG), to react immediately at the first signs of hypoxia.

Cord oedema is found in 10% of deliveries¹³. It is defined as visible oedema in a cord with a minimal cross sectional area of 1.3 sq cm. It is seen more frequently in certain maternal complications like abruptio placentae, diabetes, macerated intrauterine death and in foetal conditions like prematurity, rhesus isoimmunization, respiratory distress syndrome (RDS) and transient respiratory distress (TRD)¹⁴. There is a higher incidence in infants delivered by caesarean section. There is no significant association between cord oedema and either foetal distress or neonatal asphyxia nor is there any correlation with maternal hypertension or oedema.

Nuchal cord is usually caused by movement of the foetus through a loop of cord. One loop around the neck occurs in approximately 20% of cases¹⁵, and multiple loops occur in up to 5% of pregnancies¹⁶. Nuchal cord can be detected using color Doppler ultrasound, with a sensitivity of over ninety percent¹⁷.

Single umbilical artery occurs in less than one percent of cords in singleton pregnancies and five percent of cords in at least one twin. It also occurs more often in foetal demise than in live births¹⁸. The incidence can be overestimated if the portion close to the placenta is examined, because the arteries may fuse close to the placenta¹⁹. It is believed to be caused by atrophy of a previously normal artery, presence of the original artery of the body stalk, or agenesis of one of the umbilical arteries.

The vessels in the cord are clearly identifiable with ultrasonography, the vein being usually larger than the arteries, as seen in our case (Fig. 6). The condition may be diagnosed prenatally with the finding of only two vessels in the cord, or a vessel seen on only one side of the foetal bladder. With single umbilical arteries, a five to twenty percent perinatal mortality rate has been reported²⁰⁻²² including foetuses with severe congenital anomalies and chromosomal defects. Two thirds of deaths occur before birth, and of the neonates who die post-natally, most have associated congenital abnormalities.

Conclusion

The umbilical cord is the only, critical connection of the foetus with the placenta, through which it meets all its life requirements. Proper umbilical cord function is hence essential for the prenatal growth and development, and also the perinatal outcome. Therefore, inspection of the umbilical cord should form an integral part of the care during the first minutes of life. Any abnormality within the cord structure should be noted meticulously and proper ultrasonographic examination of both placenta and umbilical cord should be made mandatory in subsequent pregnancies. Also, more studies are required in this field, so as to help improve the pregnancy outcome.

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✪ CASE SERIES ARTICLE

Anomalous arteries in the upper limbs of a cadaver

Part III: A unilateral prominent median artery completing the superficial palmar arch

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Abstract

We observed a number of very unusual variations in the upper limb arteries, differing on the right and left sides, in a male cadaver in the dissection hall. The variations observed were many and are hence presented as a series, in four parts. The first part reported the variations in the axillary and brachial arteries, with superficial brachioradial arteries seen bilaterally, and the second part, the branches of the brachial arteries on the two sides specially focusing on the double profunda brachii and an extremely unusual cubital anastomosis. This third part is dealing with variations in the arteries of the left forearm and hand, with a prominent arteria nervi mediana taking part in the formation of the superficial palmar arch. The clinical significance of all these anomalies will be considered at length in the last part of this series article.

In the left cubital fossa the ulnar artery gave off a very prominent branch, persistent median artery of the palmar type. It was deep to pronator teres, and the fleshy bellies of palmaris longus and flexor digitorum superficialis, but anterior to the anterior interosseous artery and nerve. It entered the palm of hand passing through the carpal tunnel along with the median nerve.

The median artery anastomosed medially with the ulnar artery in the palm, thus completing the superficial palmar arch. The superficial brachioradial artery did not play any role in the formation of the arch, and its superficial palmar branch ended supplying the thenar muscles. Branches arising from the median artery were also to accompany the lateral two common palmar digital branches of the median nerve.

Key Words: Radial artery, Ulnar artery, Median artery, Superficial palmar arch, Superficial brachioradial artery.

Introduction

The median nerve normally enters the forearm passing between the two heads of pronator teres, and runs distally on the deep surface of the muscle belly of flexor digitorum superficialis (FDS). In the distal forearm it passes between and deep to flexor carpi radialis (FCR) and palmaris longus (PL) into the carpal tunnel.

A prominent median artery is believed to be due to the persistence of the embryonic pattern of upper limb arteries. It continues to maintain the superficial palmar network, though the radial and ulnar arteries would be simultaneously developing as deep

arteries. It could persist as a variant even after the development of the radial and ulnar arteries in the hand has been completed.

The superficial palmar arch is usually formed as a continuation of ulnar artery, and is completed on the lateral aspect by the superficial palmar branch of radial artery/ arteria radialis indicis/ princeps pollicis artery. Either the anterior interosseous artery or the median artery may go into the formation of the superficial palmar arch, if the ulnar or radial artery is deficient or absent.

In the present case, we observed these two arterial variations

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Part II of this Series Article appeared in PMJ Vol.2, No.2(January-June 2011) - Pg. 134-138

in the right forearm and hand, while the left side showed normal formation of the palmar arterial arches.

Materials and methods

The arterial variations were observed during the routine student dissection of a male cadaver in the Department of Anatomy, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala. The branches of the arteries of upper limb were traced carefully up to their termination; the arteries were coloured, and photographs were taken. A specific correlation of the observed variations with embryonic development of the arteries of the upper limb has also been made.

Observations

In the present study, out of the eighty upper limb specimens that had been dissected in the past few years, only one case of such wide variations in the branching pattern of upper limb arteries was observed, which in peculiar, differed on the two sides. The median artery and its contribution to the formation of the superficial palmar arch were observed only on the left side.

A. Persistent median artery

As discussed in the first part of this series article, the left brachial artery was seen to give off the brachioradial artery eight centimeters distal to the lower border of teres major. The main trunk of the brachial artery continued down into the forearm as the ulnar artery. In the cubital fossa the left ulnar artery was seen to give off a very prominent branch, the median artery at the angle between the origin of anterior interosseous artery and the ulnar artery (Fig. 10). It was lying deep to the pronator teres, and the muscular bellies of PL and FDS, but anterior to the anterior interosseous artery and nerve. It entered the palm of hand passing deep to the flexor retinaculum along with the median nerve, and hence can be said to be of the *palmar type*. The artery was not seen to give off significant muscular branches in the forearm.

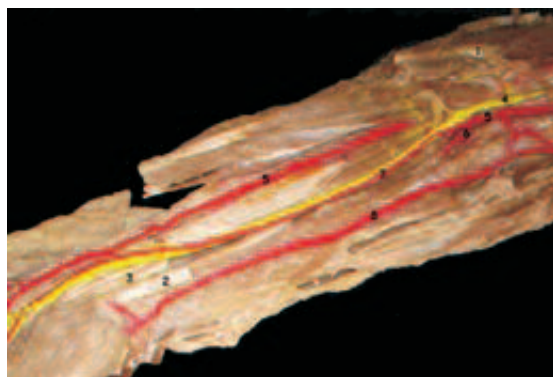


Fig. 10: Prominent median artery, branch of ulnar artery

1. Pronator teres
2. FCR
3. FDS
4. Median nerve
5. Ulnar artery
6. Common interosseous
7. Median artery
8. Superficial brachioradial artery

B. Variant formation of Superficial palmar arch

The median artery, reaching the palm through the carpal tunnel, anastomosed medially with the ulnar artery deep to the palmar aponeurosis, thus completing the superficial palmar arch (Fig. 11). Branches arising from the median artery were seen to accompany the lateral two common palmar digital branches of median nerve.

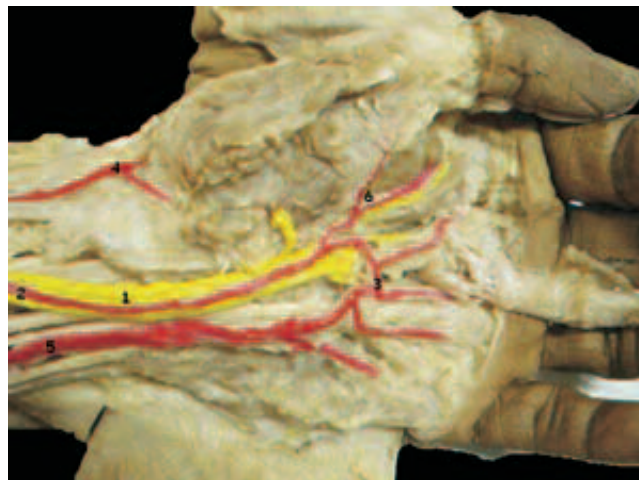


Fig. 11: Median artery traversing carpal tunnel and completing superficial palmar arch

1. Median nerve
2. Median artery
3. Superficial palmar arch
4. Superficial palmar branch of brachioradial
5. Ulnar artery
6. Branches of median artery accompanying digital branches of median nerve

The superficial palmar branch of the superficial brachioradial artery was seen to end on the thenar muscles. It did not go into the formation of the arch. The brachioradial artery was seen to get diminished in size as it curved dorsally over the lateral side of the wrist and passed into the palm through the first interosseous space. It completed the deep palmar arch on its lateral side, as the continuation of the radial artery does normally.

Discussion

The small artery that normally accompanies the median nerve usually arises from the common interosseous artery or the anterior interosseous, and ends after a short course in the forearm itself, supplying the nerve. The artery may also supply some of the flexors of forearm. As has already been discussed in the previous part of this series, the present anomaly could be due to an unusual path in primitive vascular plexus, persistence of vessels normally obliterated, disappearance of vessels normally retained, and incomplete development and absorption of some parts.

Rodríguez-Niedenführ *et al.*,²⁶ observed that median artery can persist in adult life in two different patterns, palmar and antebrachial, based on their area of supply. The palmar type represents the embryonic pattern, is large, long and reaches the palm. The

antebrachial type represents a partial regression of the embryonic artery, and is slender, short and terminates before reaching the wrist. The palmar pattern reportedly had an incidence of 20% and the antebrachial pattern, an incidence of 76%. The palmar type occurred more frequently unilaterally than bilaterally (4:1), and was a little more frequent on the right side compared to the left. In our case also the condition was unilateral, and was of the palmar type.

In the present case the median artery took origin from the angle between the ulnar artery and the common interosseous artery. The origin is reported to be variable in the two patterns of the artery²⁶. The palmar type most frequently arose from the caudal angle between the ulnar artery and its common interosseous trunk (59%) and the antebrachial pattern most frequently from anterior interosseous artery (55%). Other origins, for both patterns, were from the ulnar artery or from the common interosseous trunk. The median artery in the antebrachial pattern terminated in the upper third (74% cases) or in the distal third of the forearm (26%). However, the palmar pattern ended as the first, second or both common digital arteries (65%) or joined the superficial palmar arch (35% cases). In the present study the palmar type of median artery ended by joining the superficial palmar arch.

The median artery could pass down the forearm passing either anterior (67%) or posterior (25%) to the anterior interosseous nerve²⁶. In the present study the median artery passed anterior to the nerve. The artery reportedly pierced the median nerve in the upper third of the forearm in 41% of cases with the palmar pattern and in none of the antebrachial pattern cases²⁸. In our case the artery joined the nerve soon after its origin, but was lying over it, enclosed in a common facial sheath. It did not pierce the substance of the nerve anywhere during its distal course. So also, the median artery in our case was not seen to give rise to any muscular branch to the forearm muscles.

Based on a study of 23 cases with median artery, Rodríguez-Baeza *et al.*,²⁷ postulated that the persistence of the median artery is independent of the presence or absence of any other variation in the arterial pattern²⁷. This conforms to the observations in our case, where in spite of multiple arterial variations at different levels of both limbs, the median artery variation and its contribution to the palmar arch was unilateral.

The origin, course, supply and termination of the median artery have been studied and reported widely²⁶⁻³². The anterior interosseous, common interosseous or ulnar arteries or the caudal angle between the ulnar and common interosseous arteries have been described as the common origins of the median artery^{27,33}. Many authors have described the same course of the median artery in the forearm, between the anterior surface of the median nerve and the deep surface of the FDS. In some rare cases (1%)^{30,34} a median artery originating at the elbow and

coursing anterior to the antebrachial flexor muscles, or anterior to the flexor retinaculum has been reported^{30,31} and then it is called 'superficial median' artery, with an incidence of approximately one percent³⁴. Though rarely, the median artery can arise from the radial artery as well.

A persistent median artery of the palmar type, terminating in the hand as the princeps pollicis and radialis indicis arteries has been reported³⁵; but in our case the corresponding branches from the median artery accompanying the nerve branches were quite small. A rare case of superficial median artery having a high brachial origin and superficial course, a 'superficial brachio-median artery', has been reported to arise from the initial part of the brachial artery³⁶. In the arm it followed a superficial course, passed beneath the bicipital aponeurosis at the elbow and deep to muscular bellies of PL and FDS in the forearm.

Two rare arterial variations in a single upper limb, a brachioradial artery and a superficial median artery arising from it, were found during the routine anatomy dissection³⁷.

Eva Maria Gassner *et al.*,³⁸ conducted an ultrasonographic study in asymptomatic volunteers, to find a persistent median artery in 13 cases (26%), of which ten were unilateral and three were bilateral. In 63% cases, the persistent median artery was associated with high division of the median nerve or a bifid nerve configuration in the carpal tunnel. In our case no associated anomaly was observed in the median nerve. The mean diameter of the artery was 1.1 mm (range, 0.5 to 1.7 mm)³⁸. According to Olave and associates³⁹ the calibre of the palmar type of median artery ranged from 0.7 to 2.7 mm, mean (SD) 1.6 (0.5) mm.

Persistence of the median artery along with anomalies of the median nerve and of the palmar circulation has been reported by Sanudo and team⁴⁰. This median nerve formed a ring enclosing the median artery, which extended into the hand, providing the second common palmar digital artery and the radialis indicis. It anastomosed with the radial artery, and the superficial palmar arch remained incomplete.

In cases of palmar type of median artery, the superficial palmar arch has almost always been reported to receive a contribution from it⁴¹. The percentages of hands in which the median artery made a contribution to the superficial palmar arch were reported to be 2.2% by Janevskis⁴². According to Johnson and team⁴³, about one third of the arches are formed by the ulnar artery alone; a further third is completed by the superficial palmar branch of the radial artery; and a third by the arteria radialis indicis, often a branch of the arteria princeps pollicis, or by the median artery.

Natsis K *et al.*,⁴⁴ found two unilateral cases of median artery originating from the ulnar artery.

In both cases the it passed through the carpal tunnel, and anastomosed with the ulnar artery, forming a medio-ulnar type of superficial palmar arch. The palmar type of median artery arising from the common interosseous artery and forming superficial palmar arch with superficial branch of ulnar artery has been reported⁴⁵. Similar to our case, the superficial palmar branch of the radial artery terminated in the thenar muscles.

A case of persistent median artery with double superficial palmar arch has been reported, with the proximal one forming a complete arch situated topographically where a superficial palmar arch is usually described, and the distal incomplete arch, which provided the interdigital branches normally given off by the superficial palmar arch⁴⁶.

Coleman and Anson⁴⁷ (1961) classified the superficial palmar arch into 2 groups.

Group 1: Complete arch (78.5%) - further divided into five types:

- Type A : Classical arch formed by superficial palmar branch of radial artery and ulnar artery (34.5%).
- Type B : Formed entirely by ulnar artery (37%).
- Type C : Mediano-ulnar arch composed of ulnar and median arteries (3.8%)
- Type D : Radio - mediano - ulnar arch in which three vessels enter into formation of the arch (1.2%).
- Type E : Arch initiated by ulnar artery and completed by a large sized vessel derived from deep arch (2%).

Group II: Incomplete arch (21.5%) - contributing arteries don't anastomose; ulnar artery fails to reach thumb and index finger. It can be further divided into 4 types:

- Type A: Superficial palmar branch of radial, and ulnar arteries supply palm and fingers, but fail to anastomose (3.2%).
- Type B: Ulnar artery alone forms the arch but it is incomplete - it does not supply thumb and index finger (13.4%).
- Type C: Receives contribution from both median and ulnar arteries, without anastomosis (3.8%).
- Type D: Radial, median and ulnar arteries contribute to the arch but don't anastomose (1.1%).

Bilge and team⁴⁸ in a morphometric study on the arches found complete arches in 43/50 hands and incomplete arches in 7/50 hands.

A complex variation in the pattern of blood supply to the palm of the hand was encountered⁴⁹, with an incomplete superficial palmar arch, and superficial palmar branch of radial artery coursing superficial to the thenar muscles supplying adjacent sides of the thumb and index finger.

A cadaveric study by Nayak³² concluded that the median-ulnar type was the most common type of the arch when the median artery was encountered as a source of superficial arterial arcade of the hand, followed by the radial-median-ulnar type.

In a study on forty-five limbs from cadavers by Gellman and colleagues⁵⁰, complete arches were seen in 84.4% cases. In the most common type, the arch was formed between superficial volar branch of the radial artery and ulnar artery (35.5% of specimens). In 31.1%, it was formed entirely of ulnar artery. Incomplete arches were seen in 15.5% of specimens. In 11.1%, ulnar artery forms the arch but does not contribute to the blood supply of thumb and index finger.

In a rare case report both the vessels forming the arch, the superficial branch of ulnar and the median artery were observed to originate from the ulnar artery proper⁵¹. In such a case if ulnar artery has to be ligated for some reason, blood flow in the arch along both these routes gets completely cut off. Then the only source of blood supply is through the radial artery, the deep palmar arch and then through perforating arteries.

Two rare cases of unilateral median artery (4%) were detected⁵², the first one pierced both the median nerve and the medial branch of the anterior interosseous nerve, and formed a median-ulnar pattern of superficial palmar arch. The second case was a high origin of the radial artery which trifurcated into median, common interosseous, and ulnar arteries. The median artery formed a radial-median-ulnar pattern of the arch.

Superficial palmar arch formed solely by superficial branch of ulnar artery without any contribution from the radial artery or median artery has also been reported^{53,54}. The superficial palmar branch of the radial artery terminated in the thenar muscles. The superficial branch of the ulnar artery gave origin to four common palmar digital arteries to supply the digits. The first common palmar digital artery divided into radialis indicis and princeps pollicis arteries.

Three very rare cases of the superficial arch formation were described by Bataineh and associates⁵⁵. In the first case, superficial branch of the radial artery passed superficial to thenar muscles, and had a diameter greater than ulnar artery; it gave the princeps pollicis and radialis indicis arteries. In the second case, the arch was formed mostly by ulnar artery and was completed by a small branch of deep branch of radial artery. In the third case, incomplete arch was formed by median artery which gave off princeps pollicis and radialis indicis arteries, and ulnar artery supplied the rest of the hand except the ulnar side of middle finger and second web space, which were supplied by deep palmar arch.

Developmental correlation

In the upper limb bud, the developing single arterial trunk, the subclavian artery, continues distally forming the axillary and brachial arteries and terminates

as a deep arterial plexus in the developing hand. The persistent part of this artery in the forearm and hand normally forms the anterior interosseous artery and the deep palmar arch.

A smaller branch of this axis artery normally accompanies the median nerve into the hand, ending in a superficial capillary plexus. Later in life the developing ulnar artery links up with the superficial palmar capillary plexus, and from this plexus the superficial palmar arch is derived. Commonly the median artery loses this distal connection and gets reduced to a minute vessel.

Rodríguez-Niedenführ *et al.*,²⁶ proposed that the palmar pattern of the median artery is a remnant of the embryonic model, while the antebrachial pattern represents its partial regression. In this way it could be suggested that the involution of the median artery follows a distal to proximal direction, and in a later stage of its regression it would be present as a branch supplying the forearm flexor muscles, as this was a common characteristic in both patterns²⁶.

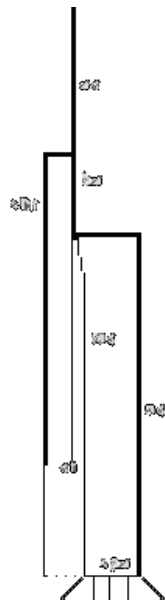


Fig. 12: Median artery forming superficial palmar arch: developmental correlation

aa: axillary artery
 sbr: superficial brachioradial
 ai: anterior interosseous
 ba: brachial artery
 ma: median artery
 spa: superficial palmar arch

In the present case the median artery arose from the ulnar artery at its junction with the anterior interosseous artery. This anterior interosseous ended proximal to the wrist, as usual. The median artery extending along the nerve reached the palm, where it anastomosed with the ulnar artery. This arrangement probably compensated for the deficient blood supply on the lateral side of forearm and hand, since the superficial brachioradial artery was diminishing rapidly in size in the distal part of the left upper limb. The same deficiency would explain the origin of the two lateral palmar digital branches from the median artery.

Conclusion

The arteria nervi mediana, palmar type, arose as a branch from the ulnar artery at its angle with the anterior interosseous artery. The artery lay within a facial sheath along with the median nerve and entered the palm of hand deep to the flexor retinaculum, and contributed to the formation of a complete superficial palmar arch. The deep palmar arch did not show any marked variation. On correlating with the stages of development of the upper limb vessels, it can be inferred that a partial regression of the axis artery has contributed to the development of such a variant pattern of arteries of forearm and hand.

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✦ CASE REPORT

Rare Cardiac tumour unraveled by skin bleeds - A case report

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Abstract

Primary cardiac tumours are extremely rare diagnostic entities. Their clinical manifestations are very variable and mimic both cardiac and non-cardiac diseases. Hence clinical diagnosis remains as a challenge for any physician. So also tumours resulting in paraneoplastic syndromes are widely recognized and well understood. However such tumours as well as their paraneoplastic manifestations are not very common in paediatric practice. We report this extremely rare case in an eleven year old boy who presented with paraneoplastic manifestations in the form of ecchymotic swellings and altered coagulation parameters. Later it was proved to be due to a life threatening right ventricular outflow tract (RVOT) mass. A high index of suspicion is essential in the timely diagnosis and management of such patients.

Keywords: Right ventricular outflow tract mass, Leiomyosarcoma, Cardiac tumour, Consumptive coagulopathy.

Introduction

It is well known that even small benign cardiac tumours¹ may have devastating clinical consequences when they are in a critical location. Recent advances in diagnostic imaging modalities and cardiac surgical procedures have altered the clinical outcome of cardiac tumours. No longer are they diagnosed at autopsy, but have emerged as a surgically curable form of heart disease. Cardiac tumours have no typical clinical manifestations. They have been described as great masqueraders. Apart from cardiac symptoms and embolic episodes, systemic manifestations described include fever, malaise, weight loss, polymyositis, hepatic dysfunction and Raynaud's phenomenon due to a possible cytokine release. A chronic disseminated intravascular coagulation as the manifestation of cardiac neoplasm, as reported in this case, is virtually unheard of.

Clinical presentation

A eleven year old boy, born of a non-consanguineous marriage presented with spontaneous painful nodular swellings over the upper arms,

chest and trunk, of one month duration. On careful examination they were found to be ecchymoses. There was no past history of bleeding tendencies, or a history of bleeding disorder in the family. His immunizations had been regular. He was well nourished and had normal growth parameters. All his systems were within normal limits, and there was no organomegaly. He was investigated in detail to rule out a haematological or connective tissue disorder. Initial investigations showed Hb 10.2 gm%, TC 8400 cells/ cmm, DC P₅₀ L₄₄ E₆, ESR 7mm/ 1st hour, absolute eosinophil count 480 cells/ cmm, and platelet count of 1.5 lakhs/ cmm. His antinuclear antibody, mantoux test and thyroid profile were negative. During this period he also had two episodes of mild epistaxis, a few days apart.

His haematological work-up showed bleeding time six minutes and thirty seconds (mildly prolonged), prothrombin time 16.7 seconds (mildly prolonged which got corrected with normal plasma), an activated partial thromboplastin time (aPTT) 45.4 seconds (prolonged, which also corrected with normal plasma), plasma fibrinogen level 84.6 mg/dl (low, both

by functional and immunological assays), D-dimer level 4521ng/ml (markedly elevated), factor VIII 51.6% (low normal), and normal levels of factors IX, XI, XII, VII, X, V, and II. An underlying consumptive coagulopathy was identified and hence the boy was investigated to find out the cause by doing a chest skiagram, ultrasonography of the abdomen and a skeletal survey, which were all normal. The child was advised follow-up with monthly monitoring of platelet count and fibrinogen levels, but he was lost from follow-up after two months.

Nine months later he presented again with giddiness and effort dyspnoea and cardiovascular examination now revealed a grade III pansystolic murmur. Detailed evaluation by echocardiography revealed a large mass in the RVOT with increased right ventricular pressure. His PT and aPTT were still prolonged and INR was 1.70. Because of impending obstructive cardiac failure, emergency cardiac surgery was done. A non-pedunculated, spherical, RVOT mass of 6.5 × 6.5 cm size was seen adherent to the RV parietal muscle (Fig. 1). It was seen extending into the pulmonary annulus, and was excised.



Fig. 1: Surgical resection of the RVOT mass



Fig. 2: Circumscribed oval whitish mass with grey-white faintly lobulated cut surface having a myxoid appearance

The semisolid mass (Fig. 2) was subjected to histopathological examination, which revealed a well-encapsulated cellular spindle cell neoplasm (Fig. 3).

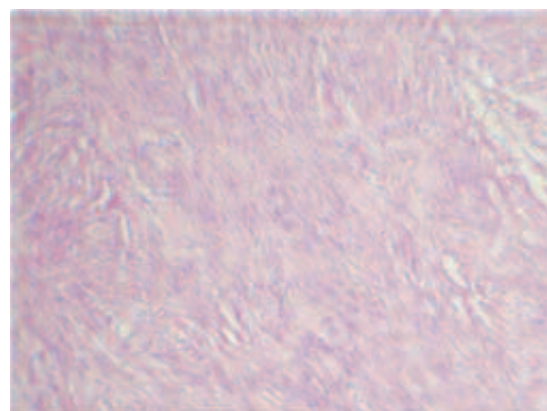


Fig. 3: Cellular neoplasm composed of interlacing bundles of spindle cells having eosinophilic cytoplasm and elongated cigar-shaped nuclei (H and E x 100X)

The possibility of a benign peripheral nerve sheath tumour was considered initially. Because of the rarity of the lesion, the specimen was subjected to further examination at a higher centre outside the country also.

Post-surgery the boy was stable and was discharged home in a week's time. His PT, aPTT, INR and fibrinogen levels became normal within ten days. After an eighteen month follow up the child remains healthy with normal haematological parameters and echocardiogram.

Discussion

In 1936, the first successful removal of a neoplasm of the heart was performed. In 1952, angiography was first used for the diagnosis of heart tumours. In 1955, bypass was used for the first time to excise an intracavitary tumour².

Primary cardiac tumours are rare, and more so in children. The majority are benign and non-invasive, although they may have significant haemodynamic consequences depending on their location³. More than 50% of primary tumours in the heart are cardiac myxomas. In most childhood cardiac tumours no treatment is necessary unless the tumour severely obstructs blood flow or causes intractable arrhythmias; most only require close follow-up care⁴.

A review of 1029 patients showed that 83% of myxomas were located in the left atrium and 12.7% in the right atrium⁵. But in children the most frequent type is rhabdomyoma, usually multiple and intraventricular. By far intraventricular tumours are reported were rarely and the possibility of a malignant tumour is highly likely⁶.

Even benign tumours in this area (RVOT) prove to be inoperable either by their location or due to technical reasons. This child had features suggestive of systemic effects due to vasoactive substances secreted by the tumour. Both these reasons make this case

special and extremely dangerous to be operated upon. Successful removal of the tumour was achieved without any structural or functional damage. The systemic effects of deranged coagulation profile also got corrected after tumour removal.

Two-dimensional echocardiography and Doppler are the best way to locate the tumour and evaluate the valvular function⁷. The histopathological examination of the mass was crucial and hence subjected to scrutiny by experts within the institute and later at Harvard Medical School by renowned pathologists who opined through their personal communication that it is one of the most unusual tumours in the RVOT. The lesional spindle cells were strongly and diffusely positive for smooth muscle actin, desmin and caldesmon, consisted with true smooth muscle differentiation. They reiterated that intracardiac leiomyomas are virtually unheard of, and that this lesion has to be best regarded as a low grade Leiomyosarcoma. This marks the importance of timely removal of this lesion saving the life of the boy who still needs careful follow up.

Some cardiac tumours including myxomas are known to secrete vasoactive substances but the exact identification of such substances are not always feasible but often disappear after resection of the tumour^{3,8}. The clinical correlation in this case is sufficient to document such an outcome.

Conclusion

Primary cardiac tumours are rare in children, and are mostly benign. Through histopathological examination did not reveal obvious sarcomatous changes, the case is considered as leiomyosarcoma, as leiomyoma in RVOT has not been reported in the available literature. Such a case presenting with

paraneoplastic features needs a high index of suspicion for prompt diagnosis and management.

Acknowledgement

Dr. Deepak Davidson and Dr. Cherian Koshy, Department of Cardiology, Dr. M. O. Annamma, Professor & HOD, Department of Pathology, for their valuable contribution in diagnosing and managing this case, are hereby gratefully acknowledged.

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✦ CASE REPORT

Spinal epidural abscess as a rare cause of acute paraplegia in adults, with a review of literature

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Abstract

Spinal epidural abscess has an incidence of approximately one to two cases per 10,000 hospital-admissions. In spite of the advances in Neuroimaging and Neurosurgical care, the incidence of the condition, especially with an unfavourable outcome like paraplegia, is on the rise. Only increased awareness, early diagnosis and swift management would improve the chances of good prognosis in such patients. Some of the patients may present clinically with a sole back pain. MRI is the choice of imaging study, which reveals spinal epidural abscess accurately and clearly. Surgical decompression and drainage of the epidural abscess combined with specific antibiotics is the treatment of choice. Here we are discussing the clinical presentation, management and follow up of a middle aged, diabetic, male patient diagnosed to have a typical spinal epidural abscess leading to paraplegia, treated promptly to have a successful outcome.

Keywords: Spinal epidural abscess, Paraplegia, Decompressive laminectomy, Neurosurgical emergency.

Introduction

A spinal epidural abscess (SEA) is considered a neurosurgical emergency as the patients are liable to deteriorate neurologically, rapidly, and unpredictably. Threats to the spinal cord from such a lesion include compression (especially in the cervical region) and vascular compromise. Impingement upon the spinal cord usually produces sensory loss, motor dysfunction, and if untreated, paralysis, septicemia and death. The diagnosis is quite frequently delayed because the initial presentation could be an isolated back pain.

Clinical presentation

A 54 year old male presented in the outpatient department with fever, followed a week later by low back pain, giving a history of total three weeks. In the last two days he developed a rapidly progressive weakness of both lower limbs, with associated bladder and bowel involvement. He was not a known hypertensive, but had been a diabetic for the past five years.

Clinical examination: On examination

the patient was totally bedridden at the time of admission, with low grade fever. In the lower limbs the weakness was more severe in the proximal group of muscles. Deep tendon reflexes of the lower limbs were found to be exaggerated. Sensory loss was observed in the umbilical region.

Investigations: C reactive protein was 47.5, and ESR was 113 mm in the first hour.

Radiological examination: Plain X-ray of the thoracolumbar spine did not reveal any bony abnormality (Fig. 1).



Fig. 1: Normal X-ray spine of the thoracolumbar region

MRI studies: Neuroimaging showed a mixed density lesion on the dorsal aspect of spine (Fig. 2), involving the posterior epidural space, extending from the T₉ - T₁₁ levels.

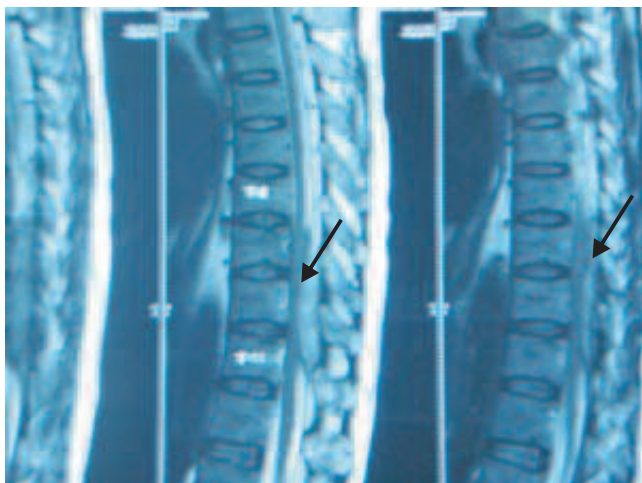


Fig. 2: MRI showing marked compression of spinal cord (arrow)

MRI contrast studies revealed a soft tissue mass, hypo-intense to the spinal cord (Fig. 3) and hyper-intense to the spinal cord (Fig. 4), as seen on T1- and T2-weighted images, respectively. With contrast MRI there was peripheral enhancement.

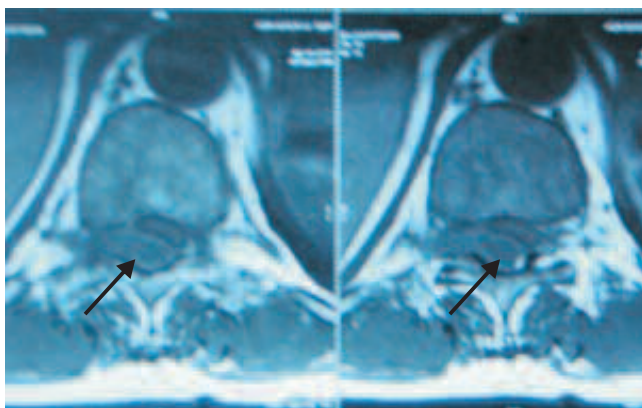


Fig. 3: T1W image with abscess compressing spinal cord (arrow)

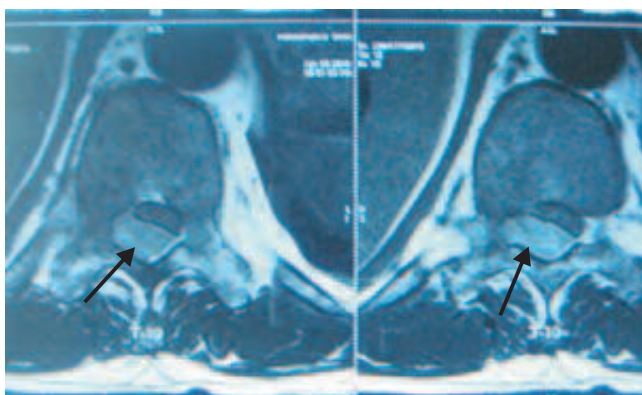


Fig. 4: T2W image showing the epidural abscess (arrow)

Differential diagnosis: The first possibility considered was an infective pathology, an epidural abscess; others included epidural haematoma, haematological disorders, angioliopma, epidural lipomatosis and secondary deposits. Investigations done to exclude these conditions were abdominal ultrasound, peripheral smear, acid phosphatase and serum electrophoresis, all of which failed to reveal any abnormality.

Surgical management: The patient underwent decompressive laminectomy from D₉₋₁₁. Operative findings included a well-circumscribed lesion in the posterior epidural space and thick granulation tissue replacing the epidural fat, along with about 10-15 ml of greenish yellow pus. Total excision of the lesion was done, and the tissue and debris were sent for pus culture and histopathological examination.

Culture and sensitivity studies revealed *Staph. aureus*, sensitive to linezolid, teicoplanin, vancomycin and septran.

Histopathology confirmed a chronic inflammatory lesion, but there was no evidence of tuberculosis.

Diagnosis: Spinal epidural abscess

Goals of post-operative management were eradication of the causative organism, improvement of the neurological status, pain relief and preservation of spinal stability.

Antibiotics given included anti-staphylococcal drugs (nafcillin + metronidazole + cefotaxime), given intravenously for six weeks. The neurological status improved by about 12 hours. Body temperature and leucocyte count came down by the fifth post operative day. He was discharged with good recovery of all neurological functions. In the third review after six months he had returned to near normal life.

Discussion

Spinal epidural abscess progressing to paraplegia is a difficult diagnosis to make in the initial stages, because it is practically such a rare condition that many physicians would never see a case during their professional life. Among all patients admitted to hospitals, the incidence is approximately one to two cases per 10,000¹.

In the beginning of the twentieth century, almost all patients with SEA used to die, in spite of all available treatment². Parallel to the improvements in the early diagnosis and decreased mortality rate, today more patients experience complete recovery from SEA². Only increased awareness and swift management of spinal epidural abscess will prevent a possibly disastrous outcome of paraplegia and subsequent morbidity in these patients.

Epidemiologically the condition is more common in adults. Most cases of SEA occur in patients aged 30 to 60 years; the youngest patient reported was only ten days old and the oldest was 87 years. The condition is more frequent in the males, the ratio of men to women being 1:0.56².

In a meta-analysis of 915 patients with SEA conducted by Reihnsaus E and team, 71% had back pain as the initial symptom and 66% had fever². Though epidural abscess most frequently presents with back pain, it is a very rare cause of back pain (0.004%), according to Deyo R A³. Localized spinal pain (89%), paralysis (80%), fever/chills (67%), and radicular pain (57%) were the common manifestations in a study of 46 patients by H J Tang and associates⁴.

Most frequent underlying condition in SEA is diabetes mellitus⁵. Diabetes predisposes to a range of different and unusual infections in relation to the spine, including psoas and spinal epidural infections. The case of a 47-year-old man, with heavy alcohol consumption and poorly controlled diabetes, on insulin therapy, with recurrent infections at different locations of spine, over a three year period, has been reported⁶.

Increase in the use of interventions on spine, especially for pain management has probably contributed to the recent increased incidence of SEA. The incidence of spinal epidural abscess appears to have increased in the United States since the 1980s, reportedly because of an increase in the age of the population, the number of spinal procedures performed,^{7,8} intravenous (IV) drug abuse, and the number of patients with acquired immunodeficiency syndrome (AIDS)⁹.

In the review of 46 patients (36 men and 10 women) with spinal epidural abscess over a 10-year period by H J Tang *et al.*,⁴ a high proportion of patients had underlying diseases of diabetes (46%), frequent venous puncture (35%), spinal trauma (24%), and history of spinal surgery (22%)⁴. Other associated predisposing conditions include a compromised immune system like chronic renal failure, alcoholism, and cancer, or it could occur as a consequence of epidural anesthesia, spinal surgery, or trauma.

The most common pathogen of spinal epidural abscess is *Staphylococcus aureus*^{4,10-13}. The organism reaches the epidural space^{4,10,13} by either contiguous or haematogenous spread¹⁰. *Staph. aureus* has been reported to be isolated from blood (39% cases) and pus drained from the site (50% cases) in an aetiological study⁴.

Clinical diagnosis of the condition is often delayed. Usually the condition presents as the classical triad of fever, back pain and neurologic deficits. Progression from one stage to another is highly variable; it may take months or could be a matter of hours. Progression of the condition is evaluated in four stages (Heusner Staging).

- Stage I - Only back pain at the level of the affected spine
- Stage II - Root pain radiating from the involved spinal segment
- Stage III - Motor/ sensory/ bowel or bladder dysfunction
- Stage IV - Paralysis

Spontaneous spinal epidural abscess is a rare^{14,15} and serious condition with a still high mortality rate^{1,15} and a considerable morbidity rate^{2,15}. The subgroup of cervico-thoraco-lumbar abscesses (also termed holocord lesions)¹⁶ is even rarer. In a review of literature from 1954 to 1997, Reihnsaus *et al.*,² found only 8 abscesses in this location among 738 cases.

A case of a 64-year-old man with psoas abscesses, epidural abscess and spondylitis, resulting in paraplegia, was reported by Bang and Lim¹⁷. It occurred after acupuncture treatment for backache. Also an unusual case of a devastating multilevel pyogenic spondylitis with paraplegia and soft tissue abscess formation in a previously healthy young man has been reported¹⁸. Methicillin-susceptible *Staph. aureus* (MSSA) was identified as causal pathogen in this case. The infection was managed by surgical debridement of all spinal lesions and a prolonged course of antibiotic therapy.

Why is lesion almost always posterior in thoraco-lumbar region?

The present knowledge on the anatomy of epidural space is based on cadaver dissection of Dandy¹⁹. The space is normally filled with fat and loose areolar tissue containing blood vessels; hence the infection can spread up and down over many vertebral levels. Epidural space is not a uniform space; in the cervical region a potential space lies between duramater and bone. Ventrally the dura is closely applied to the bone and ligaments from C₁-S₂ levels. Posteriorly a little space appears from C₇ vertebra and gradually deepens in the thoracic region from T₄₋₈ levels and then tapers to become shallow between T₁₁-L₂. The epidural space at these levels communicate with the retro-peritoneal space and posterior mediastinal space through inter-vertebral foramina; hence an abscess in the thoracic and lumbar regions is usually posterior (79%).

Abscesses in children are also more posterior in the epidural location, and usually have greater spinal column extension, but were associated with more favorable clinical outcomes than those in adults. MRI is the diagnostic procedure of choice; however, radionuclide bone scans should be considered for associated distant osteomyelitis in children²⁰.

Patho-physiology of epidural abscess: The clinico-pathological effects could be produced as a result of direct compression of spinal medulla by epidural pus or granulation tissue, or indirectly by thrombosis and thrombophlebitis of epidural veins and cord veins, and in a later stage by interruption of arterial blood supply or by focal vasculitis.

Correlation of duration of infection and gross appearance at surgery/postmortem

- a. Early (pre-suppurative) phase: inflammatory lesion, epidural mass is made of red swollen friable fat, with no pus.

- b. About two weeks: gross pus with varying amount of granulation tissue
- c. Delayed cases greyish white granulation tissue or mature fibrous tissue over the duramater.

Haematological investigations of relevance are WBC count, ESR and CRP. Lumbar puncture to determine cerebrospinal fluid protein concentrations is not needed for diagnosis, and entails the risk of spreading bacteria into the subarachnoid space with consequent meningitis; therefore, it should not be performed^[2].

The initial accurate diagnostic rate of SEA was as low as 11%, as reported by H J Tang *et al.*⁴ They observed that ESR was elevated uniformly (mean, 86.6 mm/h), and also suggested that low platelet counts ($<100 \times 10^9/L$), extremely high ESR (≥ 110 mm/h) and location of the abscess in the cervical spine would predict a poor outcome.

Myelography provided the initial means of delineating spinal epidural abscesses with some precision in the earlier decades of nineteenth century. Conventional radiographs of the spine show abnormalities in only 44 to 65% cases in the form of osteomyelitis, disc space infection and paravertebral soft tissue oedema²².

Imaging: Radionuclide studies are helpful but are non-specific. MRI scan with contrast is the imaging modality of choice; advantages include a complete evaluation over the entire length of the spine, and providing greater anatomic detail in demonstrating the rostral-caudal extent of the lesion and the degree of spinal cord compression. Virtually all recent publications on the matter agree on the value of MRI studies in the diagnosis and follow up of SEA. The degree of thecal sac compression seen on MRI correlates well with the severity of neurologic deficit. Many other entities have to be excluded, like herniated intervertebral discs, neoplasms, and spinal haematoma, tuberculoma, etc. Reihnsaus³ et al even conclude that MRI has made other diagnostic procedures essentially superfluous. However Sampath *et al.*,¹⁵ warns about a tendency to overestimate bone involvement as a disadvantage of MRI and recommend its use only in conjunction with plain radiographs and CT.

Specific management is emergency surgical decompression of the spinal cord by laminectomy and drainage of the abscess. The neurologic condition before decompressive laminectomy is an important predictor of the final neurologic outcome¹⁰.

Surgical approaches include:

- a. Immediate laminectomy
- b. Abscess secondary to vertebral osteomyelitis would need both anterior and posterior decompression
- c. In children laminoplasty is preferred to extensive laminectomy
- d. Method recommended by Hume and Dott -

small fenestrations are made between laminae to insert catheters into, and irrigate the epidural space

- e. Devilliers modified Hume technique - single level laminectomy, and introduction of catheters cranially/ caudally for irrigation

The duration of antibiotic administration after surgical intervention is usually 4-6 weeks but can be up to 12 weeks^{2,23}.

Although some cases of spinal epidural abscess may be effectively treated with antibiotics alone, a combined medical-surgical treatment constitutes the ultimate management¹⁰. Resolution with antibiotics alone can be opted in:

- patients not fit for surgery due to bad medical conditions
- abscess involving considerable length of vertebral canal
- no neurological deficit
- complete paralysis of more than three days

Deterioration of neurological status while undergoing antibiotic therapy alone has been observed, and may necessitate an emergency surgical decompression. Indications for such an interference include increasing neurologic deficits, persistent severe pain, persistent fever and leukocytosis.

Extradural administration of dexamethasone is recommended to minimize the progression of compressive oedema, cord ischemia and neurological damage²⁴. Mortality rates have been reported to be ranging from 4.6% to 31% in a recent series by Pereira C E and Lynch J C²⁵.

A case of acute paraplegia resulting from a spinal epidural abscess following a heroin injection four months prior to the presentation, has been reported²⁶. A complete neurological recovery was obtained by surgical decompression and antibiotic therapy despite the bad preoperative neurological status.

Good prognostic factors in epidural abscess include:

- a. Age below sixty years
- b. Degree of thecal sac compression less than 50%
- c. Duration of cord symptoms not more than 72 hours
- d. No co-morbid condition

The prognosis of patients who develop SEA following epidural anesthesia or analgesia is not better than that of patients with non-iatrogenic SEA, and the mortality rate is also comparable².

A study of two patients with spinal epidural abscess, but with different neurological outcomes, due to the difference in the time of intervention has been reported²⁷. The need for early diagnosis is once again stressed in this comparative study. Prompt treatment of certain infections, such as ear infections, sinusitis, and bloodstream infections could decrease the risk of an epidural abscess²⁸. Early diagnosis and treatment are essential to prevent complications.

Conclusion

Despite prompt diagnosis and intervention the mortality rate of spinal epidural abscess remains unacceptably high (18-23%), even in developed countries. In patients who survive, 20-30% will be left with significant neurologic impairment resulting in long-term disability. Patients with severe neurologic deficits rarely improve, even with surgical intervention within 6-12 hours of onset of paralysis. The functional outcome in patients who survive primarily depends on the degree and duration of neurologic impairment at the time of presentation. Increased awareness among doctors would enable timely diagnosis and rapid institution of therapy. Recognizing this relatively rare, emergent, and potentially treatable condition is a challenge for any medical professional, and it should never be allowed to progress to disastrous consequences.

Acknowledgement

The authors are specifically thankful to Dr Amol Anantarao Gautam, Department of Imageology for his co-operation towards the study and for providing the MRI pictures. We also thank all the staff members of the Department of Radiodiagnosis and Imageology.

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✦ CASE REPORT

Gender identity disorder as a comorbidity in Bipolar Mood Disorder: A report of two cases

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Abstract

Gender identity disorders involve the persistent desire or the insistence, to be one of the other sex, and a feeling of extreme discomfort with one's assigned sex and gender role. According to Diagnostic and Statistical Manual of Mental Disorders (DSM - IV - TR) patients with manic and depressive episodes or patients with recurrent manic episodes are said to have bipolar mood disorder. Reported here are the cases of two young ladies with gender identity disorder, who presented with bipolar mood disorder at a very young age. Both patients showed poor response to mood stabilizers.

Keywords: Gender identity disorder, Bipolar mood disorder, Co-morbidity

Introduction

Gender identity disorder is described as strong and persistent cross gender identification. The affective component of this disorder is commonly referred to as gender dysphoria, which may be defined as discontent with one's own biological sex and desire to possess the body of the opposite sex.

Testosterone affects the neurons that contribute to the tendency of masculinization in such areas of brain as the hypothalamus. Whether testosterone contributes much to the so-called masculine or feminine behavioural pattern in gender identity disorders still remains controversial. The quality of the mother-child relationship issue in the first few years of life is paramount in establishing gender identity. Mothers normally facilitate their children's awareness of, and pride in, their own gender. Children are valued highly as little boys and girls, but devaluing hostile mothering can result in gender problems. Father's role is also important in the early years. For a girl, the father is normally a prototype of future love object; for a boy father is a model for male identification¹.

Bipolar mood disorders can also be precipitated by hormonal changes, as evident in premenstrual dysphoric disorder, and the mood

disorders in pregnancy and post partum. It is in this perspective that the co-morbidity of gender identity disorders in females with bipolar mood disorder needs to be evaluated.

Case 1

A twenty four year old female, educated up to twelfth standard, presented with a history of over-talkativeness, decreased sleep, irritability, and excessive happiness of two months duration. The patient had a past history of episodic mental illness of seven years duration with several manic and depressive episodes.

Mental status examination showed a masculine way of dressing, short hair, and male footwear. Her psychomotor activity was obviously increased with flight of ideas and euphoric mood. Her speech and gesture were masculine. She doesn't like to menstruate and has no physical attraction to the opposite sex.

She was on regular treatment with mood stabilizers and anti-psychotics; still there were some breakthrough episodes. At the time of presenting to the OP department she was on lithium (900mg) and clozapine (100mg). History and mental status examination gave the diagnosis of bipolar mood disorder mania with co-morbid gender identity disorder.

Case 2

An eighteen year old girl with education up to eighth standard presented with history of over-activity, over-familiarity and over-talkativeness, of six months duration. The first episode was when she was in the eighth standard, and for the past four years she has had several manic episodes. Her mother also had bipolar mood disorder. Mental status examination shows flight of ideas, grandiosity and an elated mood. She had intense desire, since childhood, to be a man. She insisted on wearing eccentric, men's clothing in public. She called herself a *tomboy*; often she had a compelling desire to participate in the games and pastimes of boys. She had been staying alone in a single house. The clinical diagnosis was the bipolar mood disorder mania, with co-morbid gender identity disorder.



Fig. 1: Female patient with gender identity disorder with men's attire

Discussion

The process of becoming aware of the gender identity is an important part of the psychosocial development of the pubertal child. Gender identity disorder is a rare condition of atypical gender development, in which there is a subjective perception of self, in opposition to the individual's own gender.

The lifetime prevalence of mood disorders co-morbid with gender identity disorder has been reported to be approximately 45%.² In a study by Faycal Mouaffak *et al.*, during a six-year follow-up, a patient had been admitted eight times and had developed impulsive behaviour such as suicidal and self-injurious attempts. A broad range of antidepressants, mood stabilizers, and antipsychotic treatments were unsuccessful, until clozapine (75 mg daily) and lithium carbonate (1000 mg daily) permitted a sustained remission.³ This is similar to the present two cases where the patients showed an early onset and poor prognosis, and responded only to lithium and clozapine.

Affective disorders might also alter contentment with gender role, but the relationship is unclear. Case reports of patients with bipolar disorder suggest that gender dysphoria intensity fluctuates with affective excursions.⁴ O' Gorman however, described a bipolar patient whose gender dysphoria was mitigated during manic episodes.⁵

Estimates from the National Institute of Mental Health regarding the American population in general suggest that up to 25% may have identifiable psychiatric symptoms suggestive of anxiety disorders, depression, drug and alcohol abuse, and personality disorders.⁶⁻⁸

On the contrary Collier M Cloe and associates⁹ observed in 1997 that individuals presenting with gender dysphoria often do not have problems indicative of a coexisting psychiatric illness such as schizophrenia or major depression. At the same time they also felt that co-operation of many elements (i.e., real life test, ongoing psychiatric counseling and support) are critical in helping individuals work through the multiple psychosocial, endocrine and surgical issues associated with this diagnosis.

Conclusion

Not many studies have been reported in the available literature correlating the occurrence of bipolar disorders in patients exhibiting gender identity disorders. Further genetic exploration and hormonal studies are warranted in this area considering the younger age of presentation and the unfavourable long term outcome in such patients.

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✦ TECHNICAL REPORT

Edible Vaccines: Strides in Immunization

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Abstract

Vaccination is one of the greatest achievements of medical science and it has revolutionized the management and eradication of infectious diseases in humans and animals. Conventional vaccines bear several disadvantages from the viewpoint of production, preservation and delivery. The turn of the twenty second century has witnessed an exponential phase in the evolution of trailblazing approaches in vaccine development, including successful establishment of edible vaccines that utilize transgenic plants. This has inspired biotechnologist to explore the chances of food crops as prophylactic agents serving as vehicles for both production and delivery of vaccines.

Key words: Edible vaccines, Transgenic plants, Prophylactic agents

Introduction

Edible vaccines are emerging innovations in medical science and plant biology for the efficacious and affordable pharmaceuticals^{1,2}. Here genes encoding immunogenic proteins of pathogens are transferred into a plant and these transgenic plants then produce the desired immunogenic protein subunit vaccines.

Plants have provided humans with useful molecules for many centuries, but only in the recent past it has become possible to use plants for the production of specific heterologous proteins³. The first plant made protein was human growth hormone, in transgenic tobacco in 1986⁴. The structural authenticity of plant-derived recombinant proteins was confirmed in 1992, when plants were used for the first time to produce an experimental vaccine: the hepatitis B virus (HBV) surface antigen⁵.

Production of edible vaccine

Plant molecular farming deals with the large-scale production of recombinant proteins by using crop plants as expression hosts. Plants can be transformed via., two different multistep sophisticated strategies: 1) stable transformation, 2) transient transformation⁶.

Stable transformation refers to the insertion of foreign DNA into the

genome of the plant nucleus or plastids (eg. chloroplasts), resulting in the transfer of the genetic information to the subsequent generations of transformed plants. Two approaches are applied here a) the mostly used *Agrobacterium tumefaciens* - mediated gene transfer into the nuclear genome and b) biolistic process (particle bombardment^{6,7}). The gene containing DNA coated metal (gold) particles are fired at the plant cells using gene gun. Those plant cells that take up the DNA are then allowed to grow in new plants, and are cloned to produce large number of genetically identical crop. This method is quite attractive because DNA can be delivered into cells of the plant which makes gene transfer independent of regeneration ability of the species. But the chief limitation is the need for costly device particle gun. The advantage of using transgenic plastids is the high-level expression of recombinant protein, compared with stable transformation of the nucleus. Unlike nuclear-encoded recombinant proteins, the recombinant proteins emanating from the chloroplast genome will not be glycosylated⁸. However stable transformation is time consuming takes several months until positive transformants are obtained⁶.

Transient transformation, in which foreign DNA never enters the genome of the plant cell, hence cannot

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be inherited by the offspring. Transient transformation is mainly based on genetically modified plant viral vectors (e.g. TMV)/ agroinfiltration (Agrobacterium-mediated delivery). During agroinfiltration, leaf discs are inoculated with bacterial culture which results in transient expression of non-integrated transfer DNA (T-DNA) within the plant cells. Transient transformation only requires a few days until recombinant protein can be detected within the plant tissue^{6,7,9}, however, this procedure needs to be frequently repeated. The scheme for the construction of an edible vaccine is pre-arranged in Fig. 1¹⁰.

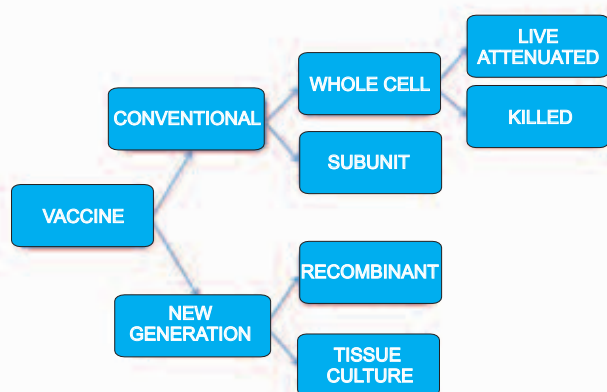


Fig. 1: Schematic representation of the evolution of edible vaccines

Transgenic plants as vaccine production units

The possibility of producing recombinant proteins in plants has opened a new field for designing innovative vaccine models. Mainly two approaches are utilized in the development of plant-based vaccines: i) plants as factories (biofactories) for potential vaccine antigens, or ii) plants as both production and delivery systems (edible vaccines).

In the first approach the recombinant protein is purified from the host plant and subsequently used for injective immunization. This has benefits compared with expression of recombinant protein in bacterial/ yeast cells. The bacterial expression system, like *Escherichia coli*, brings a pathogenic risk factor when using protein purified from such cells for use in humans while this is absent in biofactories. Moreover, the bacterial expression system lacks the ability to perform post-translational modifications of the recombinant protein. Furthermore, glycosylation of proteins produced in yeast cells differ greatly from that of proteins expressed in mammalian cells. However, the glycosylation of recombinant proteins produced in biofactories gives patterns similar to those performed by a mammalian cell⁸. When the production of edible vaccines is considered, it is desirable to select a plant whose products are consumed raw to avoid degradation during cooking like tomato, banana, cucumbers etc. A list of edible vaccines and their expression hosts are showed in Table 1^{11,12,13,14,15,16}.

Table 1. List of edible vaccines

Sl. No.	Name of the vaccine	Vector plant	Disease
1	HIV	Tobacco, tomato	AIDS
2	Rabies	Tobacco, tomato, potato	Rabies
3	Hepatitis B	Potato, tobacco	Hepatitis B
4	Vibrio cholera	Potato	Cholera
5	Enterotoxigenic E.coli	Tobacco, potato, maize	Traveller's Diarrhoea

There are several reports on development of transgenic plants that express antigenic proteins of human and animal pathogens^{17,18}. The edible vaccine approach is of interest when constructing a vaccine model against pathogens that have the mucosal tissues as their primary site of infection (e.g. HIV-1). Via an oral administration route, the mucosal surface will be exposed to the vaccine antigen and the immune cells in these tissues can be primed. The mucosal immune system is known as the first line of defense against many pathogens¹⁹. Owing to the possibility of using plants as both production and delivery system for vaccine antigens, the added advantages are i) low production costs, ii) no maintained cold chain is necessary, iii) needle-free vaccination, iv) bioencapsulation of the antigen, v) proper glycosylation of proteins, vi) local production in resource-poor settings, vii) ease for frequent boosts and viii) induction of mucosal immune responses².

Different plants have been tried as gene expression systems. Hepatitis B surface antigen (HBsAg) expressed in tobacco showed buoyant density and antigenicity similar to human- and yeast-derived HBsAg, suggesting a typical characteristics plant protein folding. Mice immunization with recombinant HBsAg (rHBsAg) demonstrated stimulation of T-cell proliferation. B- and T-cell epitopes of HBsAg were preserved when the antigen was expressed in transgenic tobacco²⁰. Constructs carrying the gene encoding *Escherichia coli* heat-labile enterotoxin (LT-B) were introduced into tobacco and potato plants²¹.

LT-B is produced by enterotoxigenic *E. coli* (ETEC) and also immunogenically interacts with the cholera toxin of *Vibrio cholerae*. Thus, LT-B is a candidate vaccine against both ETEC and cholera. The toxin protein was expressed in potato microtubers and at much higher levels in plants containing the fusion protein. The mice immunized by feeding with LT-B expressing potato tubers developed serum immunoglobulin G (IgG) and mucosal immunoglobulin A (IgA). The cholera toxin B subunit (CTB) against *Vibrio cholerae* expressed in potato tubers showed similar responses in mice. The potato was also used for the production and delivery of the human insulin antigen¹⁷ and human experiments showed enhanced serum IgG- and mucosal IgA-specific antibodies²². Norwalk virus capsid protein was expressed in potato tubers and found to be immunogenic in mice. Feeding this potato showed a modest serum level antibody increase in 19/20 healthy human volunteers^{15,16}. The first multi-component vaccine in potato against the three enteric pathogens – *V. cholerae*, rotavirus and ETEC was tried in another study and mice fed these tubers

produced both serum and mucosal antibodies against these pathogens²³. Soyabean was used for production of the glycoprotein B antibody of the herpes simplex virus 2 (HSV-2)²⁴ and corn was used for production of an LT-B subunit vaccine. Stoger *et al.*²⁵ expressed the single-chain Fv (ScFv) antibody of the human carcino embryonic antigen (CEA), a marker to diagnose tumor onset, in both rice and wheat grains.

Edible vaccines: a safer alternative

Plants provide a very attractive alternative that can be scaled up to a high production system for recombinant proteins^{18,19}. Recent advances in the understanding of transgene expression in plants allowed the development of using edible plants for delivery of antigens for immunization^{20,21,22}. Mostly edible vaccines stimulate of mucosal antibody production than injectable vaccines. Eating fruit or drinking juice of the transgenic plant should induce the mucosal immune system. Influence of the mucosal system is expected to lead serum system to produce pathogens specific immunoglobulins. Also producing and delivering vaccine in juice can curtail the costs of hypodermic needles, thereby preventing hazards from reuse of needles and, impoverished countries. There is also concern that plant material that contains recombinant proteins could inadvertently enter the food chain. Although it is apparent that pharmaceutical crops do not suffer the same acceptability problems as genetically modified food crops, the risk assessment and environmental impact studies must be carried out to the same level, to ensure the highest standards of responsibility and regulatory compliance.

Conclusion

Conventional vaccines are associated with a number of disadvantages forcing the medical community to search for alternative strategies. The use of foods as vehicles for production and delivery of vaccines is an intriguing field of Biotechnology and should provide incentives for both human health and the agricultural sector in the near future. Plant-based vaccines will provide a new use for food crops as these crops can then be grown for the sole purpose of producing and/or delivering biopharmaceutical or medicinal products. This lead to a future of safer and more effective immunization. They would overcome some of the difficulties associated with traditional vaccines, like production, distribution and delivery and they can be incorporated into the immunisation plans. But still there is lack of production and investment in this new technology but it will be likely conquered to make plant derived vaccine more efficient and dependable.

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✦ CASE REPORT

Age-specific complications of Varicella: A case report from Central Travancore

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Abstract

The burden of childhood and adult varicella in Kerala remains unknown. Primary varicella is much more severe in adults compared to childhood varicella. Here we report the case of an adult male who presented with varicella, and related complications. The advisability of varicella vaccination in developing countries like India, where the majority of young adult population is seronegative, is also discussed.

Keywords: Adult chicken pox, Varicella zoster virus, Varicella vaccine, Seronegative

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Introduction

Varicella zoster virus (VZV) causes chicken pox (varicella), a contagious disease characterized by low grade fever, malaise and a generalized vesicular rash¹. Incubation period of the virus ranges from 10 to 21 days. Malaise and low grade fever are the earliest symptoms, followed by rashes, first on the trunk and then on face, limbs and the buccal and pharyngeal mucosa. The rashes last for at least five days, and the severity differs from person to person². In industrialized countries like the United States of America, the United Kingdom and Japan it is a childhood disease and not less than 90% of the population encounter the virus by fifteen years of age. However the epidemiology of VZV in tropical countries is considerably different, with a seroconversion occurring later, during adolescence or adulthood. Primary varicella is reportedly more severe in adults than in children and is associated with significant morbidity and mortality³. Here we are reporting a case of primary varicella in an adult with the characteristic spectrum of potential complications.

presented at the Department of Medicine with a history of high grade fever and constipation for four days, and vesiculo-papular rashes, predominantly on the face, chest and abdomen, for five days. He gave a history of Type 2 diabetes mellitus for the past three years. On examination, the patient was febrile (102°F) and had extensive maculopapular lesions all over his body. He had tachycardia and mild dyspnoea; the blood pressure was recorded as 130/90mm of Hg.

On the day of admission blood samples were sent for haematological and biochemical investigations. The peripheral smear showed thrombocytopenia. Serum showed elevated levels of the enzymes alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase and bilirubin. Serum electrolyte levels were indicative of hyponatremia. Urinalysis revealed overt proteinuria. All the features when put together were suggestive of multisystem involvement complicating varicella. Diagnostic for human immunodeficiency virus, hepatitis B surface antigen, anti hepatitis C Virus, dengue and malarial parasites proved negative.

Acute phase blood sample was tested for anti-varicella zoster IgM antibodies by an immunocapture enzyme linked immunosorbent assay (Diasia, Italy). The principle of the test

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A forty two year old male was admitted to the isolation unit of Pushpagiri Medical College Hospital, Tiruvalla on 06/06/2011, when he

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is that anti-varicella zoster IgM in the patient sample is captured by anti-human IgM monoclonal antibodies found on the solid phase. Subsequent incubation with antigen complexed with monoclonal antibodies conjugated to horse radish peroxidase further selects the IgM antibodies specific to varicella antigen, revealed by the addition of peroxide substrate. The patient was positive for anti-varicella zoster IgM, confirming acute VZV infection.

The patient was treated with acyclovir and antibiotics, in addition to the necessary supportive therapy. He became afebrile and haemodynamically stable in four days and was subsequently discharged on 11/06/2011. The final diagnosis was extensive varicella zoster infection in a patient with pre-existent type 2 diabetes mellitus, complicated further by haemolytic jaundice, VZV related hepatitis, severe hyponatremia, pneumonitis and overt proteinuria.

Discussion

Primary varicella infection in adults can lead to serious complications like pneumonitis, cerebral ataxia, varicella encephalitis and bacterial superinfection of the skin lesions. Adult varicella zoster is usually associated with hepatitis, usually asymptomatic and generally characterized by elevated level of liver enzymes. In rare cases this can lead to acute liver failure which can be fatal^{2,4}. So also varicella becomes a serious infection if it occurs in pregnancy. Infected mothers may pass the virus to the unborn child; intrauterine infection of the foetus in the early stages of development may result in congenital varicella syndrome⁵.

In 1998, the World Health Organization (WHO) recommended that routine childhood varicella immunization be introduced in countries where varicella disease was a public health problem. The vaccine was more or less affordable, and high coverage could be achieved⁵. The use of the vaccine reduced the incidence of varicella and its associated complications, hospitalizations and fatality rates.

There are concerns regarding the effectiveness of vaccine induced immunity. The live attenuated varicella vaccine is 85% to 90% effective in preventing varicella and 100% effective in preventing moderate to severe disease⁶. Before the vaccine became available in 1995, there were three to four million cases of varicella in the US each year, of which 10,000 were hospitalized with complications, and approximately 100 died. While only 05% of reported cases of varicella occur in adults in US, adults account for 35% of the deaths from this disease.

There is a strong view that in developing countries disease prevention should be initiated by investing in interventions, like sanitation, safe drinking water, responsible behaviour, education, and increased funding for surveillance of vaccine-preventable infectious diseases.

In India, seroconversion largely occurs in late adolescence and adulthood. About 72% of infection occurs in the age group 15 to 25 years. The VZV infection in adolescents and adults is associated with higher mortality and morbidity. Moreover there is a good chance of pregnant women being susceptible to VZV³.

While high coverage of the vaccine among children can cause complete elimination of the disease, partial coverage can have adverse effects by shifting the age of incidence from children to adults, thus causing severe manifestations that can be life threatening.

At the same time, administration of varicella vaccine can reduce the severity of zoster during its reactivation in elderly⁵.

In India, varicella vaccine (Varivax, Merck Research laboratory) is available only in the private sector. The recommendations regarding varicella vaccination can be summarized as follows:

- It has been licensed for healthy children (more than fifteen months of age) and adults.
- People, who are not immune to varicella, thirteen years of age or older, should get the vaccine as two doses; at four to eight weeks interval⁷.
- HIV infected patients of age more than eight years, with CD4+ T - lymphocyte percentage more than 15%, are eligible for single antigen varicella vaccine.
- Vaccination of patients with leukemia, lymphoma or other malignancies should be given under expert guidance and with the availability of antiviral therapy.
- People allergic to the antibiotic neomycin and gelatin should be excluded from vaccination⁸.
- For VZV seronegative women of child bearing age, a vaccination four months prior to becoming pregnant is recommended^{3,8}.

Conclusion

Even though the range and burden of infectious diseases in India is considered enormous, surveillance studies are few, and should be initiated. Although varicella vaccine is safe and effective, issues of high cost and low vaccine coverage are deterrents to its inclusion in the routine immunization program. At the same time the vaccination should be strongly recommended for a selected susceptible sector of the population.

Acknowledgement

We acknowledge Prof. Dr. P. M. Sivan Pillai, Professor and Head, Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre for his valuable comments on the manuscript.

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✦ TECHNICAL REPORT

Renal cell carcinoma - Histological subtypes, genetic basis and prognostic factors: an overview

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Abstract

Cancer of the kidney amounts to two percent of the total human cancer burden with approximately 1,90,000 new cases diagnosed each year. They occur in all world regions, with preference for developed countries. Renal cell carcinoma (RCC) is a cause of significant morbidity and mortality. Recent advances in imaging permit early diagnosis of renal cell carcinomas and facilitate optimal management. The pattern of somatic mutations in kidney tumours has been extensively investigated and has become, in addition to histopathology, a major criterion for classification. This article gives an overview on the histological subtypes of renal cell carcinoma, including new subtypes recognized in the 2004 World Health Organization classification of renal tumours. The general applications of immunohistochemistry and cytogenetics are also reviewed with their implications in tumour diagnosis and prognosis.

Introduction

Renal cell carcinoma (Grawitz tumor) is a group of malignancies arising from the epithelium of the renal tubules. Paul Grawitz, a German pathologist first documented renal cell carcinoma in 1883. Due to the histologic resemblance to the adrenals, renal cell carcinoma was originally named "hypernephroma" because it was believed that these tumours originated from adrenal rests. In 1960, Oberling *et al.*,¹ demonstrated its origin from the proximal renal tubule based on the ultrastructural features. The tumour was renamed renal cell adenocarcinoma or simply renal cell carcinoma.

With the advent of advanced imaging technologies, the incidence of renal tumours has risen in the past 30 years. Incidentally detected tumours in the asymptomatic individuals ('incidentalomas') have been steadily rising, with frequent use of imaging techniques². They account for approximately 60% of renal tumours. The evolution of renal pathology has greatly enhanced our understanding of renal tumours. This has led to a better understanding of histologic subtypes of renal cell carcinoma.

Historical aspects of classification

Classification of renal cell carcinoma is important from the treatment and prognosis point of view as well as for understanding its histogenesis, and the molecular and cytogenetic basis, and for further improvement in its management. Keeping this view, many classification systems have been made.

In the past only clear cell and granular renal cell carcinoma were recognized. The term 'granular cell' tumours lacked precision and could be used to describe several tumour types ranging from completely benign renal oncocytoma to malignant clear cell and chromophobe renal cell carcinomas. Therefore this terminology was not used in the later classifications.

In 1986, Thoenes and colleagues from the Gutenberg University in Germany proposed a new classification for renal tumours of tubular epithelial origin known as Mainz classification³. This was based on conventional histopathologic criteria. The basic categories consist of clear cell carcinoma, chromophil type, chromophobe type and collecting duct carcinoma (Table 1). The chromophil group encompasses eosinophil type

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with abundant eosinophilic cytoplasm and basophil type with small amounts of cytoplasm.

Table 1: The Mainz classification of renal cell tumors

Tumour type	Frequency
I. Renal Cell Carcinoma	Total 92%
Clear Cell	70%
Chromophil (eosinophil, basophil)	15%
Chromophobe (eosinophil, basophil)	5%
Collecting duct carcinoma	2%
II. Oncocytoma	8%

Though Mainz Classification recognized variety of subtypes of RCC, the various categories defined in it were hampered by the confused and complex terminology.

In 1997, the consensus meetings held in Rochester, Minn., and Heidelberg, Germany defined the more common classifications of RCC in wide use today. Histologic appearance and architecture were considered instead of tumour genetics. In this classification chromophil carcinoma has been renamed as papillary renal cell carcinoma as it describes the growth pattern of majority of tumours. According to this classification, five distinct renal cell carcinoma types were identified, including clear cell (conventional), papillary, chromophobe, and collecting duct types. Those types which do not fit into other categories were termed RCC, unclassified⁴.

In 2004 WHO classified renal cell carcinoma, distinguishing several distinct tumour subtypes with distinct clinical behaviour and underlying genetic defects⁵. WHO 2004 represents an extensive revision of the classification of renal neoplasia and include several new tumour entities described since the Heidelberg/Rochester consensus conferences.

WHO classification of renal cell carcinoma

The 2004 WHO classification distinguishes three main histologic subtypes:

1. Conventional (clear cell)
[Variant - Multilocularcystic renal cell carcinoma]
2. Papillary
 - Type 1
 - Type 2
3. Chromophobe RCC
 - Classic type
 - Eosinophilic type

Rare subtypes include:

- Carcinoma of the collecting duct of Bellini
- Medullary carcinoma
- Familial renal cell carcinoma
- Mucinous tubular and spindle cell carcinoma
- Xp11 carcinoma
- Carcinoma associated with neuroblastoma
- RCC unclassified

❖ **Clear cell RCC**

Incidence: Clear cell RCC is the most common histologic subtype of malignant tumours of the kidney. It accounts for about 75% of renal masses that are evaluated.

Gross features: Macroscopically, clear cell RCCs are round tumours that bulge out of the renal cortex; they are usually clearly demarcated from the adjacent renal parenchyma by a fibrous pseudocapsule. All clear cell RCCs have golden yellow appearance due to the rich lipid content of their cells (Fig. 1A). Cysts, hemorrhages, necrosis and calcifications are commonly encountered, which results in the variegated appearance that is very characteristic of this neoplasm (Fig. 1B). Clear cell type is most prone to formation of small and large cysts.

Histology: Architecturally, most cases show evidence of glandular differentiation, hence their alternative designation as renal adenocarcinoma⁶. In the usual case, however, the pattern of growth is predominantly solid, with formation of large nests of tumour cells separated by a stroma that is characteristically endowed with prominent sinusoid-like vessels. The cytoplasm of the neoplastic cells is usually clear, due to its lipid and glycogen content (Fig 2A). A variable component of eosinophilic cells may be present. Nuclei are round to polygonal with indistinct nucleoli and finely distributed chromatin. Tumour necrosis can also be present and should be routinely reported because it is a useful predictor of clinical outcome.

Immunohistochemistry: Clear cell RCCs commonly react with epithelial membrane antigen (EMA), low-molecular-weight cytokeratins (CK8, CK18, CK19), AE1, Cam 5.2, and vimentin. Co-expression of keratin and vimentin is the rule, a feature not present in normal tubular cells. MUC-1 and MUC-3 are consistently expressed. Most clear cell RCCs react with RCC marker and CD10, which are useful for distinguishing the clear cell and chromophobe subtypes.

Genetics: Clear cell RCC often has distinct genetic abnormalities, with loss of 3p chromosome which also harbors the VHL gene. Frequent loss of chromosome 9p has also been reported.

Variant: The 2004 WHO classification of kidney tumors recognizes multilocular cystic renal cell carcinoma (MCRCC) as a rare variant of clear cell RCC with a good prognosis. This is a tumour with excellent outcome and entirely composed of cysts of variable size separated from the kidney by a fibrous capsule (Fig. 1C). The cysts

are lined by a single layer of clear to pale cells but occasionally show a few small papillae. The septae are composed of fibrous tissue that may have epithelial cells with clear cytoplasm that resemble those lining the cysts. No tumour with these features have recurred or metastasized⁶. Suzigan *et al.* proposed to rename and reclassify this tumour as *multilocular cystic renal cell neoplasm of low malignant potential*, helping urologists to conservatively manage these patients⁷.

Prognosis: Clear cell RCC has worse prognosis in comparison with chromophobe or papillary subtypes. Sarcomatoid change (5% of tumours) and histologic tumour necrosis are associated with poor prognosis.

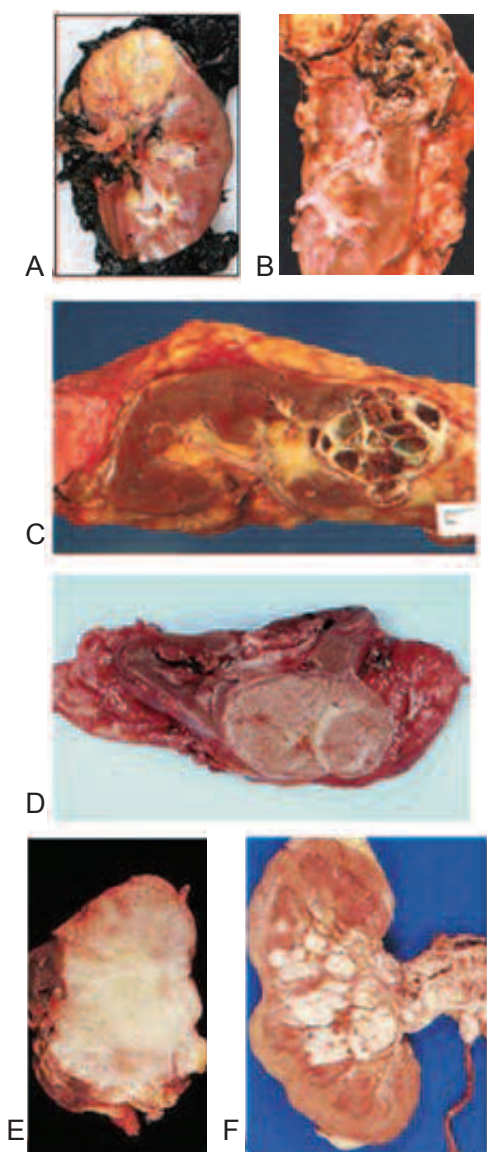


Fig. 1A: Clear cell RCC showing characteristic yellow colour
 Fig. 1B: Variegated appearance in CCRCC
 Fig. 1C: Multilocular cystic RCC
 Fig. 1D: Chromophobe RCC
 Fig. 1E: Sarcomatoid change
 Fig. 1F: Collecting duct carcinoma

❖ Papillary RCC

Incidence: It is the second most common variant, having a well-defined genetic abnormality associated with it, much like clear cell RCC. Approximately 10%-15% of renal masses are papillary RCC, with a small portion of these related to the familial syndrome. Papillary RCC was not considered a distinct subtype of RCC until the Heidelberg and Rochester consensus meetings. In 2004, WHO officially recognized two types of papillary RCC. Multicentricity or bilaterality are more common in papillary subtype than in other renal cell carcinomas. Renal tumours arising in patients on chronic haemodialysis tend to be of papillary type.

Histology: Papillary RCC is composed of a single or pseudostratified layer of cells arranged around a fibrovascular core to create the characteristic papillae (Fig. 2B). These tumours are notable for the presence of aggregates of foamy macrophages and psammoma bodies. These characteristics are common to both subtypes of papillary RCC. Papillary type I and type II are distinguished mainly by histologic and cytologic criteria. Type I tumours are more common and are composed of small cells characterized by their scanty basophilic cytoplasm and low nuclear grade. The fibrovascular cores of the papillae are lined with a single layer of cells. In contrast, type II tumours are composed of large cells with eosinophilic cytoplasm and higher grade nuclei with pseudostratification along the fibrovascular core.

Immunohistochemistry: Papillary RCCs react strongly with pancytokeratin and low-molecular-weight cytokeratin antibodies. Cytokeratin 7 reactivity has been reported for papillary RCC and is more frequent in type 1 than in type 2 tumours. RCC marker is expressed in > 90% of cases, as is CD10.

Genetics: Trisomy and tetrasomy of chromosome 7, trisomy of chromosome 17 and loss of Y chromosome in males are the most common genetic changes in papillary RCCs. Both sporadic and familial papillary RCC are associated with mutations of the *MET* gene at chromosome 7.

Prognosis: Type II papillary RCC has worse prognosis than type I, though both these tumours have a more optimistic prognosis than clear cell RCC⁸. Sarcomatoid dedifferentiation has been described in about 5% of papillary renal cell carcinomas and is considered a strong negative event. According to Moch *et al.*, the presence of extensive tumour necrosis was associated with a more favourable prognosis in contrast to clear cell RCC⁹.

❖ Chromophobe RCC

Incidence: Thoenes *et al.*,¹⁰ in 1985 described another subtype of RCC with clear cell features, which closely resembled the renal tumours experimentally induced in rats. This tumour was named chromophobe renal cell carcinoma. It accounts for approximately 5% of renal tumours.

Gross: Macroscopically, it presents as a solitary and well-circumscribed tumour. In unfixed specimens, the cut surfaces are typically homogeneously brown or tan coloured (Fig. 1D).

Histology: Chromophobe RCC derives from the intercalated cells of the collecting duct epithelium. Microscopically, they have a solid, alveolar or nested architecture. Two major patterns have been recognized, referred to as classic and eosinophilic types. The cells of the classic type usually have abundant pale vesicular cytoplasm with prominent cellular membranes due to the concentration of cell organelles at the periphery of the cytoplasm (Fig. 2C). The eosinophilic variant of chromophobe carcinoma is composed of intensely eosinophilic cells with granular cytoplasm owing to an abundance of mitochondria. Microvesicles may concentrate around the nuclei, producing prominent clear perinuclear halos. The nuclei of both variants are irregular, often wrinkled (raisin-like) and hyperchromatic. Both types of chromophobe carcinoma typically stain with the Hale's colloidal iron technique, which exhibits a homogenous blue cytoplasmic appearance indicating the presence of acidic mucins⁶.

Immunohistochemistry: Chromophobe RCC reacts strongly with pancytokeratin and EMA antibodies. About 50% of tumours react for RCC marker and CD10. Chromophobe RCCs do not react for vimentin. Recently *Kit* expression was reported in all cases of chromophobe RCC (and renal cell oncocytomas), but in no case of clear cell RCC. Thus, *Kit* reactivity can be seen as an additional diagnostic criterion to distinguish chromophobe RCC from clear cell and papillary RCCs.

Genetics: Most chromophobe renal cell carcinomas are characterized by loss of multiple chromosomes¹¹.

Prognosis: This RCC subtype has a better prognosis than both clear cell and papillary RCC, but it is worth noting that metastases from chromophobe RCC have been reported. Sarcomatoid change is associated with aggressive disease. There are some reports which indicate that chromophobe carcinoma may be the most frequent type, associated with sarcomatoid transformation.

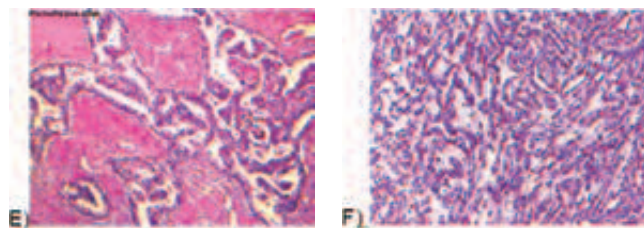


Fig. 2: Microscopic appearance of RCC types: A. Clear cell; B. Papillary; C. Chromophobe RCC; D. Sarcomatoid change; E. Collecting duct carcinoma; F. Mucinous tubular & spindle cell type (H & E)

❖ **Familial renal cell carcinoma**

Inherited or familial predisposition to renal neoplasia is present in less than 4% of renal tumours. Each of these syndromes predispose to a distinct histologic type of RCC or other kidney tumour (Table 2)¹². Clinically, hereditary renal cancers show a tendency to be multiple and bilateral, may have a family history, and present at an earlier age than the non-familial and non-hereditary renal neoplasms. There are five major types of inherited kidney epithelial tumours.

Table 2. Inherited renal cell carcinoma syndromes and related genetic defects

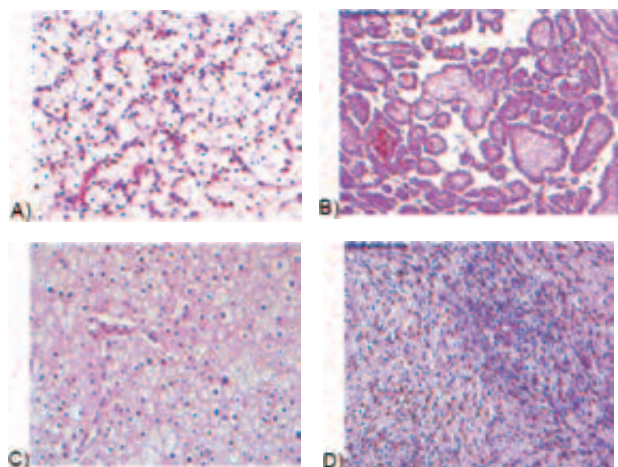
Syndrome	Gene	Chromosome alterations	Pathologic appearance
von Hippel-Lindau	VHL	3p25 loss of function mutations	Multiple, B/L CCRCC, pheochromocytoma, CNS haemangioblastomas
HPRC	MET	7q31 activating mutations	Multiple, bilateral PRCC, type 1
Hereditary leiomyomatosis & RCC	FH	1q42-43 inactivating mutations	Papillary RCC type 2, cutaneous & uterine leiomyomas
Birt-Hogg-Dubè	BDH	17p11.2 inactivating mutations	Multiple chromophobe & CCRCC & oncocytomas, skin tumours
FCCRC		3p translocations	Multiple, bilateral CCRCC

HPRC = Hereditary papillary renal carcinoma; CCRCC = clear cell renal cell carcinoma; CNS = central nervous system; PRCC = papillary renal cell carcinoma; FCCRC = Familial clear cell renal cancer.

❖ **Collecting duct carcinoma**

Collecting duct carcinoma (CDC), as the name implies, derives from the collecting duct cells in the medulla (Fig. 1F). Unfortunately, most tumours are symptomatic and patients often present with advanced stage disease. These tumours tend to have a male predominance (2:1)

Collecting duct carcinoma is characterized by infiltrating, irregular tubules and papillae of varying dimensions in a desmoplastic stromal reaction (Fig. 2E). This subtype does not have a specific staining pattern and is positive for CEA, peanut lectin agglutinin and



Ulex europaeus agglutinin. It can also be positive for cyto-keratin 34BE12 and CK7. Unlike other renal carcinomas, however, CDC does not have a clear genetic karyotype¹³.

❖ Renal medullary carcinoma

Renal medullary carcinoma was first reported to be a distinct variant of renal tumours in 1995¹⁴. This type of carcinoma is also derived from the renal collecting duct. It is considered by some to be an even more aggressive variant of CDC. It is found with overwhelming predominance in younger patients, with the mean age at presentation of 19 years. Male predominance has been reported at 2:1. This subtype is associated with sickle cell disease and in some cases sickle cell trait.

Histology reveals an infiltrative, poorly differentiated carcinoma with solid sheets of tumour cells and a significant desmoplastic response. Medullary carcinoma shows a similar pattern of immunostaining as collecting duct carcinoma. Like CDC, medullary carcinoma has proved difficult to characterize on genetic analysis.

Unfortunately, 95% of patients present with metastatic disease and prognosis is poor, with a mean survival of 18 weeks after diagnosis¹⁵.

❖ Mucinous tubular and spindle cell carcinoma

Mucinous tubular and spindle-cell carcinoma is a new addition to the WHO renal tumors classification. It is a rare tumour postulated to be of collecting duct and possibly loop of Henle origin. This tumour is named after its three components: tubules, spindle cells, and mucinous stroma. The mean age at presentation is 53 years. These tumours demonstrate a female predominance.

Microscopically, they are composed of tightly packed elongated tubules, some with a spindle-cell appearance, in a bubbly mucinous stroma (Fig. 2F). The nuclei of tumour cells tend to appear low grade and uniform. Immunohistochemical staining of this tumour is positive for CK7, racemase, and RCC antigen. Genetic analyses have suggested that trisomy of chromosome 07 and 17 as well as loss of chromosomes 01, 04, 06, 08, and 13 are characteristic of this type of tumour, though its genetic profile is poorly defined at present. These tumours have a favourable prognosis as they behave in a non-aggressive fashion.

❖ Renal translocation carcinomas

Included in the 2004 WHO reclassification are renal translocation carcinomas, which have recently been identified as a distinct subset of renal carcinoma. Most patients present at a young age (between late childhood and early adulthood) and are symptomatic. The histopathologic appearance is that of papillae lined by clear cells and cells with eosinophilic granular cytoplasm.

Genetic analysis shows that these tumours are associated with alterations of Xp11.2 gene. These alterations include both translocation and fusion of the genes coding for the microphthalmia transcription factor subfamily, which includes both transcription factors E3 and EB. The proteins overexpressed by these genetic alterations can be identified on immunohistochemical staining and help in diagnosing Xp11.2 translocation tumours. These tumours are rare but they tend to be aggressive.

❖ Neuroblastoma-associated RCC

This is a unique histologic subtype of renal cell carcinoma that occurs in long term survivors of paediatric neuroblastoma. It is believed that treatment for neuroblastoma may predispose to genetic changes responsible for renal cell carcinoma. These tumours appear histologically heterogeneous. Some tumours contain solid and papillary architecture with most cells containing abundant eosinophilic cytoplasm. The diagnosis may be suggested based on a history of treatment for neuroblastoma.

❖ Renal cell carcinoma, unclassified

Renal cell carcinoma, unclassified is a diagnostic category to which renal carcinomas should be assigned when they do not fit readily into one of the other categories. In surgical series, this group often amounts to 4-5% of cases.

Features which might place a carcinoma in this category include: 1) apparent composites of recognized types 2) sarcomatoid morphology without recognizable epithelial elements 3) mucin production 4) mixtures of epithelial and stromal elements, and 5) unrecognizable cell types. According to Zisman. et al¹⁶, this subtype is an aggressive form of RCC as most cases are at advanced stages at presentation.

❖ Sarcomatoid dedifferentiation

Sarcomatoid change can arise in all the types of carcinoma in the classification. An estimate of the percentage of sarcomatoid change should be provided, as focal sarcomatoid dedifferentiation is also prognostically significant. Sarcomatoid element has a soft, fleshy, gray-white appearance with infiltrative margins (Fig. 1 E). This is defined as a high-grade spindle cell malignancy exhibiting morphologic or immunohistochemical evidence of an epithelial origin (Fig. 2D).

In the past, sarcomatoid RCC was considered a unique RCC subtype; however, this subtype was dropped from the classification as there is no evidence to support the notion that RCC develops 'de novo' as sarcomatoid carcinoma. Therefore, the 2004 WHO classification does not consider it as an entity, but rather as a transformation or dedifferentiation of any RCC main type¹⁷.

Conclusion

Prognosis of patients with RCCs is most accurately predicted by TNM stage. Within stages, Fuhrman grade has a strong predictive value.

Histologic subtypes of RCCs are not independent prognostic factors comparable with TNM stage and Fuhrman grade. However it may reflect the underlying genetic features of RCC. So the evaluation of this parameter is fundamental in the pathology report of RCC.

Clear cell renal cell carcinomas are associated with worse prognosis compared with papillary and chromophobe RCC. It is also well known that collecting duct and renal medullary carcinomas are associated with aggressive clinical behaviour and poor prognosis. Among the papillary renal cell carcinomas, longer survivals were reported for type 1 when compared with type 2 tumours, suggesting that this distinction has prognostic significance. It is also important to recognize the sarcomatoid component in these tumours because of its consequential adverse prognosis for the patient.

Recently histologic coagulative tumour necrosis is shown to be an independent prognostic factor of outcome for clear cell and chromophobe RCC, and its presence should be routinely reported and used in clinical assessment. It is significantly associated with death from clear cell and chromophobe RCC, but not with death from papillary RCC.

Thus, novel RCC classification systems based on gene expression offer a promising approach for diagnosis, prognosis, and possibly therapy.

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✦ TECHNICAL REPORT

Prezi - A better presentation tool

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Prezi is a new web-based presentation application tool that uses a single canvas instead of traditional slides eg. *Microsoft (MS) Powerpoint*^(R). The text, images, videos and other presentation objects are placed on the infinite canvas and grouped together in frames, which gives a natural flow for the presentation. The canvas allows the users to create non-linear presentations, where users can *zoom in*, to get the details of an image and *zoom out* for a larger image of a visual map¹.

Prezi was originally developed by architect Adam Somlai Fischer to show large architecture drawing as well as details, has been used in a great variety of ways for personal purpose as well as for industrial drawings. Adam Somlai Fischer and Peter Halacsy improved on *Prezi* during 2007-2009, as they felt slides limited their ability to develop and explain ideas.

Advantages of *Prezi* over classical digital presentations:

Prezi is a web-based, canvas style presentation with embedded objects, which allows a group collaboration, whereas *MS Powerpoint* or similar presentation software are installed applications with sequential slides consisting of mere texts, pictures and animations².

The most important aspect of *Prezi* that makes it different from the traditional slide show is that it is not linear. Here we can think of the presentation as a big black board or canvas where we can put out our ideas and then *zoom in* and *out* through them.

Here we are explaining our experience with *Prezi* being used for a presentation on a simple topic on *Sulfonamides* in Pharmacology, which was tried out in a class room of medical

students in Pushpagiri Medical College, Tiruvalla, Kerala, India.

Before starting we need to *sign in* by creating an account in the *Prezi* homepage www.prezi.com. The *learn* tab on the top left corner of the page may be used to access the tutorial on *Prezi*. We start by a double click and typing as much matter we want to be included in the presentation.

Before using *Prezi* all the images may be placed in a single folder for easy access.

1. The *Prezi* has a transformation 'zebra' which can be used to transform the text (bigger, smaller, changes in font style). The text will move in the direction of the zebra lines. The text can be rotated with the outer ring and scaled using the inner ring. This centers the text too.

The menus for *zoom in/out* and others are available on the bottom and sides (Fig. 1).

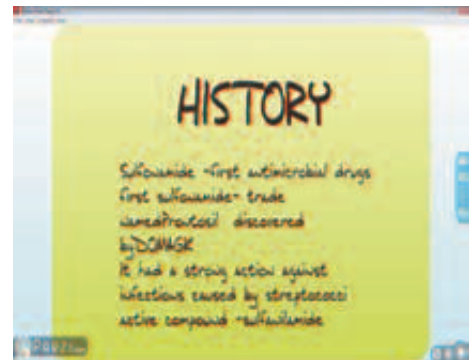


Fig. 1: The *start up* screen of class on *Sulfonamides*

2. Fig. 2. depicts the presentation as a whole in a single canvas. Here the different images that are to be presented can be visualised as a single entity. The different images under the section of *Sulfonamides* can be visualized together.



Fig. 2: Bird's eye view of the topic on Prezi

3. In Prezi we have a giant work area where we can write texts, insert images and videos. We can zoom in to the important points and zoom out for an overview. Figure 3 shows the zoom in feature where the image on the spectrum of *Sulfonamides* is visualized clearly. So with the zoom in feature the focus of the audience is now on this single image.

The zoom out feature will show the adjacent images as a whole (Fig. 4).



Fig. 3: Zoom in feature

4. An image can be added by clicking on the INSERT icon on the upper left of the Prezi home page. Figure 5 shows the image of the fingers involved in burns, and demonstrates the use of topical *sulfonamides* like *Silver sulfadiazine*. By clicking on the image and moving the zebra we can alter the size, move or rotate the image.



Fig. 4: Zoom out feature



Fig. 5: An image inserted in figure

5. The image or text may be deleted by clicking on the plus sign (+) on the zebra and then choosing 'delete'.

6. Some menu key board short cuts used in Prezi are:

- The *path* menu is drawn by using the 'P' on the keyboard.
- The *frame* menu by pressing 'F' on keyboard. The different frames options including circle and rectangle are available (Fig. 6).
- Shapes and Inserts by 'S' and 'I' respectively.



Fig. 6: The frame feature which envelopes a group of items

7. After all required matter (texts and images) are added, we need to provide the correct path for the presentation. Using the zoom buttons, we can zoom in and out and locate the exact one that is needed.

After clicking on *path*, we should click on *text* or *images* in the order we want it to be presented. The *path* may be altered by *delete all* and started again by dragging the unassigned *path ball* to the object that is required.

The presentation, when ready can be viewed by moving into presentation mode by clicking on 'show'. The arrow keys on the keyboard are used to move through *Prezi* and to *zoom in* and *out* through the texts and images of our presentation. An executable version of the presentation can be downloaded freely for future use.

Conclusion

Prezi can be used to put all our interconnected ideas in a linear path that can be visualised in a sequence without losing the links. *Prezi* can prove as a good alternative to the classical powerpoint-like presentations with added advantages. It could prove

enormously useful for doctors, teachers and medical students alike.

Acknowledgement

Dr. Rajeev A, Professor, Dept of community Medicine is being acknowledged for his valuable inputs in this article.

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1. <http://office.microsoft.com-us/powerpoint>. Accessed on September 1,2011
2. Adan Somlai Fischer. About perspective of prez. Available at <http://www.prezi.com>. Accessed on September 1,2011



✦ TECHNICAL REPORT

Towards custom-made oral rehydration solutions: Principles and practice

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Water is fundamental to our existence. Approximately 5-10% of total body water is turned over daily via obligatory (non-exercise) fluid loss avenues. Water balance is achieved and maintained by matching input and output from the body¹. As can be ascertained from the data by Bossingham MJ *et al.*, urinary loss is essentially matched to intake from food and beverages (Table 1), whereas the stool losses could be covered by metabolic water production which is about 41 gm, 55 gm and 107 gm water, produced for every 100 gm protein, carbohydrate (starch), and fat consumed². Insensible water losses in the form of sweating and respiration are 13 ml and 30 ml water, respectively, given off for every 100 kcal energy consumed, which is substantially

underestimated by people and hence ignored³. If allowed to consume *ad libitum*, a normal person can make up for this loss more than adequately (Table 1), provided, awareness is built into people regarding water balance. Physical activity increases sweat rate and hence insensible water loss too. Most of the people are voluntarily dehydrated, which means that they are averse to making up the insensible water loss especially when they are physically active. This is usually because of the choice of drinks they favour as per the taste which they are accustomed to. Voluntary dehydration was even practiced to reduce diarrhoea in order "to rest the gut" before the introduction of concept of Oral Rehydration Solution (ORS) by the World Health Organization (WHO).

Table 1: Water balance in a young active individual under experimental conditions¹

Output (ml ± SD)		Input (ml ± SD)	
Urine	2095 ± 514	Food and beverages	1997 ± 419
Stool	138 ± 37	Metabolic	394 ± 67
Insensible	1325 ± 223	Ad libitum	1116 ± 537

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The present concept of oral rehydration was born in the backdrop of diarrhoeal deaths in developing world⁴. The concomitant loss of electrolytes was sought to be corrected using appropriate proportions of electrolytes as was lost according to the given condition. As can be seen in the Table 2, the cholera stools had the highest loss of electrolytes among the infective diarrhoeas. Hence, the original ORS from the stable of WHO had higher concentrations of electrolytes which was reconstituted with proportionate amounts of water as was needed⁵. As the diarrhoeal spectrum changed, the need to change the composition of ORS emerged because of the differential loss of electrolytes as is illustrated (Table 2)

Diarrhoea is not the only situation in which water can be lost from the body. Vomiting is obviously a concern in gastro-enteritis and is a well known cause of dehydration.

In addition to such obvious reasons of dehydration, the rising summer temperatures have brought upon us to think the need to consider the increased insensible losses through sweat and respiratory water which also need to be corrected as urgently as the other causes of dehydration. Another route of loss of water is urinary loss can vary according to various pathological situations. These have to be dealt with in a different manner because of additional medical issues including medications.

PIMS & RC

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Table 2: Electrolyte losses in various situations (per litre)

	Sodium	Potassium	Volume loss
Stool - No growth	38.1	22.5	Ranges from 5 ml/kg/hour or 100 ml per stool to 10 - 15 ml per kg body weight per hour i.e., 500 - 1000ml/ hour
Stool - Rotaviral diarrhoea	29.7	21.0	
Stool - Salmonella enteritis	37.2	20.5	
Stool - E.coli diarrhoea	38.8	23.7	
Stool - Shigella gastroenteritis	60.4	40.9	
Stool - Cholera	95.3	25.3	1-2 ml/kg/hour
Gastric secretion - vomitus	60.0	15.0	
Sweat - acclimatized persons	8.6	4.4	Upto 1.2 litre per hour
Sweat - unacclimatized individuals	79	15.6	

The amount of water lost varies depending on the condition involved. Whereas there is a definite system for assessing water loss in diarrhoeas, at least in children, other instances are somewhat complex.

Moderate dehydration in diarrhoea and vomiting is assessed by a sticky oral mucosa, lethargy and in kids no tears while crying when body has lost at least 5% of its original weight in water. Pronounced sunken eyes and markedly diminished skin turgor would mean about 6 to 8% dehydration. Weak thready pulse would indicate about 9-10% dehydration⁶. Obvious shock (tachycardia, hypotension, cool extremities) and loss of skin tenting could indicate higher levels of dehydration⁷. The volume lost is directly visible and can be roughly assessed and extrapolated to the electrolyte loss as well.

In insensible water loss, the assessment would be more complex and could involve fairly large amount of loss in the form of sweating between 0.5 to 2 L/hour⁸. The electrolyte loss in this situation would be highly variable depending on the environmental and personal characteristics such as acclimatization.

Mismatch of the replaced solution and lost fluids could end up in electrolyte disturbances such as hyponatremia.

While replacing lost fluids the most obvious choice used to be an isotonic solution which matches the ECF concentration of sodium which is 135 to 145 mMol/L, copied adequately by Ringer lactate and Isotonic saline. Dextrose 5% as a replacement solution in dehydration has been shunned by various authorities because of obvious lack of logic and contra-indications.

Addition of glucose, however, to ORS is because of its unique action in the gut aiding the active absorption of electrolytes and water. This has brought us to various other formulations for oral rehydration

custom-made to suit the kind of losses one is facing as per indications (Table 3).

The transition from a high osmolar ORS to a low osmolar ORS by WHO has already been substantiated widely. However, it appears appropriate that, scientific institutions are brought up-to-date to be able to formulate different combinations of custom-made ORS for replacement of fluids not only for Paediatric use, but, even for adults suffering from, say, heat illnesses.

Knowledge of what is lost from the body in terms of water and electrolytes can be easily made use of in the custom construction of a suitable ORS formulation in the given situation. This can suitably be extended to the design of better intravenous solutions as well.

Table 3: Formulations of Oral rehydration salts

	Na	K	Glucose	Indications
WHO original ORS	90	20	111	Cholera/ Diarrhoea
WHO Reduced Osm. ORS	75	20	75	Diarrhoea/ Vomiting
Pedialyte ⁹	45	20	140	Diarrhoea/ Vomiting
Gatorade sports drink ⁹	23.5	<1	(40)gm/L	Sweat loss

The required maintenance amount of the water to keep the balance is another issue which has to be enforced to be able to reduce voluntary dehydration among people.

An allowance of 50 ml/100 cal/day will replace insensible loss of water in the normal course of daily routines, and 66.7 ml/100 cal/day will replace the average renal loss so that the total requirement is about 116.7 ml/100 cal/day.

As water of oxidation will supply approximately 16.7 ml/100 cal/day, the remaining 100 ml/100 cal/day must be supplied to meet the remaining water losses especially among patients on parenteral fluid therapy.

It is generally agreed that the maintenance requirements for water of individuals is determined by their caloric expenditure. By means of the following formulae, the caloric expenditure of hospitalized patients can be determined from weight alone.

For weights ranging from 0 to 10 kg, the caloric expenditure is 100 cal/kg/day; from 10 to 20 kg the caloric expenditure is 1000 cal plus 50 cal/kg for each kilogram of body weight more than 10; over 20 kg the caloric expenditure is 1500 cal plus 20 cal/kg for each kilogram more than 20¹⁰.

Conclusion

The net body water balance is regulated remarkably well day-to-day as a result of thirst and hunger in most normal people. Fluid loss from human body can occur under various circumstances. For designing an ideal rehydration solution we need to know the type and amount of fluid lost with its electrolyte content. It may be possible to customize the replacement fluid for our local epidemiological conditions.

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✦ QUIZ

Paediatric Dermatology

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1. A seven year old child with history of recurrent respiratory infections is admitted with pneumonia. He has grade III malnutrition, 2nd degree stunting, generalized lymphadenopathy and mild hepatosplenomegaly. He has peculiar skin lesions mainly in both upper and lower limbs (Fig. 1 & 2). Biopsy pictures (low power and high power) are also shown (Fig. 3 & 4). What is the most probable diagnosis?



Fig. 1



Fig. 2

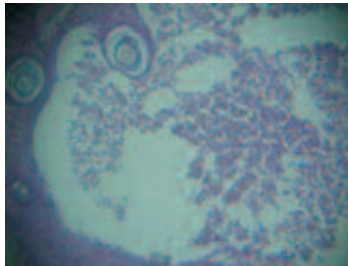


Fig. 3

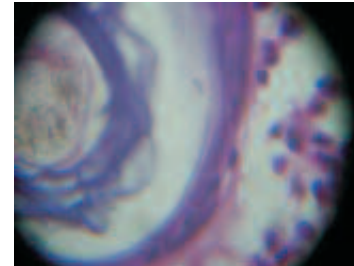


Fig. 4

2. A five year old boy is admitted with pain in both ankles for four days, reddish rashes on lower legs, feet (Figure 5, 6) and hands for three days, and severe abdominal pain of one day duration. What is the probable diagnosis?



Fig. 5



Fig. 6

Answers

1. Eosinophilic folliculitis associated with HIV; also known as sterile eosinophilic pustulosis. Itchy rash of unknown aetiology, occurs most commonly in children with positive HIV serology. It is one of the AIDS defining illnesses occurring in HIV positives with advanced immunosuppression. Similar problem occurring in HIV negative individuals is known by the name 'Ofuji's disease' and is seen in Japanese people.
2. The classic triad of Henoch Schonlein purpura, also known as anaphylactoid purpura. It is a systemic small vessel vasculitis of unknown aetiology.

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ISSN 0976-402X



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