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IJOPARB

Indian Journal of Perinatology and Reproductive Biology

Official Journal of Indian Society of Perinatology and Reproductive Biology

ISOPARB MIDTERM CONFERENCE at CHENNAI

From L-R: Dr Ratna Kumar, Dr Meena Samant, Dr Milind Shah & Dr Vijayalakshmi Seshadri

On 7th and 8th October, was attended by nearly 200 delegates.

The two workshops

- Risk Fetus
- Practical Skills in Obstetrics

The important topics in the Scientific sessions on Perinatology were very interactive. The Dr Rajkishori Jha Oration was delivered by Prof S Ratnakumar, Expert Advisor, Maternal Health, National Health Mission, State Health Socoety, Tamilnadu. The topic of oration was "Obstetrician as a perinatologist - is there a paradigm shift?" The Key Note Address on "How to Reduce Perinatal Morbidity in Twin Pregnancy" was delivered by Dr Milind Shah, President, ISOPARB.

Dr Savithri Subramaniyam, Prof of OBSTETRICS & GYNECOLOGY, Vijaya Hospitals, Chennai, was the Guest of Honour in the inauguration.

There were 22 outstation faculty from Kolkata, Cuttack, Patna, Bhubaneswar, Guwahati, Thrissur, Vijayawada, Hyderabad, Bangalore, Varanasi, Lucknow, Mumbai, Sholapur.

It was a satisfying academic meet of ISOPARB. The Organizing Chairperson was Dr Vijayalakshmi Seshadri. It was a great success with the leadership of President of ISOPARB, Dr Milind Shah and the Secretary General Dr Meena Samant, the Immediate Past President Dr Manju Gita Mishra and all the Senior and active members of ISOPARB.

Members & Faculties of ISOPARB at Midterm Conference in Chennai

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Editor's Choice

Indian Journal of Perinatology and Reproductive Biology (IJOPARB)

The months of September-October 2017, were the festive season for the whole country. To start with Ganesh Chaturthi, Id-Ud-Joha, Durga Puja, Dassera, Maharrum, Lakshmi Puja, Dipawali and ….. were the so many. Everybody was in the festive mood and away from daily routine. The main part of it was, relaxation, to enjoy good food, to meet friends, relatives and to exchange good wishes. However, at the end of this long holidays, we all are again back to work.

The next issue (2017) of the IJOPARB is at our hand. We have the professional responsibility to remain updated and competent where ever we do our clinical practice. IJOPARB is an important media of continuing professional development (CPD). This issue of IJOPARB has come out with a significant change in its contents when compared to its predecessors. In this issue we have got few articles of inter disciplinary nature. All these are away from the traditional boundary of Obstetrics and Gynecology.

It is our great pleasure to read the article "***Neonatal sepsis –a major challenge***" (p.14). The author, Prof Banerjee, is in charge of the neonatal unit at Calcutta National Medical College. His expertise and experience, I am sure, are to add more to our knowledge and understanding in diagnosing and managing such neonates with sepsis.

The editorial under, 'views and reviews' is the article entitled: "**Anti-Mullerian Hormone in Reproductive Biology**". It is a pertinent area of reproductive biology that we all, the specialists and the post graduates need to know. The other article '***Comments by the expert***', is very interesting and informative. When I stepped in, to work in the field of reproductive endocrinology under the able guidance of my Sir, Prof B N Chakravarty, I could see assessment of ovarian function is an important issue. During the session of laparoscopic pelvic evaluation, in such cases with sub fertility, I was told by him to comment on the ovarian morphology and volume also. In the early nineties, it was a routine with Sir to measure the D3 serum level of FSH, prolactin and thyroid function. I remember, we used to document the information of ovarian volume and the morphology as a routine. We learned, smaller the ovarian volume, lesser is the ovarian reserve. Presence of corrugations on the ovarian surface specially in a woman with age ≥ 36 yrs, suggest reduced ovarian reserve. Every one of us presently know plethora of information are available in the area of ovarian reserve assessment. However, controversy still remains. We need to know, how to rationalize our investigations in respect of an individual patient. Should we combine all the test results or consider the most important one? Is there a protocol to optimize our result for an individual woman while going for ovarian stimulation? Should we refuse treatment to a woman when the value of anti Mullerian hormone (AMH), is below the optimum level? Is AMH, the absolute biomarker to guide us?

We are too fortunate to have our Sir, Prof B N Chakravarty for his opinion as an expert in this difficult field of reproductive biology. Many areas are yet ill understood. We all must read the article '***Comments by the expert***' to enhance our understanding.

In the previous issue of IJOPARB, we had an article on '***Acute kidney injury in obstetrics***'. In continuation to that, we have included a review on '***Chronic Kidney***

Disease (CKD) and Pregnancy' by Dr Kataruka, DM (Nephrology), from Calcutta National Medical College, Kolkata. It will surely enrich our readers.

We look forward more such articles from the interdisciplinary field to update ourselves. Over all this issue of IJOPARB is very informative. We sincerely thank our teachers, Prof B N Chakravarty, Prof S Banerjee and Dr M Kataruka for their contributions in the Journal. We do expect the feedback from our readers, in the section '***Letters to the Editor***' for any query and comment.

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Views and Reviews

Anti-Mullerian Hormone in Reproductive Biology

Professor (Dr) Hiralal Konar

Anti-Mullerian Hormone (AMH) is a member of the transforming growth factor beta (TGF β) family. This peptide is produced by the granulosa cells of the preantral and small antral follicles up to 6 mm diameter. It is also secreted by the sertoli cells of the fetal testis. In male, it causes regression of the mullerian duct system. In a female fetus, it is detectable as early as 36 weeks of gestation.[1] Levels of AMH progressively rise after birth and it reaches the highest level after puberty. The secretion of AMH from the growing pre-ovulatory and the atretic follicles is low. It decreases progressively with age and is undetectable at menopause. Serum AMH levels are found to correlate strongly with the number of antral follicles in the ovary.[2]

With this observation, the levels of AMH is taken as an indicator of ovarian reserve.[3,4] Serum levels of AMH is cycle independent and can be measured at any phase of the menstrual cycle. This means that the intercycle and intracycle variations in AMH levels are insignificant. This is a distinct advantage compared to estimation of serum FSH, estradiol (E2) (as the follicle number declines). Inhibin (A & B) are secreted from the granulosa cells. Inhibin regulates FSH secretion through a negative feedback response to the pituitary. FSH is the main hormone to control folliculogenesis. However simultaneous LH secretion is essential as explained in two cell two gonadotropin theory of ovarian steriodogenesis.

AMH levels can vary in certain clinical situations.[5] Any woman using combined oral contraceptives (COCs) or GnRH analogues for a long period (more than an year), has been found to have low levels of AMH. However this change is a reversible one. Physiological function of AMH is difficult to assess. Experimental observations suggest that AMH is a negative regulator of follicular growth. It reduces follicular sensitivity to FSH. FSH exerts a negative influence on AMH secretion in small follicles.

Assessment of ovarian reserve is an important issue before any ovarian stimulation protocol or prior to any Assisted Reproductive Technique (ART) cycles.

Several parameters of ovarian reserve assessment are carried out before predicting any woman as poor responder. Commonly used parameters are: 1) Levels of cycles D_3 serum FSH (>10-15 IU/L), 2) D_3 serum levels of estradiol (>60-80 pg/ml), 3) Levels of AMH (≤3.6 to 7.8 P mol/L), 4) Low levels of basal serum inhibin B (< 40 pg/ml) 5) Antral follicle count (AFC) using TVS – low count. (total <3 from both the ovaries and ovarian volume <3 Cm^3, 6) Ovarian volume measurement (OVM) - decreased.

The main interest in measuring AMH before any controlled ovarian stimulation protocol (ART cycle) is to assess : a) Ovarian follicular pool reserve, b) Ovarian responsiveness to FSH and c) Prediction of any excess ovarian response to ovarian hyperstimulation syndrome (OHSS).

Studies have shown that AMH and AFC have high accuracy for prediction of poor ovarian response. However the superiority of AMH or AFC in terms of better predictive ability is unknown. AMH measurement is cycle independent. For AFC ultra sonographer must be a skilled one, for correct assessment of follicle detection, size determination

and counting. On the other hand, assay procedure of AMH present some problems. For that reason AMH values should be interpreted according to the reference ranges of specific laboratory. Values above and below the optimal range should be viewed and interpreted carefully. Presently two assay systems are available. Values below the optimal range are suggestive of low antral follicular pool with poor reproductive ability. Values above the optimal range suggests, poly cystic ovarian syndrome (PCOS). These women are associated with high antral follicular count. These women run the higher risk of ovarian hyperstimulation syndrome (OHSS).

Anti-Mullerian Hormone as a Predictor of Ovarian Reserve, Ovarian Responsiveness to FSH

Reproductive ability of a woman is strongly correlated with the chronological age as well with the ovarian reserve. Women with advanced age are generally considered as poor responder due to depleted follicular pool. Combined together, age (≥ 36 years) and reduced ovarian reserve (AFC < 5-7 follicle and low levels of AMH, (<0.5-1.1 ng/ml) are associated with poor reproductive outcome. However AMH values have shown wide variation in an individual with different age ranges. Studies have shown ovarian reserve may not always match with the chronological age. Variations may be due to genetic, autoimmune, ethnic or environmental factors and it may or may not be reversible.[6] Decreased AMH levels indicate reduced antral follicle count and hence reduced ovarian reserve, and it is independent of woman's age. It is relevant to mention that most studies, correlating AMH with reproductive outcome, have been done in the setting of an infertility clinic, IVF clinic or an ART center. These reports do not necessarily reflect the results of association for the population in general. However a good correlation exists when the different parameters of woman's age, AMH levels and AFC are combined together.

AMH as a Reliable Marker of Ovarian Reserve

AMH is thought to have the potential of predicting ovarian reserve. Studies are awaited to correlate the values of AMH with reproductive performance in general population rather than attending the fertility clinic.

Many centers have considered the cut off values of AMH for defining poor responder in clinical practice. This value ranges from 0.5 to 1.1 ng/ml (≤3.6 to <7.8 pmol/L). Whereas for AFC the values range from <5 to < 7 follicle.

Another interesting observation is over the same age range, AMH is positively but less closely correlated with the non-growing follicle (NGF) recruitment. However, this does not mean that an individual with high AMH levels will have lowest number of non-growing follicles. Over the years, several studies have established that basal D_3 serum AMH levels and number of oocyte retrieved in a woman with stimulation protocol is significantly better compared to a woman observed with D_3 serum FSH, estradiol (E2) or inhibin B.

AMH and Ovarian Responsiveness: AMH is commonly used now a days, in the setting of an fertility clinic, for controlled ovarian stimulation as it has a strong positive correlation with the number of oocyte retrieval. AMH can predict quantitative ovarian response. ***AMH values can be a guide for selection of stimulation protocol in respect of an individual patient***. Main advantage with this approach is AMH level can optimize the response and can avoid the over response and the complications like Ovarian Hyper Stimulation Syndrome (OHSS).

AMH and Optimum Ovulation Stimulation Protocol

Pretreatment AMH values can ensure optimal treatment protocol (long agonist, antagonist, flare or the other).

Women with Poor Ovarian Reserve (AMH: <5 pmol/L, DSL assay) : Optimal treatment strategy for the poor responder, is yet to be achieved. The woman need to be counseled. Maternal age is an important determinant. Options may be to use GnRH agonists with long acting gonadotropins (FSH) and hCG for oocyte maturation and the luteal phase support. The woman with low AMH have low risk of OHSS and therefore long acting gonadotropins, hCG can be used safely.

Women with Normal Ovarian Reserve (AMH: 7-20 p mol/L) are preferably considered for antagonist based approach. Risks of OHSS are there but to some extent small it is less.

Women with High Ovarian Reserve (AMH: >20 p mol/L) antagonist protocol is preferred. However these women run the higher risks of OHSS compared to the previous two categories.

AMH and AFC have been considered to have the similar predictive value. It is yet uncertain whether a woman could be refused treatment based on the ground that her AMH values are low. Low AMH values are the predictor of poor ovarian response. Many centers have evaluated the cut off value for AMH. It is yet unknown whether the variation due to genetic, auto immune, environmental and ethnic factors and the chronological age make the nomogram different and also the cut of value.

AMH Levels in Woman with PCOS

The association of high AMH levels in women with PCOS is not well understood. It is not yet clear whether it is the increased antral follicle numbers or the increased population of follicular granulosa cells per antral follicle or mainly the cellular sensitivity pattern, is the cause for rise. Women with PCOS with high AMH levels are associated with high androgen (hyper androgenemia) as well as high insulin (insulin resistance) levels. Attempts to reduce the androgen and insulin levels (metformin therapy) failed to reduce the AMH levels.[7]

Clinical approach of management in such women revealed that women with normal range of serum androgens and insulin sensitivity have normal range of AMH. These women do better with ovarian stimulation protocol compared to their counterpart with PCOS, hyper androgenemia and insulin resistance. It is yet to understand the reason for AMH gene over expression in such a subset of population with PCOS.

AMH, and Correlation with Controlled Ovarian Stimulation, Oocyte Retrieval and Implantation

Serum D_3 AMH levels have been strongly correlated with ovarian reserve and quantitative ovarian response to controlled ovarian stimulation (COS) than serum inhibin B, FSH and E2 levels.[8] Studies revealed a positive correlation between serum AMH around the time of hCG administration and the number of oocytes retrieved, fertilization rate, embryo score and the implantation rates.[9] Recent studies further related positively to the levels of AMH in follicular fluid with oocyte quality and embryo implantation. Follicular fluid AMH is found to be the better predictor of oocyte quality than the serum AMH.[10]

Summary

The peptide Anti-Mullerian hormone is secreted by the granulosa cells of the pre antral and antral follicles upto 6 mm diameter. Levels of serum AMH correlate strongly with antral follicle pool in the ovary.

This positive association is much superior than any other associations like patients' age, day 3 serum FSH, estradiol or inhibin B.[11] Woman's age, though considered as an important predictor of ovarian response, is not an absolute one. Women with similar age have wide variability in the pool of recruitable follicles.[12] AMH is an important biomarker and its role in reproductive endocrinology is multiple. It can reliably predict the ovarian response to different stimulation protocol for an individual woman. It is observed that basal D_3 serum AMH levels and number of oocyte retrieved in a woman with stimulation protocol is significantly better compared to a woman observed with D_3 serum FSH, estradiol (E_2) or inhibin B. However it is yet unknown whether AMH in isolation or combined with antral follicular count, improves the predictive value. AMH cut off value:- (0.5-1.1 ng/ml or ≤3.6 – 7.8 pmol/L)----- and for AFC is <5 - <7. Even with extreme cut off value, (women identified as poor responder), women still become pregnant and may have live births. Young woman defined as poor responder have better prognosis compared to the older woman.

Follicular fluid AMH values is observed to be a better predictor for oocyte quality compared to its serum values. Despite the promising role role of AMH in reproductive endocrinology, further studies are awaited for its wider clinical use.

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Comments by the Expert

Anti-Mullerian Hormone in Reproductive Biology – Utility in Clinical Practice

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Introduction

Even 15 years ago, AMH was considered primarily as a mullerian inhibitory substance (MIS) and its function was mainly concerned with mullerian regression and sexual differentiation in males only. But recently its role in controlling and prediction of ovarian function during women's reproductive period is gaining interest very fast. It is secreted in the female as a protein hormone by ***small pre-antral, large pre-antral and small antral follicles in the ovaries***. Apart from predicting ovarian reserve and ovarian responsiveness to stimulation, serum AMH values are being utilized for the diagnosis of pathophysiology of PCOs. Association between AMH and obesity has also been described. The level of serum AMH has also been utilized for recognition and diagnosis of granulosa cell tumor in females. In male, it has a specific indication for recognition of clinical situation of male hypogonadism in the pre-pubertal period.

Importance in Reproductive Medicine

ART, a revolutionary treatment for infertility has brought remarkable popularity of AMH in the field of reproductive medicine. ART is an expensive treatment. Each step of treatment of ART demands precision and perfection. Controlled ovarian stimulation (COS) is an integral step of ART. Since its introduction in early 1980s, there have been many modifications in the stimulation protocol but an ideal protocol has not yet been standardized. Current move is to personalize or tailor the protocol as it suits individual patient's requirement. For this, correct identification of a dependable 'marker' or 'predictor' is desirable for that patient for whom the stimulation protocol is expected to be designed. This refers to 'markers' for identification of 'ovarian reserve' and 'ovarian response' of the particular patient for whom the stimulation regime is to be formulated.

In the initial years of experience of ART practitioners, various markers were used and some of them are still being used today. These are – age of the patient, short menstrual cycle (ovarian ageing), baseline FSH, E2, inhibin, CC challenge test, previous ovarian surgery, poor response in previous IVF (if there is a history of previous IVF) etc. But none of these parameters either alone or in combination could precisely identify the marker of 'ovarian reserve' or type of 'ovarian response' – the patient will have following the specific type of stimulation she is expected to receive.

Changing Concept in 'Marker' Selection

Since identification of anti-mullerian hormone in the pre-antral and small antral follicles of the ovaries and their role in folliculogenesis, serum AMH has been found to be a dependable marker (at least more precise than other known markers) for identification and prediction of the patient's ovarian reserve and more importantly her 'ovarian response' to a particular

stimulation protocol. There are various reasons for this confidence. AMH, - unlike E2 or inhibin, is an autonomous product – not dependant on 'feed-back' mechanism like other ovarian hormones and therefore can be assessed on any day of menstrual cycle and the results of assessment on different day of menstrual cycle will not vary. Therefore, based on this result of AMH, patients may be classified as – ***(a) normal responder (b) hyper responder (c) poor responder***. However, it must be realized that in order to have a more convincing criteria, in addition to AMH, AFC and to a certain extent age of patient should be combined together for the categorization of patients with regard to their ovarian reserve and response. 'Reserve' and 'response' indicate 'quantity' and 'quality' of follicles respectively. AFC (antral-follicle count) denotes quantity (number of follicles) and AMH indicates quality of follicular response. The following table (Table-1) illustrates the practical utility of AMH and AFC for individualization of the treatment protocol in three categories of responders with different ovarian reserve.

Table-1

Low Ovarian Reserve AFC < 5 AMH < 1ng/ml		Normal Ovarian Reserve AFC : 5-15 AMH : 2-5ng/ml		High Ovarian Reserve AFC > 15 AMH > 5ng/ml	
Minimizing treatment burden		Maximizing success rate		Minimizing OHSS risk	
GnRH antagonist	Maximal FSH stimulation	Antagonist/ Agonist Protocol	Average Gn Stimulation	Antagonist protocol	Minimizing FSH stimulation

Comparative Role of Age, AFC and AMH in Predicting Markers as Ovarian Reserve and/or Ovarian Response as well as Selection of Starting Dose of Gonadotropins

The role of AMH and AFC in determination of ovarian 'reserve' and 'response' has already been emphasized. However, it is still recognized that apart from AMH, FSH and AFC, woman's age is also a commonly used clinical marker based on which the starting dose of gonadotropin has so long been calculated. Because with advancing age, women's ability to respond to ovarian stimulation usually declines. But women with similar age may have a wide variability in the pool of recruitable antral follicles. Therefore, age may not be the only important criteria based on which the dose of gonadotropin can be calculated. Other markers of ovarian reserve namely FSH, AFC and AMH are equally and may be better predictors for ovarian response. Amongst these three, the performance of AFC and AMH are superior than FSH in predicting the size of 'primordial follicle pool' and 'follicular recruitment rates'. Though age as marker of ovarian reserve has several advantages like lack of variability between cycle and perhaps it is an easy and inexpensive marker but age has the least ability to predict poor and hyper responders. Therefore for all practical purposes both AMH and AFC still stand out to be the most reliable predictors for ovarian response, - and therefore for selection of gonadotropin starting dose. The following diagram illustrates the starting dose of gonadotropin programming based on response predicted by AMH and AFC and classified as hyper, normal and poor responders.

Selection of protocol (antagonist/agonist) and starting dose of gonadotropin based on AMH and AFC values

Prediction based on AHM and AFC (same values as above (pmol/L)		
Expected high response	Expected normal response	Expected poor response

Suggested Treatment		
GnRH antagonist + 150IU FSH	GnRH antagonist + 200IU FSH	GnRH antagonist + 300IU FSH

Reasons for Selecting AMH and AFC Rather than Age and FSH as Superior Quality Markers

- The predictive markers commonly used to select the correct protocol and selection of drug and its dose are decided by:- age of the woman, outcome of previous attempt (if she had any), FSH, AMH, AFC and previous history of ovarian surgery etc
- Of these, who are having the first attempt, - age, FSH, AMH, AFC have been considered to be the reliable markers for ovarian response
- Though woman's ability to respond to stimulation declines with advancing age, age alone is not a dependable marker
- Because women with similar age may have wide variability in the pool of recruitable follicles
- A correlation between individual response to other three markers like FSH, AMH and AFC appears to be stronger and amongst these, AFC

and AMH appear to be more effective than FSH. Therefore, AFC and AMH have been accepted as the most dependable markers of ovarian response

Limitations

Having said so much in favor of AMH, - yet it must be admitted that there are limitations as well.

Even a few years back, AMH values were not standardized. Recently there has been an evolution of AMH assessment from laboratory versions to the commercially available diagnostic systems lab (DSL) and Immuno-tech Beckman Coulter (IBC) assessment. Currently published studies have used either the DSL or IBC assessment methods. But using these two different assay procedures have also created problems because values reported by different authors have varied substantially. IBC assay provides values of AMH which are higher than those provided by the DSL assay. Currently, the problem has been solved to a large extent as Beckman Coulter has purchased the patents of all previous versions and initiated AMH Generation-II assay. AMH Generation-II assay is highly specific and has been developed to standardize the measurement of AMH-between methods. A similar precision and excellent correlation between-assay agreement should be obtained when laboratory change from the DSL (diagnostic system laboratory) to AMH generation-II Elisa assay. Therefore it has been suggested that performance of AMH generation-II assay is ideal for determination of physiological role of AMH in men and women.

At present, in clinical practice, the normal level of plasma AMH has been accepted as 1-3 ng/ml. Levels between 0.7 to 0.9 ng/ml is recognized as low normal, while levels below 0.3 ng/ml is considered as very low level. Level above 3ng/ml is considered very high and may be a diagnostic marker for PCOS women. But still there is a wide variation in the level for clinical interpretation.

Ongoing Research for Better Ovarian Response Predictor

In future, in addition to conventional markers like AMH and AFC, genetic polymorphism, - such as single nucleotide polymorphism (SNP) may be more dependable marker of ovarian reserve or response. Information about polymorphism of the FSH receptor gene (FSH-R) is already available; and they may help in predicting the appropriate dose of FSH for individual woman. Other PCOS genes so far identified include AMH and AMH-receptor genes. From these studies, it appears that future attention in clinical research should focus on genetic prediction for individualization of COS protocol.

Review Article

Neonatal Sepsis—A Major Challenge

Prof (Dr) Syamal Kumar Bandyopadhyay[1]

Introduction

Sepsis is the commonest cause of neonatal mortality. Despite advances in maternal and neonatal care, infections remain a frequent and important cause of neonatal morbidity and mortality, particularly in a developing country like India.

Although blood culture is the gold standard for the diagnosis of sepsis, culture reports are available only after 48-72 hours. In this era of multidrug resistance, it is mandatory to avoid unnecessary use of antibiotics to treat non-infected infants. Thus, rapid diagnostic test(s) that include Interleukien-6 (IL-6), neutrophil CD64 index, procalcitonin and nucleated RBC count– and differentiate the infected infants from the non-infected, particularly in the early neonatal period– have the potential to make a significant impact on neonatal care. The aim of this review is to give a summary of recent diagnostic tests of sepsis, along with the preventive measures.

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, and accompanied by bacteremia in the first month of life.[1] Sepsis is the most common cause of neonatal mortality, and is responsible for 30-50% of total neonatal deaths, each year in developing countries.[2–4] The term neonatal sepsis, refers to the systemic infection of neonates including septicemia, pneumonia, meningitis, arthritis, osteomyelitis, and urinary- tract infection. As per National Neonatal-Perinatal Database (NNPD) (2002-2003), infection is the primary cause of mortality in 18.6% of intramural neonates, among which Klebsiella pneumonia is the most frequent bacterial isolate (32.5%), followed by Staphylococcus aureus (13.6%). Sepsis is 12 times more common in extramural admissions (39.7%). In extramural admissions, Klebsiella is the most prevalent bacteria responsible (27.5%), preceding S. aureus (14.9%). Sepsis is responsible for deaths in 38.0% of these extramural babies.[4,5]

Definition

National Neonatal Forum of India has defined neonatal sepsis as follows:4

1. Probable (clinical) sepsis: It is found in an infant having a clinical picture suggestive of septicemia if any one of the following criteria are present:

Existence of predisposing factors: Maternal fever, foul smelling liquor, prolonged rupture of membranes (>24 hrs), or gastric polymorphs (>5 per high-power field). The septic screen would be positive due to the presence of two or more of the five parameters namely, TLC (< 5000/mm), band to total polymorphonuclear cells ratio of >0.2, absolute neutrophil count < 1800/ml, C-reactive protein (CRP) >1mg/dl and micro ESR > 15 mm-first hour.

Radiological evidence of pneumonia.

2. Culture Positive Sepsis: In an infant having a clinical picture suggestive of septicemia, pneumonia or meningitis, if either of the following criteria are found:

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Isolation of pathogens from blood or CSF or urine or abscess(es)

Pathological evidence of sepsis in the autopsy.

Classification

Early Onset Sepsis (EOS)

EOS appears within 72 hours of age. Presence of foul smelling liquor or 2 or more of the perinatal risk factors should be investigated with sepsis screens, and be treated, accordingly.[6]

Late Onset Sepsis (LOS)

LOS usually appears after 72 hr of age. The source of infection is either nosocomial or community-acquired, and is present in neonates with septicemia, pneumonia or meningitis.[7,8]

Investigation

HRC monitoring [heart rate characteristics]

Research about cardiac electrical patterns has revealed that reduced variability and transient decelerations in heart rate may be early indicators of clinical instability, and are hypothesized to be mediated by the cholinergic anti-inflammatory pathway.[9] The HRC index is a statistically-derived interpretation of the beat-to-beat variation in a patient.10 A low index indicates normal variation, but as normal variation is lost, the index rises, and so does the risk of clinical deterioration.

A recent randomized controlled trial of >3,000 very-low-birth-weight infants revealed that the use of HRC monitoring significantly decreased the 30-day mortality rate after a septic-like event, without a significant increase in antibiotic days.[9] The mechanism by which mortality decreases in the monitored cohort remains unclear. In a separate study, neonates with culture-proven sepsis had a statistically higher HRC during the 24 hours leading to the septic episode, compared with the healthy control group.[10] However, neonates with culture-negative, septic-like events had a statistically similar rise in HRC. Therefore, HRC is able to serve as an early warning sign of impending clinical instability. Additional research is needed to determine if it can differentiate between true sepsis and a culture-negative, septic-like event.

Blood Culture: Blood culture is the gold standard for diagnosis of septicemia. It should be done in all cases before starting antibiotics.

Sepsis Screen:[11,12]

This is a panel of tests consisting of

Component	*Abnormal value*
TLC	< 5000/mm3
ANC	< as per Manroe chart for term and Mouzinho's chart for very LBW (VLBW) infants
Immature/total neutrophil	> 0.2
Micro-ESR	> 15mm in 1st hr
CRP	> 1mg/dl

All neonates, suspected to have sepsis, should have a septic screen to corroborate the diagnosis.

However, the decision to start antibiotics need not be conditional to the sepsis screen result if there is a strong clinical suspicion of sepsis. Sepsis screen is considered positive if two or more of these are positive. If the screen is negative but clinical suspicion persists, it should be repeated within 12 hours. If the screen is still negative, sepsis can be excluded with reasonable certainty. The absolute neutrophil count varies considerably in the immediate neonatal period, and normal reference ranges are available from Manroe's chart.[13] For very-low-birth-weight infants, the reference ranges are available from Mouzinho's charts.[14] Presence of two abnormal parameters in the screen is associated with 93-100% sensitivity, 83% specificity, and positive and negative predictive values of 27% and 100%, respectively in detecting sepsis.

In a recently published paper, the authors have evaluated the SNAP-II score for the assessment of illness severity which consists of 6 physiological parameters, namely lowest mean arterial pressure (MAP), worst ratio of partial pressure of oxygen (PaO2) to fraction of inspired oxygen (FiO2), lowest temperature (in ºF), lowest serum pH, occurrence of multiple seizures, and urine output (<1mL/kg/hr). They found that SNAP-II can predict mortality as well as organ dysfunction in severely septic neonates; Individual components of the score do not have equal predictive abilities.[15]

Lumbar Puncture (LP)

The incidence of meningitis in neonatal sepsis varies from 0.3-3%, in various studies.[4,7] In EOS, lumbar puncture is indicated in the presence of a positive blood culture, or when the clinical picture is consistent with septicemia. In case of LOS, LP should be done in all infants, prior to starting antibiotics. LP should not be done in the following cases:[16]

Asymptomatic babies being investigated for maternal risk factors; However, LP should be performed in these cases as well, if blood culture becomes positive, subsequently.
Premature neonates afflicted with respiratory distress syndrome (RDS); In this case, LP should be postponed in critically-ill and hemodynamically unstable babies; if traumatic, it should be repeated within 12-72 hours.

Urine culture

The rate of positive urine culture in infants with EOS is low. Given the low yield of positive urine culture results, and costs of processing the specimens, urine culture should not be part of the traditional sepsis evaluation in the first 72 hours of life.[17]

Radiology

Chest X-ray is done in cases of respiratory distress or apnea. Abdominal X-ray should be done for diagnosis of necrotizing enterocolitis.

The Most Recent Diagnostic Tests of Neonatal Sepsis

Isolation of bacteria from blood is the most specific and standard method used to diagnose neonatal sepsis. The drawback of culture based diagnosis is the 24–48 hour assay time. Newer diagnostic tests can be grouped into:

1. Acute phase reactants
2. Cell surface markers
3. Granulocyte colony-stimulating factor
4. Cytokines
5. Molecular genetics
6. Mol cell proteomics

Acute Phase Reactants

These groups of endogenous peptides are produced by the liver as part of an immediate response to the infection or tissue injury. These reactants are C-reactive protein, procalcitonin, fibronectin, haptoglobin, lactoferrin, neopterin and oromucosoid.

C-reactive Protein (CRP)

In a study, it was concluded that CRP, IL-6 and IgM are helpful in the early diagnosis of Gram-negative neonatal sepsis, although CRP continues to be the best single test. A CRP value of 5 mg/dl was the best among the three parameters with 95% sensitivity and 98% negative predictive value. The best combination was CRP ≥ 5 mg/dl and/or IgM ≥ 20 mg/dl. The use of both CRP and IgM in combination was the most helpful method in predicting Gram-negative neonatal sepsis which had a significant role in making decisions regarding antibiotic treatments.[18]

Another recent study was carried out in order to compare the efficiency of Serum Amyloid A(SAA) with that of C-reactive protein (CRP) and procalcitonin (PCT), in diagnosis and follow-up of neonatal sepsis in pre-term infants. The results showed that SAA is an accurate and reliable marker for the diagnosis and follow-up of neonatal sepsis. It is especially useful at the onset of inflammation for the rapid diagnosis of neonatal sepsis, and can be safely and accurately used in combination with other sepsis markers, such as CRP and PCT in diagnosis and follow-up of neonatal sepsis in pre-term infants.[19]

Procalcitonin

Both procalcitonin (2.3 ng/ml) and CRP (3 mg/dl) had high specificity and positive predictive values (97%, 91% and 96%, 87%, respectively), though with low sensitivity (48% and 41%, respectively) for sepsis diagnosis. The conclusion was that procalcitonin >2.3 ng/ml or CRP > 3 mg/dl indicates a high likelihood for neonatal sepsis, and antibiotic therapy should be continued even in the presence of sterile cultures. However, it is not a readily available diagnostic assay in most institutions.[20]

Cell Surface Markers

Neutrophil CD11b and CD64 appear to be promising markers for the diagnosis of early- and late-onset infections. For culture-positive sepsis episodes, the CD64 index had the highest area under the curve (0.852) of all hematological variables, with a

sensitivity of 80%, a specificity of 79%, and a cutoff value of 4.02. Therefore, neutrophil CD64 is a highly sensitive marker for neonatal sepsis. Prospective studies incorporating CD64 into a sepsis scoring system are warranted.[21] There is a 2–4 fold increase in neutrophil CD11b expression in infants with blood culture positive sepsis. The sensitivity and specificity of CD11b for diagnosing EOS are 96– 100% and 81-100%, respectively.

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (GCSF), a mediator produced by bone marrow, facilitates proliferation and differentiation of neutrophils, and has been proposed to be a reliable infection marker for early diagnosis of neonatal sepsis. A concentration ≥ 200 pg/ml has a high sensitivity (95%), and negative predictive value (99%) for predicting early onset neonatal bacterial and fungal infections.[22]

Cytokines

Newborn infants display a higher percentage of IL6 and IL8 positive cells than adults do. There is a sharp rise in IL6 concentration on exposure to bacterial products, which precedes the increase in CRP. Umbilical cord blood IL6 has consistently been shown to be a sensitive marker for diagnosing early-onset neonatal sepsis at the onset of infection, compared with other biochemical markers, including CRP, IL1ß, TNFα, and Eselectin, although sensitivity is reduced at 24 and 48 hours, since IL6 concentrations fall rapidly and become undetectable after 24 hours. The measurement of IL6 (early and sensitive) along with CRP (late and specific) in the first 48 hours of presumed septic episodes, improves the sensitivity compared with either of them alone.[23]

IL-6 levels may be useful in the initiation, as well as early termination of antibiotic therapy in late-onset neonatal sepsis.24 IL8 is considered to be a highly accurate marker with its sensitivity ranging from 80% to 91%, and specificity from 76% to 100.

Newborns developing early-onset infections are born with higher TNF-α concentrations than non-infected infants. Other markers studied over the last few years include adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, Eselectin, L-selectin), complement activation products (C3a-desArg, C3bBbP, SC5b-9), and IL-1alpha, IL-1beta, and IL-receptor antagonist (IL1RA), which have been found to significantly increase during sepsis, though these findings require further evaluation for clinical application in the diagnosis of newborns' infections. The combination of TNF-α and IL-6 provided a sensitivity of 98.5%, and it is a highly sensitive marker of sepsis in the immediate postnatal period.[25]

Molecular Genetics 26-29

Polymerase chain reaction (PCR) analysis relies on the fact that the bacteria specific 16S rRNA gene is highly conserved in all bacterial genomes, and so it can be useful for identification of bacteria in clinical samples. Amplification targeting of this 16S rRNA gene is a potentially valuable clinical tool in samples with low copy numbers of bacterial DNA, as this gene is present in 1 to more than 10 copies in all bacterial genomes. The gene also has a number of divergent regions nested within it, so PCR can be targeted for species-specific detection of bacteria in clinical samples. This technology has also been reported to be a very sensitive (100%), and rapid method for detecting potential pathogens in amniotic fluid, commonly involved in the pathogenesis of pre-term labour and adverse neonatal outcomes.[26]

Early exclusion of bacterial infection could help to reduce overuse of antibiotics. It is predicted that eventually real time PCR combined with DNA Micro Array technology will allow not only identification of the organism but also the antimicrobial sensitivity pattern, which is so critical to clinical care. It has been revealed from an Indian study that PCR is useful, and superior to blood culture for early diagnosis of sepsis in neonates with 100% sensitivity and 100% specificity. Once available in most tertiary centres, PCR can help in early and accurate diagnosis.[29]

Role of Proteomics for Diagnosis of Neonatal Infection[30–31]

In a study, it was found that significant alterations in levels of eight serum proteins in infected pre-term neonates (specifically P- and E-selectins, interleukin-2, soluble receptor α, interleukin-18, neutrophil elastase, urokinase plasminogen activator, its cognate receptor, and C-reactive protein) were observed at statistically

significant increased level.[30] The presence of S100A12 and S100A8 in amniotic fluid is predictive of early-onset neonatal sepsis and poor neuro -developmental outcome.[31]

Management

Along with supportive general management and appropriate and specific antibiotic therapy the newer advances and adjuvant therapies in management of sepsis are-

Intravenous Immune Globulin (IVIG)
IgM enriched IVIG
Granulocyte colony-stimulating factor (G-CSF)
Exchange transfusion
Pentoxifylline.

Advances in Prevention

Before Delivery

Maternal immunisation is an important method of providing neonates with appropriate antibodies, as soon as they are born.[32] This approach, in comparison with other approaches, is less sensitive to obstacles in accessing the health care system. Examples of successful interventions include maternal tetanus toxoid, and influenza immunisations. Studies of maternal immunisation with S. agalactiae type III conjugate vaccine have demonstrated excellent placental transfer and persistence of protective levels in 2-month-old infants.[32] Encouraging results are also emerging from studies of maternal immunisation with pneumococcal polysaccharide and conjugate vaccines.[33] The vaccines all have excellent safety profiles. However, barriers to maternal immunisation include: liability issues for vaccine manufacturers in developed countries; education of the public and health care providers regarding the benefits of maternal immunisation; and poor ascertainment of data from low-income countries.[32] Although they are not yet commercially available, several vaccines are close to being released and will hopefully prove to be efficacious in decreasing the rates of EOS and LOS, caused by GBS.[34]

During Labour and Delivery

There is strong evidence that clean delivery practices and handwashing during delivery reduces rates of neonatal sepsis both at home and in health facility settings.[35] Interventions to improve hand washing rates have been remarkably successful in research settings.[35] New studies from Malawi and Nepal indicate that maternal antisepsis interventions such as vaginal chlorhexidine during labour may have a significant impact on rates of neonatal mortality and sepsis in developing countries.[36] Intrapartum antibiotic prophylaxis has been highly effective in reducing both early-onset neonatal bacterial and maternal sepsis in developed countries.[37]

After Delivery

There is also strong evidence that hand washing by health care providers after delivery can reduce neonatal sepsis and infection rates, especially in hospitals.[35] Umbilical stump chlorhexidine cleansing has recently been shown to substantially reduce neonatal deaths in Nepal.[38] There is emerging evidence that neonatal skin antisepsis preparations, such as sunflower seed oil provides cheap, safe, and effective protection against nosocomial infections in hospitalized pre-term neonates, and infants in studies in South Asia. Application of chlorhexidine to neonatal skin has also been shown to be effective in reducing neonatal sepsis in studies from South Asia.[39] Neonatal immunisation has long been considered an important method of reducing neonatal infections. However, the response varies according to the antigen.[40] BCG, polio, and hepatitis B vaccines are highly immunogenic when given at birth.[41] Breast milk contains secretory IgA, lysozymes, white blood cells, and lactoferrin, and has been shown to encourage the growth of healthy lactobacilli and reduce the growth of E. coli and other Gram-negative pathogenic bacteria.[40] RCTs that focused on increasing early initiation and exclusive breastfeeding rates demonstrated significant reductions in diarrhoea and acute respiratory infections in neonates and older infants in India.[42] Neonatal micronutrient supplementation trials have focused on vitamin A supplementation. Older studies have shown significant reductions in respiratory disease in low-birth-weight infants after the administration of parenteral vitamin A.[43] More recently, trials of vitamin A supplementation in newborns have shown encouraging reductions in neonatal mortality, and more trials are underway.[44]

Conclusion

As the cost of management of neonatal sepsis is a burden to a developing country and even after treatment of sepsis a child can have an adverse neurodevelopmental outcome, it is always better to prevent the sepsis by proper maternal care, early accurate diagnosis, proper hand hygiene, awareness, proper antibiotic administration policy.

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Review Article

Chronic Kidney Disease and Pregnancy

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Introduction

Incidence of chronic kidney disease (CKD) is increasing worldwide. Similarly, incidences of renal diseases are increasing in pregnant and pre-pregnant women. Presence of renal disease may alter the course of pregnancy and both maternal and fetal outcome. Increase in incidence may be due to increase use of screening test and increase in risk factors in the pregnant and pre-pregnant women. Many women are diagnosed to have renal disease during their pregnancy due to universal screening of blood pressure and proteinuria. Pre-eclampsia and eclampsia occurs at a higher incidence in patients with kidney disease and occurrence of both the condition may deteriorate the pre-existing kidney disease in pregnant women. Incidence of CKD in women aged between 20-39 years in United States is estimated to be 3%[1] and in UK, it is estimated to be 2% (Health and Social Care Information Centre, 2010). Incidence of CKD in Indian subcontinent is not available due to lack of central registry. Pregnant women with CKD are at a higher risk of complication and adverse outcome compare to healthy pregnant women.[2] Advances in the knowledge and treatment of CKD has resulted in better pregnancy and fetal outcome compared to past, consequently now most of the pregnant females with CKD had a successful pregnancy outcome.

Factors Affecting Pregnancy Outcomes

Predictors of adverse events in pregnancy include:

- Severity of renal disease
- Severity of proteinuria
- Severity of hypertension
- Development of pre-eclampsia
- Previous poor obstetric history

Proteinuria

Proteinuria is a known risk factor for CKD progression. In pregnancy, proteinuria in CKD patients is associated with worse outcome. During pregnancy, in CKD patients, proteinuria may aggravate due to increased renal blood flow and cessation of ACE inhibitors. About 30% of non proteinuric CKD patients may start excreting significant amount of protein during pregnancy. Both pregnancy and proteinuria are a pro-thrombotic condition. So anti-coagulation therapy with low molecular heparin may be considered in patients with proteinuria more than 2gm per day or serum albumin less than 2gm/dl.

Hypertension

Incidence of chronic hypertension in general population is increasing. Pregnant women with chronic hypertension have a poorer pregnancy outcome compared to the normotensive pregnant woman. New onset hypertension is common in normotensive CKD patients during pregnancy. Previously hypertensive CKD patients have higher incidence of pre-eclampsia and eclampsia during pregnancy compared to non CKD control. Target blood pressure control in CKD pregnant patient has not been validated but experts

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recommends a target of 120-139/70-85 mmHg for all women with CKD.[3]

Pre-eclampsia

Diagnosis of pre-eclampsia in pregnant lady with pre-existing CKD is difficult. Both CKD and pre-eclampsia can present with hypertension and proteinuria. Diagnosis of superimposed pre-eclampsia in CKD patients can be suspected by sudden aggravation of proteinuria or decline in renal function after 20 week of pregnancy. Decrease platelet count and deranged LFT also points towards superimposed pre-eclampsia in CKD patients.[4] Soluble fms-like tyrosine kinase receptor (s-Flt) and placental growth factor (PlGF) concentration ratios are lower in women with CKD compared to women with pre-eclampsia.[5] Thus, these markers can be used to distinguish between CKD and pre-eclamsia.

Incidence of pre-eclampsia increases with decline in renal function. Presence of hypertension and proteinuria also increases the risk of superimposed pre-eclampsia in CKD patients. Women with lupus nephritis, renal transplants and reflux nephropathy appear to be at increased risk of pre-eclampsia. Women with CKD and pre-eclampsia should be admitted for assessment of maternal and fetal assessment. Multi disciplinary consultation should be done to assess the maternal risk of deteriorations of renal function and neonatal risk of preterm delivery.

Management

Pregnant women with CKD should be managed by obstetrician along with consultation of nephrologist and neonatologist. When a CKD woman becomes pregnant, her medication prescription should be reviewed and all teratogenic medicines like ACE inhibitors and others should be stopped. Blood pressure should be strictly controlled with systolic blood pressure less than 140 mm of Hg. Diastolic pressure less than 70 mm of Hg should be avoided due to risk of fetal compromise. All patients should be started on aspirin 75mg daily. Thrmboprophylaxis with low molecular heparin should be considered in patient with serum albumin less than 2 gm/dl or having proteinuria more than 2 gm per day. Vitamin D deficiency should be corrected with cholecalciferol and/or 1-alpha-calcidol depending on level of renal impairment. UTI should be promptly treated with proper antibiotic and antibiotic prophylaxis should be continued after one episode of UTI. Hemoglobin should be maintained around 10-11 gm/dl. Oral or intravenous iron therapy should be continued. Erythropoietin should be started or dose should be increased if desired haemoglobin is not maintained with iron therapy. Creatinine should be monitored every monthly till 32 week and then every fortnightly. Dialysis should be initiated if blood urea > 20 mmol/L, or problems with hyperkalemia or acidosis or fluid balance are detected. Hemodialysis in pregnant women are given in gentle manner with slower blood flow and dialysate flow. More frequent hemodialysis (5 times a week) are required in pregnant patients. Patients who are on peritoneal dialysis can be continued in peritoneal dialysis if there is no problem in inflow and outflow. Babies of women on peritoneal dialysis have higher birth weights and patients have fewer episodes of pre-eclampsia but are more likely to deliver preterm.[6] If there are problems in flow or adequate dialysis is not achieved with peritoneal dialysis, patients can be shifted to hemodialysis. Fetal growth should be regularly monitored. It is unusual for CKD to be a valid indication for cesarean section; however, operative deliveries are common. Water soluble vitamins including 5 mg folic acid should be prescribed to compensate dialysis loss. New fistula should not be created during pregnancy due to possible combined effects of increased cardiac output and circulating relaxin.

In the post natal period, aspirin can be stopped if no other indication. Thromboprophylaxis should be continued up to 6 week post natal. Blood pressure should be rigorously controlled and post natal renal function should be monitored.

Maternal Renal Outcomes According to Pre-pregnancy Serum Creatinine

Pre-pregnancy serum creatinine <1.5 mg/dl (130 μmol/l): Progression to dialysis and remain dialysis dependent post nataly is seen in less than 10% women. Uncontrolled hypertension, proteinuria > 500 mg/day and GFR < 40ml/min/ m2 are associated with higher renal decline. There is 40% risk of pre-eclampsia if baseline proteinuria >500 mg/day.

Pre-pregnancy serum creatinine 1.5-2.5 mg/dl (130-220 μmol/l): Decline or permanent loss of GFR in 30% of women. Chances of renal function

decline increases to 50% if hypertension remains uncontrolled. Nearly 10% will have ESRD soon after pregnancy.

Pre-pregnancy serum creatinine more than 2.5 mg/dl (>220 μmol/l): Almost every patients progresses to ESRD near term or post pregnancy and remains dialysis dependent.

Fetal Outcomes According to Pre-pregnancy Serum Creatinine

Pre-pregnancy serum creatinine <1.5 mg/dl (130 μmol/l): 90% of female will have a live birth. But 50% will have premature labor and 60% will have small for gestational age babies.

Pre-pregnancy serum creatinine 1.5-2.5 mg/dl (130-220 μmol/l): 85% will have live birth but 60% will have premature birth. Complication rates are higher with uncontrolled hypertension and pre eclampsia.

Pre-pregnancy serum creatinine more than 2.5 mg/dl (>220 μmol/l): Fetal loss is high but exact estimate is unknown.

Pre-pregnancy Counselling

Advances in treatment had improved the outcome of pregnancy in CKD patients with successful outcomes. However, all women with CKD remain at higher risk of complications compared with healthy pregnant ladies. all women with CKD should be offered pre-pregnancy counselling in order to discuss the following:

- The effect of renal disease on pregnancy, including maternal and fetal complications
- The effect of pregnancy on renal disease
- Safety of medication

All women with CKD who are preparing for pregnancy should be counselled for accelerated decline in renal function, increased risk of pre-eclampsia and flare up of glomerulonephritis. They should also be counselled for risk of pre maturity and other fetal complications. Women with advanced renal failure (serum creatinine more than 2.5 mg/dl) should be counselled for use of contraceptives.

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Original Article: Obstetrics

A Randomized Controlled Trial to Evaluate Efficacy of Moderate versus High Dose Ursodeoxycholic Acid in Treatment of Intrahepatic Cholestasis in Pregnancy

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ABSTRACT

Objective: Intrahepatic cholestasis in pregnancy (ICP) is a hepatic disorder of pregnancy associated with maternal morbidity and adverse foetal and perinatal outcomes. Ursodeoxycholic acid (UDCA) in varying doses has been used for treatment of ICP but limited information about the optimum dose is available. This randomized controlled trial was undertaken to evaluate the comparative efficacy of 600 mg versus 900 mg of UDCA in ICP.

Methods: Diagnosed ICP cases with <34 weeks of gestation at presentation were randomized to receive either 600mg (300mg twice daily) or 900 mg (300 mg thrice daily) of UDCA daily. Changes in pruritus score, serum AST, ALT and bilirubin levels from baseline to delivery were noted. Perinatal outcomes monitored were gestational age at delivery, preterm delivery, birth weight, meconium stained amniotic fluid and NICU admission rates.

Results: Fifty four subjects were recruited with 27 evaluable per group. Within group comparison showed significant reduction in pruritus score, AST, ALT and bilirubin levels following therapy. Between groups comparison showed a statistically significant difference in ALT (p=001); birth weight (p=0.006), gestational age at delivery (p=0.001), NICU admission rates (p=0.003), meconium stained amniotic fluid (p=0.001) and LUCS deliveries (p= 0.010).The 900 mg dosing group showed better effects compared to the 600mg group with regard to the above.

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Conclusion: This RCT has demonstrated that 900 mg of UDCA was better than 600 mg of the drug in reducing risk of fetal and neonatal adverse outcomes. Improvement in pruritus scores and some liver enzymes though better in the higher dose group were comparable.

Key words: Intrahepatic cholestasis of pregnancy, ursodeoxycholic acid, efficacy, randomized controlled trial

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder of pregnancy observed during the late second or third trimester of pregnancy. It is characterized by significant pruritus of varying intensity with or without associated systemic symptoms. Impaired liver function with raised liver enzymes and bile acids levels are noted in the absence of other underlying liver diseases.[1,2] The clinical symptoms and biochemical abnormalities spontaneously resolve shortly after delivery but may rarely be delayed by a few months postpartum. The disease is associated often with obstetric complications and adverse fetal and perinatal outcomes.

The incidence of ICP varies widely and a published report of 2009 estimated it to be about1.5-4%.[1–3] The pathophysiology of ICP is not well defined and include genetic, environmental, hormonal and oxidative stress related factors.

The treatment of ICP is also not well defined and include both systemic agents like antihistaminincs, phenobarbitone, cholestyramine, dexamethasone, s-adenosylmethionine (SAM), ursodeoxycholic acid (UDCA), herbal agents and topical preparations like emollients and calamine lotion.[4,5]

UDCA is a naturally occurring bile acid which is hydrophilic in nature. It has been suggested that UDCA treatment significantly reduces serum taurocholic and taurodeoxycholic acid concentrations by displacing more hydrophobic endogenous bile salts from the bile acid pool which thereby protects the hepatocyte membrane from the damaging toxicity of these bile salts. It also enhances bile acid clearance across the placenta as evident by lower cord blood bile acid levels which leads to reduction of the deleterious effects of the raised bile acids in the fetus.[5,6] Other proposed mechanisms include stimulation of biliary secretion by post-transcriptional regulation of BSEP and the alternative exporters MRP4 and MRP3. It has also been proposed to have antiapoptotic effects and to reduce mitochondrial membrane permeability to ions and cytochrome c expression.[7,8] UDCA also results in a normalization of the cholic acid: chenodeoxycholic acid and glycine: taurine ratios.[9] UDCA treatment has also been shown to reduce the bile acid level in cord blood,[74] amniotic fluid[10] and colostrum.[11] Additionally in vitro model studies suggest that UDCA may protect cardiomyocytes from bile acid-induced arrhythmias.[12]

The safety profile is good as serious maternal and fetal adverse effects are infrequent with UDCA treatment. However, though rare, at high doses women may complain of gastrointestinal effects like indigestion, abdominal discomfort, vomiting and diarrhea.

Some trials have demonstrated the beneficial role of UDCA singly or in combination with other drugs for the management of ICP.[4,5,13,14] However, various doses ranging from 500 mg to 2000 mg have been tried but the most frequent being 600 mg daily. In clinical practice it has been observed that effective control of symptoms and reduction of liver enzymes are often not achieved with this dosing strategy so this study was conceived to compare the efficacy and safety of 600 mg versus 900 mg daily of UDCA in the management of ICP. Accordingly, the study objectives of this randomized, parallel group, open label controlled trial were:

1) To compare the effects of 600 mg versus 900 mg daily ursodeoxycholic acid in controlling pruritus and reducing raised liver enzymes in subjects with ICP.
2) To compare the maternal and foetal outcomes of these two dosing strategies.

Accordingly, the study assessment outcomes were:

1) Pruritus score on a 0 to 4 likert scale where 0 (absence), 1 (occasional), 2 (discontinuous

pruritus every day with prevailing asymptomatic lapses), 3 (discontinuous pruritus every day with symptomatic prevailing lapses), 4 (constant pruritus).

2) Changes from baseline to delivery, of liver enzymes (AST and ALT) and serum bilirubin.
3) Maternal outcome assessment by noting preterm delivery rates, operative delivery rate and incidence of postpartum hemorrhage in each study group
4) Fetal outcome assessment by comparing the gestational age at delivery, late fetal heart rate deceleration, meconium stained liquor, APGAR, birth weight and duration of NICU stay.

Methods

This randomized, parallel group, open label, controlled trial was undertaken at a tertiary care teaching hospital of eastern India with prior approval from the Institutional Ethics Committee.

The subject inclusion criteria were: patients with < 34 weeks of pregnancy (as determined by LMP or dating USG scan) presenting with moderate to severe pruritus and raised liver enzymes with a clinical diagnosis of ICP. The exclusion criteria was gestational diabetes, pregnancy induced hypertension, preexisting liver, gall bladder diseases and dermatological diseases, viral markers positive for active or chronic hepatitis B,C virus infection and multiple pregnancy.

Randomization sequence was generated online random number generator software and allocation concealment by SNOSE (serially numbered opaque sealed envelope) method. Unstartified randomization method was adopted. The study was open label but in order to eliminate bias it was assessor blinded i.e. one of the investigators who assessed pruritus score and monitored the other study outcomes were unaware of the allocation group. Similarly biochemical laboratory personnel were unaware of treatment assignment groups. The test group subjects received ursodeoxycholic acid tablets at a dose of 300 mg thrice daily versus the control group which received 300 mg twice daily orally till delivery. The manufacturer of the medication was Win-Medicare Company Limited, New Delhi, and it was provided free of cost from the hospital to all study participants. Subjects were not allowed to take any systemic antihistaminic, or other drugs known to have beneficial effects in ICP. Application of topical preparation was not allowed.

The subjects were screened and those fulfilling the study selection criteria were enrolled after taking written informed consent. Baseline clinical and liver function reports were noted and the study drugs were dispensed and all subjects were asked to attend follow up visits at weekly intervals. Pruritus score was assessed at each visit. All subjects were followed up till delivery and the maternal and fetal outcomes were recorded. Liver enzymes were assessed at regular intervals and the last report done prior to delivery was considered as the study end levels.

Adherence to treatment was assessed by pill counting method and only those subjects who had more than 90% compliance rate were considered assessable.

The sample size was calculated on the basis of anticipated difference in serum total bilirubin between two study arms at the end of 2 weeks treatment period. 21 subjects were required per group in order to detect a difference of 0.5 mg/dl in this parameter with 90% power and 5% probability of type 1 error. The standard deviation of serum total bilirubin was assumed to be 0.5 mg/dl on the basis of earlier study and 2 sided testing. Assuming 20% drop out rate, the recruitment target is being kept at 27 subjects /group.

Results

During the study period (May 2015 to June 2016), 65 subjects were screened for study eligibility and 54 were randomized to the two study groups. There were no loss to follow up and all subjects who were randomized were evaluable and an Intention to Treat (ITT) analysis strategy was adopted.

The baseline profile of the study population is enlisted in Table 1. The maternal age, gravida, gestational age at enrolment were comparable between the groups. 7.41% in test group, while none in the control group had a family history of ICP and this difference was statistically not significant (p= 0.49). Similarly, comparison of history of ICP in previous pregnancies showed that 11.11% patients in group A and 3.7 % in group B had a past history. This difference was not

statistically significant (p= 0.07). Both the groups were comparable in terms of gravidity.

Table 1:Baseline profile of study subjects

Variable	Group A (control) n=27	Group B (test) n=27	P value
Maternal age, yr (mean ±SD)	24.70	25.00	0.79
Gestational age at presentation (wk) (mean ±SD)	30.41 ±1.31	30.70 ±1.49	0.44
Family history of ICP n (%)	0	2 (7.41%)	0.491
Past history of ICP n (%)	3 (11.11%)	1 (3.71)	0.07

Group A: 600 mg UDCA; Group B: 900 mg UDCA;
ICP- intrahepatic cholestasis in pregnancy

Comparison of pre versus post treatment liver enzymes (serum alanine transaminase and aspartate transaminase) in both groups are shown in Table 2. The pre-treatment values in both groups were raised and comparable. In both the groups, when the pre-treatment ALT and AST levels were compared with the post treatment levels of the respective groups there was a statistically significant (p<0.001) reduction. However, between group comparison of post treatment levels of ALT were significantly lower in group B compared to group A (p=0.046) but the post treatment AST levels were comparable (p=0.30).

Table 2: Between group comparison of pre versus post treatment serum ALT, AST levels

Variable	Group A n=27 Mean ± SD	Group B n=27 Mean ± SD	P value
ALT Pre treatment	180.85±69.02	171.85± 60.27	0.612
ALT Post treatment	97.7±42.69	75.63± 36.50	0.046*
AST Pre treatment	152.44±65.4	134.44±60.97	0.30
AST Post treatment	68.93± 36.25	55.59±35.70	0.179

Group A: 600mg UDCA; Group B: 900 mg UDCA;
ALT- alanine transaminase; AST aspartate transaminase
*statistically significant

Comparison of median pruritus scores and bilirubin levels are shown in Table 3. In both groups there was reduction in the pruritus scores and bilirubin levels post treatment and it was statistically significant but when the comparison was made between the two groups, the reduction was not statistically significant with a p value of 0.10 for pruritus scoring and p value 0.06 for bilirubin level.

Table 3: Comparison of pruritus score and serum bilirubin between groups

Variable	Group A n=27 Median (IQR)	Group B n=27 Median (IQR)	p value
Pruritus Score Pre treatment	2 (1 to 2)	2 (1 to 2)	0.74
Pruritus Score Post treatment	2(0 to 1)	0 (1 to 1)	0.10
Serum bilirubin^ Pre treatment	0.8 (0.6 to 1.2)	0.6 (0.5 to 1.1)	0.06
Serum bilirubin Post treatment	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.6)	0.61

^Median values were compared as bilirubin data was not normally distributed. IQR: interquartile range

Comparison of maternal, fetal and perinatal outcomes are depicted in Table 4. In majority of the parameters the higher dose group showed better outcomes compared to the lower dose group.

The mean gestational age at delivery was significantly higher in group B compared to group A (p value of 0.001). Similarly, preterm delivery rates were also lower in group B compared to group A (p value of 0.012) which was also corroborated by higher mean birth weight of babies in group B versus group A (p value of 0.006).

Table 4: Comparison between groups of various maternal and perinatal outcomes

Variable	Group A (control) n=27	Group B (test) n=27	P value
Mean gestational age at delivery, wk (±SD)	36.81 ± 1.71	38.37 ±1.45	0.001*
Mean birth weight, g (±SD)	2600.74 ± 338.49	2806.11 ±155.24	0.006*
Preterm delivery	16 (59.26%)	6 (22.22%)	0.012*
LUCS delivery	17 (62.96%)	8 (29.62%)	0.010*
PPH	3 (11.11%)	1 (3.7%)	0.610
Meconium stained amniotic fluid	13 (48.15%)	1 (3.7%)	0.001*
Late fetal heart rate deceleration	2 (7.41%)	1(3.7%)	1.00
NICU admission	14 (51.85%)	3 (11.11%)	0.003

LUCS- lower uterine segment caesarean section; PPH- postpartum hemorrhage; NICU- neonatal intensive care unit; Numbers shown are n (%) unless otherwise specified. *statistically significant

Mode of delivery was compared between the groups. In the control group the spontaneous vaginal delivery rate was 25.92 % while in the group B it was 66.67%. The higher spontaneous vaginal delivery rate in

group B is indicative of better delivery outcomes. The percentage of LUCS was significantly higher in group A (62.96%) compared to group B (29.62%) and this difference was significant (p value of 0.010).

Only 3.7% of deliveries in group B were meconium stained indicating better fetal outcomes compared to the control group. However, babies who had late fetal heart rate deceleration were comparable between groups. There were no still births or neonatal deaths. The NICU admission rates were also lower in group B compared to the control group. The safety profile was comparable. There were only 2 subjects in the 900 mg group who reported indigestion and loose motion of mild intensity after 3 to 4 days of treatment initiation which resolved spontaneously while in the 600 mg group one subject complained of diarrhea which did not need any treatment (p>0.05). Thus the study results show better fetal and perinatal outcomes in the 900 mg dose group compared to the 600mg dose levels.

Discussion and Conclusion

The use of UDCA in the treatment of ICP has been empiric and published data from observational and interventional studies have documented its efficacy in controlling maternal pruritus and reducing elevated liver enzymes but data of its effects on fetal and perinatal outcomes is limited. UDCA has been used in varying doses globally, but no optimum dose finding studies have been undertaken in Indian population. This randomized controlled trial was undertaken in a tertiary care hospital of eastern India to compare two dosing levels of UDCA in diagnosed cases of ICP to evaluate their effectiveness in controlling maternal pruritus and raised liver enzymes. Additionally, its beneficial effects on fetal and perinatal outcomes were also evaluated.

The results of our study indicate that there was a significant reduction in both pruritus and raised liver enzymes from pre-treatment levels in both groups. The 900 mg daily dose produced greater reduction of pruritus, serum bilirubin and AST level compared with 600 mg dose but the difference was not statistically significant. However, a statistically significant lowering of ALT levels was observed in the group comparison indicating that compared to the 600mg daily dose the 900 mg dose was more effective in lowering the elevated ALT levels.

Comparing the maternal and fetal adverse outcomes in the two groups have yielded better effects for the 900mg daily dose levels with comparable safety profile. The rates of meconium stained amniotic fluid, preterm delivery and caesarean deliveries were lower in the 900 mg dose group. The gestational age at delivery, birth weight were higher in the 900 mg dose compared to the 600 mg group and the difference was statistically significant. The neonatal intensive care unit admission rate was significantly lower in the higher dose group.

We compared the results of our studies with other published studies where UDCA was used as the primary treatment in ICP. Majority of the published trials have evaluated the effects of UDCA with either placebo or with SAM and the daily doses of UDCA used in these studies was variable and ranged from 500 mg to 2000 mg.

A Cochrane systematic review[13] published in 2013, on ICP has highlighted that in five trials where UDCA was compared to placebo as treatment there was a significant reduction in pruritus but no significant difference was observed in fetal distress. There were fewer preterm births but spontaneous pre term birth rates were also not significant.

Diaferia et al[14] had conducted a placebo controlled trial of 600 mg twice daily UDCA in a small sample of 16 patients and all newborns had an Apgar score >7 and normal postnatal growth. Another placebo controlled randomized controlled trial by Palma et al[5] where a mean daily dose of 16 mg/kg body weight of UDCA was administered in three divided doses for a period of three weeks. The results showed that subjects on UDCA had a statistically significant improvement in pruritus, serum bilirubin, and AST and ALT levels. The pregnancy outcomes were also better than the placebo group. A semifactorial randomized controlled trial conducted in UK[15] evaluated the effects of UDCA (500mg twice daily) versus placebo with early term delivery versus expectant management. The results showed significant better outcomes in the UDCA group compared to placebo. Our study was mainly directed to provide insights about the optimal dose of UDCA in Indian pregnant mothers with ICP and the results were comparable to several international studies.

To the best of our knowledge this is perhaps the only randomized controlled clinical trial which was undertaken in Indian population to compare two dosing schedules of UDCA in ICP. The study was also adequately powered to detect any significant difference between these two doses. The study has few limitations which include our inability to do blinding due to logistic constraints but we tried to minimize bias by having it observer blinded. Secondly, we could not evaluate all biochemical parameters suggested to have a diagnostic or prognostic role associated with ICP.

We can conclude that this RCT has provided insights in the use of UDCA in ICP. The 300 mg thrice daily dose had similar efficacy in controlling maternal pruritus compared to 300 mg twice daily but the higher dose had better effects with regard to pregnancy and fetal outcomes.

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DECLARATION:

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Original Article: Gynecology

Prevalence and Presenting Features of Genital Tuberculosis among Gynecology Inpatients: a Retrospective Study in a Teaching Hospital

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ABSTRACT

Objective: The objectives of this study were to study incidence of tuberculosis of female genital tract with various clinical presentations, and evaluate treatment outcomes.

Method: All newly diagnosed patients of genital tuberculosis over a period of 5 years were studied retrospectively. During study period, 30 cases were diagnosed as and treated for genital tuberculosis. Diagnosis was confirmed by tissue culture or histopathology.

Results: Incidence of genital tuberculosis was 0.16% of all gynecological admissions over 5 years. The most frequently encountered clinical presentation was infertility (37%). Of the 30 patients, 25 (83%) underwent combined hysterolaparoscopy. Open surgery was done in rest 5 (17%). Lap dye test showed no spillage in 23 cases (76.7%). Hysteroscopy was abnormal in 9 patients (30%). The commonest operative finding was presence of peritoneal nodules (43.3%). Histopathology showed 21 cases positive for tubal infection (70%) while endometrial disease was seen in 9 (30%). Out of 30 patients, 12 were lost to follow up. Five out of 18 patients (27.8%) conceived spontaneously after completion of antitubercular therapy.

Conclusion: Genital tuberculosis is rare. It is imperative to not miss a single case as tuberculosis of reproductive tract is a treatable chronic infection with good outcomes.

Keywords: Tuberculosis, genital tract, hysterolaparoscopy.

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Introduction

Genital tuberculosis in females is believed to be a rare entity. However as India continues to be a major repository of tuberculosis infection, genital tuberculosis (TB) may be commoner than believed. According to 2015 data, India has 2.8 million cases of TB.[1] WHO also estimates that our country was among those six nations which accounted for 60% of the 10.4 million new TB cases in 2015. Unnotified cases of TB in India continue to be of grave concern for health agencies. In this background, we have attempted to record the incidence of new cases of female genital tract TB in our teaching institute from records maintained for the last 5 years.

Most surveys have reported prevalence of genital TB in women from less than 1 to about 20% in different countries.[2] However, scanty data is available regarding incidence of genital TB in India, considering the fact that it is less commonly diagnosed. It is always secondary to TB infection in other organs (commonest primary site being lung), which are easier to diagnose. Primary genital TB is also believed to be due to transmission of infected semen from male partner. Infection is disseminated to reproductive system either by bloodstream or lymphatics. Common symptoms are menstrual irregularity, amenorrhea, menorrhagia, lower abdomen lump or pelvic pain. Cases also remain asymptomatic till advanced stages when female fertility is severely compromised. Hence primary or secondary infertility is another common presentation in young female patients with occult TB. Fallopian tubes and endometrial cavity are most prevalent sites of disease.[3]

Due to inconsistent symptomatology, it is difficult to pinpoint diagnosis of genital tract TB. Even manifold diagnostic tests like USG, HSG, serology and bacterial culture have certain limitations as regards to sensitivity and specificity of disease detection. The gold standard of diagnosis of genital TB is mycobacterial isolation by culture—either using conventional culture medium or liquid culture methods (BACTEC). But disease yield by bacteriological detection method is poor in case of genital TB because of scanty mycobacterial presence in female reproductive tract. Also it takes not less than 5-6 weeks for bacteria to grow in culture media prolonging time of diagnosis and delay in treatment initiation. It is more feasible to obtain tissue diagnosis by open surgical or laparoscopic method. Alternatively, molecular biological method for DNA detection (viz. PCR technique) is a fast, sensitive and convenient diagnostic modality.

In our hospital, we relied on either laparotomy or diagnostic laparoscopy along with hysteroscopy to detect genital TB lesions. Classical manifestations of tubercular disease like nodules, peritubal adhesions, hydrosalpinges and tubo-ovarian masses were looked for. In hysteroscopy, endometrial bands, synechia, tubercles or blocked tubal ostia and stenosed cavity were noted. Tissue diagnosis was established by presence of typical epitheloid granulomas in histopathology. Presence of caseation was not deemed to be mandatory, more so in case of endometrial samples. This is because of the insufficient time to develop caseation in tuberculous granulomas of endometrium as a result of periodic shedding. Presence of AFB in tissue samples or culture positivity further clinched the diagnosis in few cases.

Both open and laparoscopic surgeries are established modes of treatment of tubercular lesions in female genital tract, particularly with regard to infertility patients. All patients diagnosed with genital TB were referred for antitubercular medical therapy. Thus early disease detection is mandatory for initiation of timely therapy for genital TB to prevent irreversible changes.

Materials & Methods

This retrospective observational study was conducted including patients of genital tuberculosis over a period of 5 years who were admitted in NRSMCH Kolkata for diagnosis and definitive management. The following group of patients were suspected to have tuberculosis and admitted for further investigations and management:

i) Unexplained infertility
ii) Tubo-ovarian masses
iii) Chronic pelvic pain
iv) Non-resolving menstrual irregularities

The investigations included routine hemogram, chest radiography, ultrasonography (TAS, TVS or both) and hysterosalpingography (HSG) in selected and indicated cases. Subsequently depending on test

findings, patients underwent appropriate surgical procedure for diagnosis and treatment. Diagnosis was confirmed by tissue culture, presence of AFB or most commonly histopathological examination of samples obtained from hysterolaparoscopy or laparotomy.

The clinical history of patients diagnosed with genital TB and other necessary data for study were obtained from Medical Records section. Notes regarding results of diagnostic tests conducted of all cases were obtained. Types of diagnostic and curative surgical procedures performed and results of culture and histopathology reports were noted. All the patients were referred to Chest department for anti-tubercular medication. Records of treatment outcomes were studied in patients who followed up in OPD.

Results

Thirty women were diagnosed with genital TB in our study series in a 5 year period out of a total 3610 patients admitted in our unit for various gynecological disorders (incidence of 0.16%). The most common age group of affected women was between 20 and 30 years (60%). Genital TB was least common in patients exceeding 40 years (only 3%). Twenty two patients were married (73%). Out of 30 cases, 20 (67%) were nulliparous, 8 primi (27%), and rest 2 multiparous women (Table 1).

Table 1

Study Parameters	Number of patients (n=30)	Percentage
Age in years		
<20	6	20
20-30	18	60
31-40	5	7
>40	1	3
Marital status		
Married	22	73
Unmarried	8	27
Parity		
Nulliparous	20	67
Primi	8	27
Multipara	2	6
Presenting complaints		
Infertility	11	37
Chronic pelvic pain	7	23
Menstrual irregularity	5	17
Menorrhagia	5	17
Fever	1	3
Weight loss	1	3

The most frequently encountered presentation in our study of genital TB was infertility (11 out of 30 cases). Of 37% of patients of genital TB complaining of infertility, 80% belonged to primary infertility group. Ten patients (34%) presented with menstrual cycle irregularity, amenorrhea or menorrhagia due to unexplained causes. Chronic pelvic or lower abdominal pain was the chief complaint in 7 women (23%).

Hysterosalpingographies were done in selected cases where HSG was not contraindicated e.g. T.O. masses, irregular menstruation, highly suspicious of genital tuberculosis or malignancy. Data obtained from case records showed positive findings in 18 women out of total 20 patients of genital TB who underwent HSG. Of 90% cases with abnormal HSG, absent contrast spillage from either or bilateral uterine tubes was present in 8 patients (40%). Five more patients (25%) had only restricted or loculated spillage in peritoneal cavity. Irregular outline of endometrial canal or narrow cavity was seen in 3 HSGs (15%). Two of these HSGs were reported as Asherman's disease. Hydrosalpinx was documented in 2 patients (10%) (Table 2).

Table 2

HSG FINDINGS	No. of patients (n=20)	Percentage
Normal	2	10
Irregular and/ or narrow cavity	3	15
Suboptimal/loculated spillage	5	25
Absent spillage	8	40
Hydrosalpinx	2	10

Of the 30 patients, 25 (83%) underwent combined hysterolaparoscopy. Lap dye test showed free spillage in 7 cases (23.3%) while rest showed no spillage. Hysteroscopy was abnormal in 9 patients (30%). Atrophic endometrium was seen in 2 cases. Five cases showed narrow endometrial canal. Synechiae was observed in 3 of these patients. Frank tubercles were seen in only 2 patients. Open surgery was done in 5 patients (17%) due to contraindication or non-feasibility of laparoscopy. The commonest operative finding in both groups combined was presence of classical tubercles/peritoneal nodules (seen in 13 of 30 cases). Tubo-ovarian masses were found in 5 patients

(16.7%). Flimsy peritubal adhesions, extensive pelvic and bowel/omental adhesions were reported in a total of 14 patients (46.7%). Tubal anatomy distortion with beaded appearances, rigidity of tubes, or hydrosalpinges were seen in 12 cases (40%). Four patients (13.3%) showed straw colored free fluid in peritoneal cavity (Pouch of Douglas region in particular) (Table 3).

Table 3

LAP DYE TEST	Number of patients (n=30)	Percentage
Free spill	7	23.3
No spill	23	76.7
HYSTEROSCOPY		
Normal	21	70
Abnormal	9	30
LAPAROSCOPY/LAPAROTOMY FINDINGS		
Nodules	13	43.3
Tubo-ovarian masses/hydrosalpinges	8	26.7
Mild to moderate adhesions	9	30
Severe adhesions	5	16.7
Peritoneal free fluid	4	13.3

Appropriate interventions were performed in adnexal and endometrial tubercular pathologies. Synechiotomy was attempted under hysteroscopic guidance in patients with adhesions in endometrial cavity. Resection of tubo-ovarian masses, adhesiolysis, salpingectomy in hydrosalpinx cases, and peritoneal toileting after fluid drainage were done in patients with ascites. Patients were referred to Chest OPD on discharge for 6 month anti-tubercular drug therapy (DOTS). Out of 30 patients, 12 were lost to follow up. Five out of 18 patients (27.8%) conceived spontaneously after completion of treatment (Table 4).

Table 4

TREATMENT OUTCOME	No. of patients (n=18)	Percentage
Symptoms relieved	12	66.7
Conceived spontaneously	5	27.8
Referred for ART	6	33.3

Tissue samples obtained from endometrial canal or abdominal cavity were sent for AFB staining, culture and histopathology. Tubercular granulomas were seen in 9 endometrial histopathology sections. None of the sampled endometrial tissues showed culture positivity. In 21 cases, tubal and peritubal/peritoneal biopsy materials tested positive for caseating or non-caseating granulomas. Of these, only 3 cases showed mycobacterial growth in cultures and 2 biopsies stained positive for acid fast bacilli.

Discussion

In our study out of a large number of patients attending Gyne OPD over a period of 5 years, 30 cases were conclusively diagnosed as genital TB and managed accordingly. The incidence of genital TB was 0.16%. (30 out of 3610 patients). This incidence rate is in concordance to previous study finding by Arora et al published in 1992, who reported 0.75% incidence amid women in India.[4] In another old study by Agarwal, disease frequency was reported to be 1.8% in 1974 which dwindled to 0.89% in 1989 and onwards.[5]

The most common age group of patients affected was between 20 and 30 years (60%). Schaefer also in a very large study population reported 68% of cases of genital TB in the young age group.[6] Nagpal showed similar 74% incidence in 21-30 years' age group.[7]

Our study showed infertility to be the most common presenting complaint of genital TB (37% of patients). Most of the literature confirms this.[8] Menstruation-related complaints including menorrhagia were the next important presenting features (34% in our study). Similar findings were reported by Sharma et al in their study which showed only 57.6% of patients with normal menstrual cycles.[9] Chronic pelvic pain was chief complaint in 7 of 30 cases (23%). In his study, Schaefer found pelvic pain as an important symptom in 50% of patients.

HSG was abnormal in 90% (18/20) of our study cases. Sharma et al and Ohri et al reported 27.5% and 33.3% of abnormal HSGs in their studies.[9,10] This difference is likely due to meticulous case selection done in our study including only those patients strongly suspicious of having genital TB.

Hysteroscopy was performed in combination with laparoscopy in 25 cases with abnormalities in 28% of patients (7 out of 25 genital TB patients). All these patients showed histopathologic findings positive for tuberculosis infection of endometrium. A study by Arpitha VJ et al also showed positive hysteroscopic findings in 26.1% of 51 cases.[11] Endometrial

involvement is much less common than tubal disease due to the fact that cyclic shedding and regeneration of endometrial tissue makes it less vulnerable to tubercular bacilli proliferation.

Laparoscopy was done in 25 patients of our study group and rest 5 underwent laparotomy. Classical findings of tubercular infection viz, peritubal adhesions, tubal or cornual blocks, beaded tubes and peritoneal nodularity were demonstrated in combination or isolation in 21 cases (84%). In total, 28 out of 30 patients (93.3%) demonstrated positive findings by combined hysterolaparoscopy or laparotomy. Shende P et al also reported positive findings in 94% of their patients (30/32) who underwent hysterolap.[12] In another study by Bapna N et al, 72.4% of patients had tubal involvement out of total 58 cases of genital TB, while 17.2% had frozen pelvis due to dense adhesions.[13]

In our study, 5 patients out of 18 reporting for follow-up conceived spontaneously after completing 6 months of ATD therapy. No data was available regarding rest 12 patients. Conception rate is 27.8%. Similar post treatment conception rate of 28% post was reported by Shende P.[12]

Conclusion

Our study shows incidence of genital tuberculosis to be 0.16% of all gynecological inpatients. Only high degree of suspicion can lead to identification of at risk cases. Infertility, chronic pelvic pain and unexplained long-standing menstrual cycle aberrations are the most encountered symptoms. These subset of patients along with those having non-resolving tubo-ovarian masses need proper work up dedicated towards diagnosis of tubercular infection of reproductive tract. Tubes (70%) and endometrium (30%) were the commonest sites of involvement in our study. Either hysterolaparoscopy or laparotomy was used to both diagnose and treat positive cases followed by antitubercular drug therapy. Symptomatic recovery as well as spontaneous conception is possible in compliant patients.

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Original Article: Gynecology

Endometriosis in Abdominal Surgical Scar Tissue

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ABSTRACT

Aim: To evaluate abdominal wall surgical scar endometriosis.

Methods: Case series of 6 patients who presented to our institution with abdominal scar endometriosis were evaluated over a period of 3 years and review of literature was done. The diagnostic approach and treatment is discussed.

Results: All patients had a painful mass located at abdominal scars with history of previous obstetric surgeries. The ages ranged from 25 to 41 years and ultrasonography detected hypoechoic mass in the scar region. Initial diagnosis was scar endometriosis in 5 and incisional hernia in 1 out of 6 patients. Treatment was achieved with surgical excision in all 6 patients and patients were followed up for recurrence.

Conclusion: Scar endometriosis is a rare and often misdiagnosed condition. Though diagnostic modalities like Doppler ultrasound, computed tomography, magnetic resonance imaging and FNAC can be used for differential diagnosis, definitive diagnosis is by histopathological examination. The treatment should be careful wide local surgical excision.

Introduction

Endometriosis is the presence of functioning endometrial tissue outside the uterine cavity. It generally occurs in pelvic sites such as ovaries followed by the uterine cul-de-sac, uterosacral ligaments, posterior surface of uterus, broad ligament, pelvic peritoneum, bowel, and rectovaginal septum. Extra-pelvic endometriosis can be found in unusual places such as the nervous system, thorax, urinary tract, gastrointestinal tract, and in cutaneous tissues, and its most frequent location is in the abdominal wall.[1,2,3] Meyer first documented a case of Scar endometriosis in 1903. Gynecologic surgeries are the most common inciting factors of which hysterectomy [2%] and caesarean section [< 0.4%] are the most common ones. Tubal ligation, laparotomy for ectopic pregnancy, salpingectomy, hysterotomy and episiotomy, etc. are the uncommon causes.[4,5] It has multiple differential diagnoses like incisional hernia, hematoma, neuroma, suture granuloma, lipoma, abscess, sebaceous cyst, and neoplastic tissue or even metastatic carcinoma[6] and patients reach the general surgeons first. Scar endometriosis patients are often referred to the general surgeons because the clinical presentation suggests a surgical cause. We present 6 cases of scar endometriosis. By presenting this paper,

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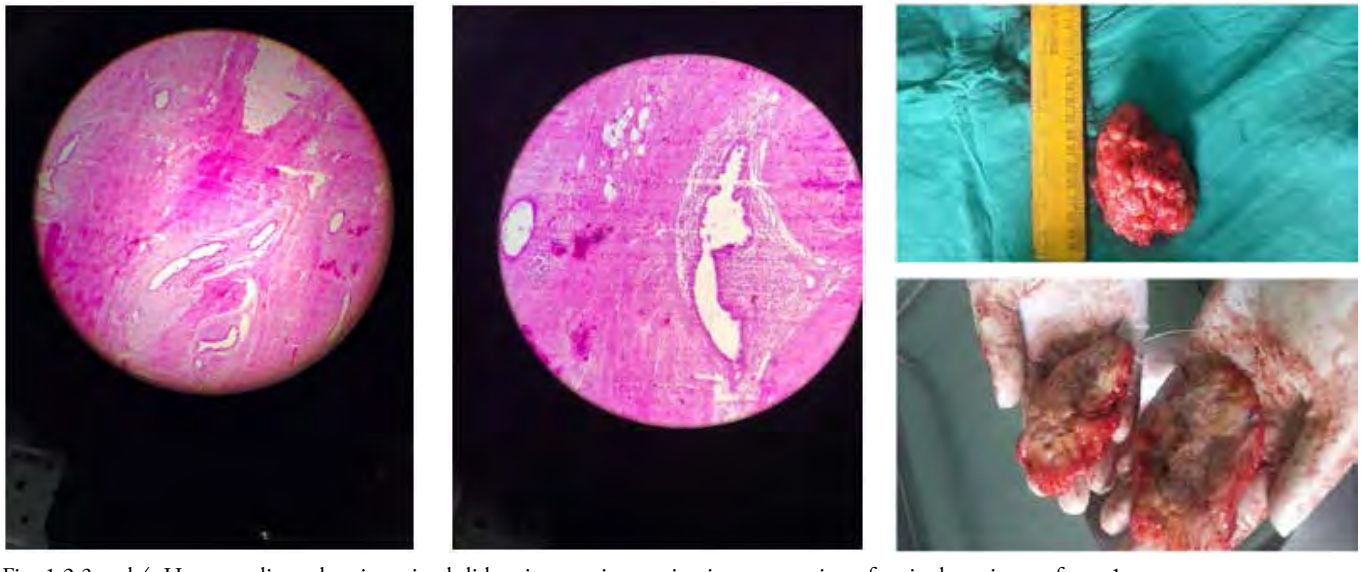
and conducting a review of the literature, we intend to increase the awareness of scar endometriosis to be included in the preliminary diagnosis, by both obstetricians and general surgeons.

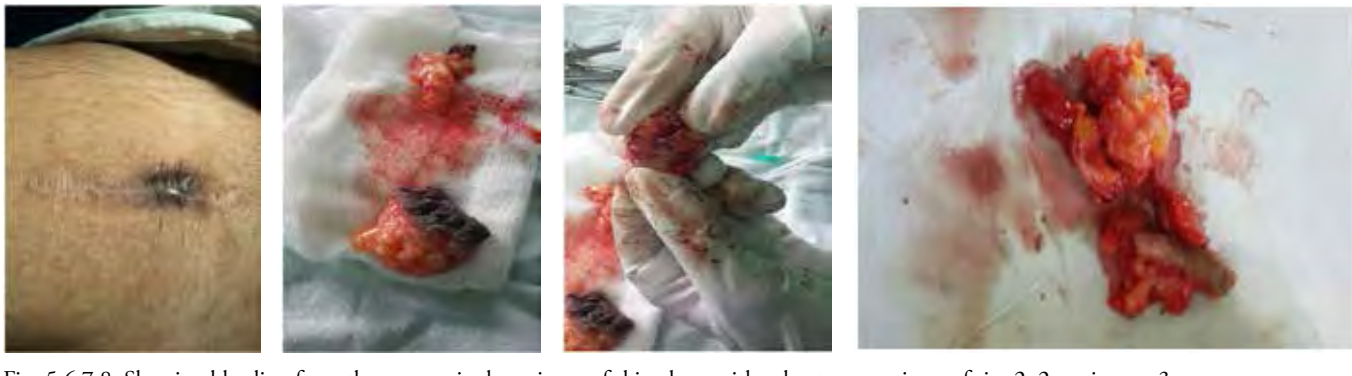
Case Reports

CASE 1: SK, a 37-year old woman, P2L2, presented on March 2017 with complaints of painful swelling over left margin of previous cesarean section scar since 3 years. The swelling was initially small, gradually increased in size. The pain had a cyclic character accompanying her menstruation. She had two cesarean sections, 4.5 and 11 years ago. She had history of surgical excision of scar endometriotic mass 2 years back and developed recurrent symptoms with a one year symptom free interval. On abdominal palpation, a firm mass was felt under the left side of pfannensteil cesarean section scar. Ultrasonography showed ill-defined hypoechoic mass in the anterior abdominal wall. Surgical exploration revealed a firm mass of 6×4 cm above the external oblique aponeurosis, surgical excision of the mass was done. Histology described fibro-collagenous tissue along with endometrial glands, endometrial stroma with areas of congestion, hemorrhage and edema, few chronic inflammatory infiltrates are also seen. The endometrial glands lacked nuclear atypia, consistent with scar endometriosis. Patient is on follow up.

CASE 2: RD, a 38-year old woman, P3L3, presented with non-cyclical symptoms of painful mass over abdomen. She was a known case of endometriosis since 2 years and was on medical management of pain relief. she was admitted on Dec 2016 for surgical exploration of a painful mass over upper margin of midline vertical scar below umbilicus with a pre-operative diagnosis of pelvic endometriosis with incisional hernia. She had three cesarean sections 3, 5 and 7 years ago. During the operation, surgical excision of a mass of 3×2 cm from subcutaneous fatty layer below midline abdominal scar was done. Uterus was adherent to anterior abdominal wall over previous scar matrix. Hysterectomy and bilateral salpingo-oophorectomy for endometriosis with cysts in ovaries had to be done, then mesh repair of incisional hernia



Figs 1,2,3 and 4: Hematoxylin and eosin-stained slide microscopic examination, cut section of excised specimen of case 1.



Figs 5,6,7,8: Showing bleeding from the scar, excised specimen of skin along with subcutaneous tissue of size 2×2 cm in case 3.

was done. Microscopically the mass showed fibro-fatty tissue with few glands lined by benign columnar epithelium, the stroma is edematous and few chronic inflammatory infiltrates, consistent with tissue of endometrial origin. There was no recurrence in 6 months follow up period.

CASE 3: RD, a 31-year old woman, P2A1L2, presented to outpatient department on May 2016 with a painful swelling on the lower abdominal scar since 5 months. She had undergone hysterotomy and bilateral tubal ligation 3 years ago. She had menstrual cycle related symptoms of pain, brownish discoloration and bleeding from the mass. She was admitted and a mass of 2×2 cm from the subcutaneous fatty layer during surgical procedure, mass was resected. Histology confirmed tissue of endometrial origin.

CASE 4: SK, a 37-year G2P1L1 was admitted on May 2015 at term pregnancy with history of one previous cesarean section 8 years back. She gave history of resection for scar endometriosis 5 years ago and developed recurrence 2 years back with a painful mass in scar which was not cycle related. On examination, she was at term pregnancy with a small palpable mass over right side of previous Pfannensteil incision. She was admitted for elective cesarean section and intra operatively, surgical excision of a firm mass was done from subcutaneous tissue below the cesarean scar, sized 2×2 cm. Tissue was sent for histopathological diagnosis, which confirmed the diagnosis of scar tissue with endometrial origin.

CASE 5: MD, a 41-year P2L2 presented on March 2015 with a cyclic painful mass over cesarean section scar and heavy menstrual bleeding since 5 years, for which she was on medical management. She had two previous cesarean sections 9 and 15 years back. Ultrasound showed a hypoechoic mass of 4×4 cm below cesarean scar and normal pelvic scan. Patient was admitted for excision of scar lesion and hysterectomy was done as patient insisted even after counselling. Histologically the scar tissue was consistent with scar endometriosis. Patient did not develop recurrence in 2 year follow up.

Fig 9: Specimens of case 5 after surgical excision of a 4×4 cm mass below the scar (right side of picture)

CASE 6: MK, a 25-year old para 2 (P1A1L1) Woman, who underwent lower segment cesarean section 2 years back presented on Dec 2014 with a painful mass at the stitch line (Pfannenstiel incision) for the last 6 months. The lesion used to be more painful and hyperaemic during menstruation. On per abdominal examination, a painful lesion of about 4 cm × 2 cm was found at the left side of the stitch line, which was firm in consistency. Excision of a 3×2 cm raised reddish firm mass in the subcutaneous tissue was done, at the left side of scar Histopathological report confirmed it to be scar endometriosis. The patient had no recurrence in 2.5 year follow up period.

Discussion

Endometriosis is defined as finding endometrial gland and stroma outside the uterus. There are many theories on endometriosis development but the subject is still controversial. Direct mechanical implantation seems to be the most appropriate theory for explaining scar endometriosis.[6] During cesarean section, endometrial tissue might be seeded into the wound and under the same hormonal influences, these cells proliferate.[7] The most evident risk factor for the presence of scar endometriosis is history of obstetric procedures, especially caesarean sections.[8] Accurate diagnosis of scar endometrisis may be challenging due to its nonspecific nature of clinical presention and also due to multiple differential diagnoses, such as suture granuloma, incisional hernia, hematoma, abscess, cysts and lipoma.[6] Under the influence of Ovarian hormones, ectopic endometrial cells (stromal and glandular cells) during the menstrual period bleed slightly at the scar location, with an inflammatory reaction and subsequent tissue repair. With each menstrual cycle, the lesion increases in volume and behaves like an invasive tissue, which can be seen in histopathological examinations. Such invasion might compromise the skin, subcutaneous cellular tissue, muscles, aponeurosis and peritoneum. At certain

points during the cycle, areas of focal hemorrhage can be identified, along with areas of active chronic endometriosis with fibrosis and cellular infiltration that is rich in macrophages and histiocytes loaded with hemosiderin pigments, all of which may not be evident.[8]

The most evident clinical manifestation is a painful subcutaneous nodule in surgical scar area. Other complaints include perception of mass, cyclical bleeding. Pain and increase in the size of mass may be cycle related or noncyclical. The clinical diagnosis becomes impaired when complaint is not cyclical. The interval between onset of symptoms to past surgery varies from few months to 10 years.[6] Mass lesion at the scar site which is gradually increasing in size may be associated with skin discoloration and may or may not have cyclical periodicity. However presence of cyclical periodicity is pathognomonic. Non-invasive imaging modalities like USG with color Doppler, CT scan, MRI and FNAC can be highly suggestive but not diagnostic. Histology is the hallmark of diagnosis. It is satisfied if endometrial glands, stroma, and hemosiderin pigment are seen.[9]

Taking the iatrogenic implantation of scar endometriosis as the main theory, several measures have been proposed for prevention of scar endometriosis, but without any proper scientific evidence. Failure to close the parietal and visceral peritoneum in the cesarean section may be related to greater rates of scar endometrioma.[10] It is recommended not to use the same surgical material and the same instruments as used in hysterorraphy, when suturing other abdominal wall layers.[8,11] Some authors have reported ongoing use of high doses of progesterone in order to decrease the occurrence of endometriosis at the surgical site, during the first six months after hysterotomy.[11] Other authors have recommended washing the abdominal wall as a prophylactic measure, using irrigation with a salt solution before closing the wall.[8,12] Although there is no evidence to support any preventive measure, taking proper care during the surgical procedures involving uterus is highly recommendable.

Medical management with oral contraceptive pills, progestogens and GNRH analogues provide alleviation of symptoms, with recurrence after cessation of therapy and also have side effects. Wide surgical excision with at least 1 cm margin is the treatment of choice.[13,14] In our study, five patients had history of previous caesarean section and one patient had history of hysterotomy. Five of them presented with complaint of perception of a painful mass and one had painful lesion over scar with cyclical bleeding from the scar. Cyclical symptoms were observed in three out of six patients. Careful surgical excision of mass was done in all patients and sent for histopathological examination, which confirmed scar endometriosis in all patients. Two out of six patients underwent hysterectomy along with excision of scar endometriosis, one of which had associated pelvic endometriosis and although hysterectomy with bilateral salpingo-oophorectomy is a radical surgery, it was carried out along with surgical excision of mass to prevent recurrence in both of them. Three of them had no recurrence in follow up period, one lost follow up and the other two are on follow up. So, we recommend wide local surgical excision as the treatment of choice for scar endometriosis as it is a superficial lesion mostly limited to abdominal wall and there is less chance of recurrence unlike medical management.

Conclusion

Scar endometriosis is a rare and misdiagnosing condition due to multiple differential diagnoses. It has to be kept in mind while evaluating any patient presenting with mass in previous surgical scar. Proper clinical history of the patient may aid to the diagnosis. Imaging modalities and FNAC can help to some extent, but diagnosis is confirmed histopathologically and the treatment of choice is wide local surgical excision.

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Review articles

Reviews based on the recent and relevant subjects of clinical interest should be considered.

4. LAYOUT OF MANUSCRIPTS

Manuscript text should be in English (US spelling), double-spaced, font size 12, in Arialmmes New Roman font.

First page

The first page of the manuscript should contain the following: (1) title; (2) full names of authors (6 maximum, although listing more authors may be considered on an individual basis if authorship requirements have been met and a request has been included in the cover letter); (3) affiliations of authors (i.e. department, section or unit of

an institution, hospital or organization, city, and country (4) full contact details (postal address. email address) of the corresponding author ; (5) a list of up to 8 keywords for indexing and retrieval:

Footnotes linking author names to affiliations should be listed as 1,2,3 etc..

The first page should also list the type of article: Clinical Article; Brief Communication: or Review Article.

Abatract

Cllnlcal artlclea

A structured abstract not exceeding 200 words is required for all full-length clinical articles. It should contain all and only the following headings: Objective; Methods; Results; and Conclusion.

The Objective reflects the purpose of the study: that is, the hypothesis that is being tested. The Methods should include the setting for the study, the participants (number and type), the treatment or intervention, and the type of statistical analysis. The Results include the outcome of the study and statistical significance, if appropriate. The Conclusion states the significance of the results.

Review articles

An abstract not exceeding 200 words is required for all review articles.

Narrative reviews require an unstructured abstract. Systematic review articles should have a structured abstract with the headings; Background; Objectives; Search strategy; Selection criteria; Data collection and analysis; Main results; and Conclusions.

Brief communications

Brief communications should not include an abstract.

Main text

In full-length articles, subject matter should be organized under the following headings, with no subheadings: Introduction; Materials and methods; Results; Discussion; Acknowl edgments; Conflicts of interest; and References.

Brief communications should not have any headings separating the text.

Clinical articles

The main text of clinical articles should not exceed 2500 words, excluding the first-page information, abstract (no more than 200 words), author contributions, acknowledgments, Conflicts of interest, references (no more than 25), figure legends, and tables and figures. Please include the word count in the cover letter and on the first page of the manuscript.

Review articles

Review articles should have no more than 3000-3500 words in the main text and 40 references. Please include the word count in the cover letter and on the first page of the manuscript.

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Brief communications should be no more than 400 words, excluding the first-page information, synopsis, keywords, author contributions, acknowledgments, conflicts of interest, references, figure legends, and tables and figures. There should be no more than 4 references and no more than 1table or 1figure.

Power calculations, statistics, and reporting of numbers.

Power calculations

Where appropriate (e.g. for clinical trials), power calculations should be performed as part of the study design, and a statement providing the power of the study should be included in the Materials and Methods. Authors should state how the power calculation was determined, including what type of difference the calculation was powered to detect and on what studies the numbers are based.

Statistics

The statistical tests used and the significance level set should be listed in the methods for all studies that employed statistical analysis. Information regarding the statistical software programs used should be included in the methods: for example, "SPSS version 20 (IBM, Armonk, NY, USA)." This information should not be included in the reference list.

P values should be provided where calculated. The largest P value that should be expressed is $P>0.99$. The smallest P value that should be expressed is $P<0.001$.

For measures of effect (e.g. relative risks, risk ratios, odds ratios), authors should also report confidence intervals (e.g. 95%) so that the precision of the effect estimate can be assessed.

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Studies of patients, patient records, or volunteers require Ethics Committee approval and informed consent.

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Include a statement in the methods that the research protocol was approved by the relevant Institutional Review Board or Ethics Committee before the study began; if such approval was not needed/obtained, include an explanation. Authors must provide copies of the appropriate documentation if requested.

Informed consent

Include confirmation in the methods that all human participants gave written informed consent before the study began; if consent was not needed/obtained, include an explanation. Authors must provide copies of the appropriate documentation if requested.

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Sources of funding should be acknowledged by the author(s), along with the names of individuals who do not fulfil the criteria for authorshi p, but who have made a substantial contribution to the manuscript.

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Journal article

[1] Vellacott ID, Cooke EJ, James CE. Nausea and vomiting in early pregnancy. Int J Gynecol Obstet. 1988;27:57-59 .

Book

[2] Speroff L, Glass BH, Kase NG. Clinical Gynecologic Endocrinology and Infertility. Baltimore: Williams and Wilkins; 1982.

Chapter in a book

[3] Disaia PJ, Creasman WT. Invasive Cancer of the Vulva. In: Disaia PJ, Creasman WT, eds. Clinical Gynecologic Oncology. St Louis: C.V. Mosby; 1984:214-219.

Web reference

[4] World Health Organization. WHO Recommended Surveillance Standards, Second Edition [WHO website). 1999. http://www.who.int/csr/resources/ publications/ surveillance/whocdscsrisr992.pdf.

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Each table should be titled, numbered (with Arabic numerals), and placed on a separate page after the reference list (not embedded within the main text).

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ISOPARB PG CLASS

20th & 21st December, 2017

Organised by **Institute of Obstetrics & Gynaecology, Sir Ganga Ram Hospiral, New Delhi**
Under the Aegis of ISOPARB, IMS Delhi Chapter and AOGD
Venue: Hospital Auditorium

The coordinators of the programme were Dr Mala Srivastava and Dr Harsha Khullar from Sir Gangaram Hospital, New Delhi. About 50 students all over Delhi and surrounding areas attended the 2 days program of Gurukul. It was moderated by 24 faculties members comprising of senior teachers from Sir Ganga Ram Hospital, AlIMS, Maulana Azad Medical College, Lady Hardinge Medical College, Safdurjung Hospital and Army Hospital & R R. The students were very happy to have Dr. Hiralal Konar as a visiting faculty from National Medical College Kolkata. There were

- Didactic lectures on dummy pelvis
- Breech Delivery
- Cardiotocography
- Case presentation covering both obstetrics & gynaecology

There were sessions on contraception, instruments and discussion with practical viva-voce.

Two days program was widely appreciated by all the students.

They requested for more such sessions with ISOPARB in future.

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