"DIAGNOSTIC VALUE OF POLYMERASE CHAIN REACTION FOR DETECTION OF GENITAL TUBERCULOSIS IN EVALUATION OF FEMALE INFERTILITY"

By

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ABBREVIATIONS

AFB	: Acid fast bacillus
ARC	: Assisted Reproduction Centre
CT	: Computerized tomography
ESR	: Erythrocyte sedimentation rate
GTB	: Genital tuberculosis
HSG	: Hysterosalphingography
LJ media	: Lowenstein Jensen media
MRI	: Magnetic resonance imaging
PCR	: Polymerase chain reaction
POD	: Pouch of douglas

ABSTRACT

Title: "Diagnostic value of Polymerase Chain Reaction for detection of Genital tuberculosis in evaluation of female infertility".

Objective :To evaluate the diagnostic value of PCR in detection of genital tuberculosis in infertile women.

Material and methodology

This prospective clinical study was carried out at Assisted Reproduction Centre of KLES PRABHAKAR KORE Hospital, Belgaum.96 infertile women who underwent hysterolaparoscopy as a part of their infertility workup were investigated for genital tuberculosis by PCR and histopathology of endometrial curettings.

Results

Of the 96 women enrolled,21(21.8%) women were found to be positive for genital tuberculosis .TB PCR was positive in 20(95.3%) cases and histopathology in 2(9.5%) cases.

Conclusion: PCR is a rapid and a highly sensitive diagnostic modality in diagnosis of genital tuberculosis.

Keywords: Genital tuberculosis, Polymerase chain reaction, Female infertility

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INTRODUCTION

Tuberculosis is an important health problem worldwide. Its one of the most important cause of infectious morbidity and mortality. The disease is a major barrier to social and economic development.

One third of the worlds population is currently affected with tuberculosis. In India every year 1.8 million people develop the disease, of which 80,000 are infectious,1000 die of it everyday, with 2 deaths occurring every three minutes due to it. A single infected person is known to infect 10 or more people per year.[1]

Lately an increase in the trend of the disease is noted due to emergence of drug resistant tuberculosis, particularly in settings where there is a coexistence of HIV infection and disease pattern has also changed with a higher incidence of disseminated and extra pulmonary tuberculosis. Genital tuberculosis represents 15-20% of extra pulmonary tuberculosis and is the second most common site infected after pulmonary tuberculosis.[2] Genital tuberculosis is seen in 13% of all patients with pulmonary tuberculosis.[3] It accounts for 5-10% of all pelvic infections. Its an important cause of infertility ,being an etiological factor in 1-18% of the cases .[4]

The cause of infertility is predominantly tubal block, adhesions in the endometrial cavity and ovulatory dysfunction. It is almost always secondary to a tubercular lesion elsewhere in the body, with fallopian tubes being affected most commonly (90%), followed by the endometrium (50%), ovaries (10-30%), vagina and vulva (<1%).[5]

However, its incidence is underreported due to latency of the organism, asymptomatic and varied presentation in majority of the cases and paucity of an accurate diagnostic modality. For decades it has been a diagnostic dilemma for clinicians but lately with the advent of newer diagnostic modalities more cases of genital tuberculosis are being recognized. Places where diagnostic facilities are not available diagnosis is made mainly by high index of clinical suspicion and use of appropriate investigations. However, majority of the cases have asymptomatic presentation.[5]

A high ESR and a positive mantoux test are usually non specific.

AFB culture has a low detection rate and takes a long time to give positive results (4-8 weeks). However, liquid culture with radiometric growth detection such as BACTEC allows more rapid detection of the bacteria (10-14 days) and rapid drug susceptibility testing .[6]

HSG is usually avoided in a known or highly suspicious case of genital tuberculosis for the fear of exacerbating the disease. Tubal occlusion is the most common HSG finding in genital tuberculosis usually seen at the isthmo ampullary junction followed by hydrosalphinx, peritubal adhesions, rigid pipe appearance &irregularity of the tube.[7]

Abdominal and pelvic ultrasound, CT and MRI are usually employed in circumstances where an abdominal or pelvic mass is present. It helps to identify ascites / loculated fluid, adnexal mass, peritoneal thickening, omental thickening and endometrial thickening.[2]

Hysterolaparoscopy is a well recognized procedure for the diagnosis of genital tuberculosis and detects macroscopic changes like tubercles, peritubal, periovarian adhesions, hydrosalphinx, tuboovarian mass though it may miss subtle changes found in the early stages of the disease.[8]

Histopathology can provide the diagnosis of this condition with certainty however a report based on a single sample could result in a high false negativity .This can be either due to technical failure, inability to obtain adequate sample or wrong time of sample collection in relation to the disease.[7]

Rapid nucleic acid amplification techniques such as PCR is currently the most sensitive and rapid method for the detection of genital tuberculosis. It can detect fewer than ten organisms in clinical specimens, an important feature since genital TB is paucibacillary. However, it doesn't distinguish live from killed bacilli.[6]

In the present study an attempt has been made to assess the diagnostic value of PCR in routine evaluation of infertile women and also a correlation has been done between the positive cases of genital tuberculosis and hysterolaparoscopy findings.

AIMS AND OBJECTIVES

PRIMARY OBJECTIVE: To evaluate the diagnostic value of PCR in detection of genital tuberculosis in infertile women.

SECONDARY OBJECTIVE: Correlation of the clinical and hysterolaparoscopic findings in cases found positive for genital tuberculosis by PCR or/and histopathology of endometrial curettings.

REVIEW OF LITERATURE

Tuberculosis is an age old disease but is amongst the foremost killers of the 21st century. The highest rates of TB are in some of the world's poorest countries, and the economic toll taken by the disease is enormous. It is a major health problem in India and it is responsible for a significant proportion of women presenting with infertility. The actual incidence of genital tuberculosis cannot be assessed accurately, since the disease is discovered incidentally in many patients, and in a large number of symptomless patients, this disease remains undiscovered. It is estimated that 5-10% of infertile women all over the world have genital tuberculosis although this varies from less than 1% in the United States to nearly 18% in India [4] It is found in 0.75 to 1% of all gynaecological admissions in India .[5]

The disease is responsible for 5% of all female pelvic infections and occurs in 13% cases of pulmonary tuberculosis. [3]

Although genital tuberculosis can occur in any age group,75% of the patients are in the reproductive age group(25-45 years).Postmenopausal women account for 7-11% cases of GTB. [2]

High risk groups: Tuberculosis of the genital tract is comparatively common in women who have a family history of tuberculosis, chronic pelvic pain associated with infertility, past history of tuberculosis, secondary amenorrhea associated with infertility, and an adnexal lump, alone or associated with infertility .[9]

Symptomatology: Genital tuberculosis is a chronic disease and often has a low-grade symptomatology with very few specific complaints. Sutherland reported the varied symptomatology seen in patients with genital tuberculosis. Infertility was reported in 44% of patients with genital tuberculosis, pelvic pain in 25%, leucorrhea in about 5% of cases, and abnormal vaginal bleeding in 18% cases. Post-menopausal bleeding accounted for 2% of patients presenting with genital tuberculosis. Rare symptoms included an abdominal mass or unexplained ascites. Most cases of confirmed genital tuberculosis had perfectly normal clinical examination (43%) and about a quarter of cases presented with an adnexal mass (23.6%)while Tripathy et al observed primary and secondary infertility in 58% cases, pelvic pain in 18% and leucorrhoea in 26% of cases. The predominant menstrual symptom noted was secondary amenorrhea in 43% cases followed by menorrhagia in 17% cases& oligomenorrhea in 11% cases. The most significant signs were pelvic mass in 21% cases and an unhealthy cervix in 17% cases. Tuberculous lesions elsewhere in the body were seen in about 18% of cases . [9]

Organs involved : Regarding frequency of involvement of the different parts of the genital tract, the tubes are involved in 90 to 100% cases, uterus in 50 to 60%, ovaries in 20 to 30%, cervix in 5 to 10%, vagina and vulva in 1 to 2% of the cases. Myometrial involvement is very rare.[5]

Mode of spread : Genital Tuberculosis is invariably secondary to a primary lesion elsewhere in the body, the latter usually being quiescent by the time pelvic involvement is diagnosed. The spread of genital tb is mainly by hematogeneous, lymphatic or direct extension from a contiguous focus. Hematogenous spread is mainly from the lungs while lymphatic spread is known to occur from a primary abdominal lesion in the

intestine or kidneys. Rarely, direct involvement of vulva and cervix occurs from an infected male sexual partner. [10]

Pelvic tuberculosis may present in three clinical forms: tuberculous salphingitis, tubercular peritonitis and endometrial tuberculosis.

Tubal involvement: The tubal pathology varies according to the mode of infection. If infection is lymphatic borne, the tubercles are formed on the surface, with adhesions all around. In haematogenous spread, the tubercles are deeper and look red, oedematous and swollen in the acute infection phase and fibrosed in the chronic cases. In 50% of the cases, the tubes get blocked; blockages being multiple and the tubes appear thickened and shotty. Sometimes, a localized blockage at the outer end results in the formation of hydrosalpinx or pyosalpinx with thick fibrous walls. Both fallopian tubes are involved in majority of the cases.[11]

Endometrial involvement: From the tubes, the infection reaches the endometrium where it either persists in the basal layer, which is not shed during menstruation, or it gets reinfected from the tubes following menstruation. Tuberculous endometritis is common affecting 60-70% of the women presenting with genital tuberculosis. Even in advanced pelvic tuberculosis, evidence of caseation, fibrosis and calcification are rarely seen in the uterine cavity. Occasionally the endometrial cavity is obliterated by extensive adhesions. Total destruction of the endometrium can result in amenorrhea. Tuberculous pyometra can also develop in post menopausal women with an occluded internal cervical os.[12]

Peritoneal involvement: Tuberculous peritonitis is often associated with tuberculosis of the pelvis. Clinically tuberculous peritonitis can be divided into two groups. In the wet peritonitis there is an outpouring of the straw coloured fluid into the peritoneal cavity, producing ascites. The peritoneum of the parietal wall and viscera are covered with tubercles. The tubes in addition to being covered with tubercles are enlarged and distended. This pattern is usually associated with hematogenous spread of the tuberculous organism to the peritoneal surface and pelvic organs.

Another type of tuberculous peritonitis encountered is the dry or adhesive type. In this condition the bowel adheres to the bowel by innumerable dense adhesions that blend with the musculature. The muscle is also invaded to some degree by the tuberculous process.[13]

Ovarian involvement: A tuberculous infection of the ovary is seen in about 25% cases of genital tuberculosis and usually involves only the surface of the ovary and represents an extension of the infection from the peritoneal cavity and the adjacent fallopian tubes. The infection is occasionally limited to perioophoritis, extension to the ovarian parenchyma is prevented by the tunica albugeniae. Often, the ovaries have normal macroscopic appearance and the diagnosis is made only on histopathological study. However a break in the tunica caused by ovulation may cause the bacilli to gain access into the ovarian parenchyma and so ovaries may have tubercles, adhesions, thickening of the capsule and sometimes even caseating abscess/cavities in the ovarian substance.[12]

Cervical involvement: In cervix, the tuberculous lesion can be ulcerative or proliferative. In ulcerative form, the ulcers have serpigenous outline, clean cut edges and a yellow

base. Early ulcers are often seen near the external os The proliferative lesion has papillary formations which may be pedunculated or sessile. Finally, caseation occurs which leads to progressive destruction of the cervix.[12]

It is uncommon to have tuberculosis involving the vagina or the vulva. It is seen only in 2% of the cases with genital tuberculosis. The gross appearance may be ulcerative or hypertrophic with the presence of multiple sinuses.[2]

Diagnostic dilemma: The diagnosis of the disease is difficult. Apart from varied clinical presentation, a past history of tuberculosis or a history of contact may not be forthcoming and an evidence of tuberculous lesion elsewhere in the body may be lacking. The abdominal and vaginal examinations may be normal. A high erythrocyte sedimentation rate and a positive Mantoux test are non-specific. The chest skiagram is normal in most cases. A pelvic ultrasound and hysterosalpingography examinations may be of some help. Histopathological evidence in biopsy of premenstrual endometrial tissue or demonstration of tubercle bacilli in culture of menstrual blood or endometrial currettings can only provide the diagnosis of disease with certainty. [5]

Various Diagnostic modalities:

Mantoux test: It may be useful in populations where tuberculosis is a rare disease. The Mantoux test may show sensitivity of up to 55% for the accurate diagnosis of genital tuberculosis in populations with a low incidence. The Mantoux test may be negative in patients with active tuberculosis if the patient has overwhelming clinical disease, is severely immune compromised, has co-incidental viral infection or is malnourished. The validity of the Mantoux test, therefore, is variable. In populations with a high incidence of tuberculosis and where BCG is given routinely, the Mantoux test is often falsely positive. The Mantoux test may, in rare cases of genital tuberculosis, elicit a systemic reaction, while a local abdomino-pelvic reaction in the form of lower abdomen pain, tender adnexa and increased discharge from the cervix may be noted for 24 to 48 hours after the injection of tuberculin.[14]

Chest X-ray: More than 75% of the patients with active, culture-proven genital tuberculosis have a normal chest X-ray. It is important not to use a chest X-ray as exclusion for the diagnosis of genital tuberculosis. [15]

Culture: The gold standard remains the proof of acid-fast bacilli in biological specimens or culture. In patients presenting with sub-fertility and/or abnormal bleeding, a culture of menstrual fluid may be the most useful strategy. Culture of mycobacterium tuberculosis on Lowenstein-Jensen medium is the most accurate diagnostic method. Culture is more sensitive, requiring only 100 organisms per milliliter. However, culture may take up to eight weeks to grow on LJ medium.[6] (Figure No. 1)

BACTEC has a sensitivity of 80-90 percent compared to Lowenstein-Jensen medium, which has sensitivity of 30-40 percent. Whether cultured by LJ medium or BACTEC, the detection of a positive culture depends on various factors like (1) Number of organisms in the specimen – heavy smear positive specimens may turn positive as early as 48 hours, but if the bacterial load is low, it takes longer to grow the bacilli (2). Treatment status of the patient – if the patient is already on treatment; the bacilli are debilitated and may require a longer time to grow. All BACTEC cultures are maintained for 6 weeks and LJ culture for 8 weeks before being reported as negative. Besides technical drawbacks in demonstrating Mycobacterium tuberculosis in laboratory, a substantial number of TB lesions of genital tract are bacteriologically mute. Culture is the gold standard for diagnosis of genital TB. Rapid culture techniques like BACTEC can be used to save time in diagnosis. [16]

Histopathology of the premenstrual tissue can provide the diagnosis of this condition with certainty. Histopathology demonstrates the typical caseous granulomatous lesion with giant epitheliod cells with or without Langerhans's giant cells. Caseating necrosis is rare in specimens from the genital tract.(Figure No. 2) The lesion is highly suggestive of but not diagnostic of genital tb. However it has got a high false negative rate due to either technical failure, inability to obtain adequate sample or wrong time of collection of the sample in relation to the disease stage.[i]

The only source of material generally available for culture or biopsy of the female genital tract without resorting to diagnostic laparoscopy or laparotomy is the endometrium and menstrual discharge .However, the often small innoculum and slow growth of mycobacteria reaching the endometrium from the tubes reduces the chance of

obtaining a positive result from a single endometrial biopsy or menstrual culture. Multiple samples may have to be collected. The best time for examining the endometrium is several days before the expected menstrual period, at which time the tubercles reach their maximum growth. The portion of endometrium most likely to show tubercles is in the region of the uterine cornua, where spread from the tubes first occurs. Part of the endometrium obtained should be examined bacteriologically, as this may be positive for tuberculosis when histologic examination is negative. [17]

Abdominal and Pelvic Ultrasound ,CT And MRI are performed in circumstances where an abdominal or pelvic mass is present. Sonographic features of wet tuberculosis include septated ascites, particulate ascites, loculated fluid, thickened peritoneum, endometrial involvement and adnexal mass. Features of dry tuberculosis include adnexal mass, adhesions and loculated fluid. When compared with laparoscopy ultrasound was able to identify ascites/loculated fluid in(100%), adnexal mass in (93%),peritoneal thickening in (69%), omental thickening in (61%) and endometrial thickening in (83%) cases. Awareness of these features may improve diagnostic accuracy and avoid misdiagnosis and unnecessary surgical interventions. CT findings of abdominal tuberculosis may mimic diffuse peritoneal malignancy. These features include ascites, omental and mesenteric infiltration and smooth thickening of the parietal peritoneum.[18]

Hysterosalphingography : HSG continues to be an important diagnostic procedure to evaluate infertility in women. It can reliably diagnose unsuspected genital tuberculosis to help provide early intervention and timely treatment for a better outcome .HSG has been found to help diagnose asymptomatic cases of genital tuberculosis in many cases in India. The various abnormalities depend on the involvement of the fallopian tubes,

endometrium, and the severity of the disease. Endometrial tuberculosis has nonspecific appearance on HSG characterized by synechiae formation, a distorted uterine contour, and venous and lymphatic intravasation. The synechiae and intrauterine adhesions are characteristically irregular, angulated, and stellate-shaped with well demarcated borders. Scarring endometrial tuberculosis may convert the uterine cavity into a T-shaped cavity or asymmetric small shrunken cavity.[19] (Figure No. 3,4,5) Chavhan et al. observed genital TB in 7.5% of the HSGs performed for infertility. In their series the most common features were isthmo ampullary tubal occlusion in 81% cases, terminal hydrosalphinx in 16% cases, synechiae and intrauterine adhesions in 16% of women and venous and lymphatic intravasation of dye in 27%of women.[6]

Laparoscopy is now a well recognized procedure in the diagnosis of tuberculosis in infertile women with various findings,being the presence of miliary tubercles, whitish yellow or opaque plaques surrounded by hyperaemic areas in the tubes and uterus in acute stages.In chronic stages the tubes show nodular salphingitis, patchy salphingitis, hydrosalphinx, caseosalphinx or adhesions.[20] (Figure No. 6, 7, 8)

Laparoscopy picture of Tripathy and Tripathy observed adhesions ,tubercles and hyperaemia in 59.6% cases as well as adhesions in the POD in 11.3% cases,[12]while Bhide et al observed pelvic adhesions in 48% cases,tubercles in 33.8% cases,unilateral adnexal mass in 11.3% cases,and bilateral adnexal mass in 22%,encysted ascites in 8.5% and lesions in bowel or omentum in 24.5%.[21]

Hysteroscopy should be combined with laparoscopy to exclude/ confirm endometrial involvement. Tubercles, microcaseation, distorted ostium, caseous material coming through the ostium, distorted uterine cavity are some of the findings on hysteroscopy. Yasmin et al reported the presence of thick fibrous adhesions in 45%, flimsy adhesions in 40% and muscular adhesions in 15% of the cases found to be positive for genital tb. It was seen that 65% had adhesions in the body,25% at the site of the internal os and 1% had adhesions in the cervical canal,[22]while Bhagwan et al reported normal hysteroscopic findings in 20.5% cases; grade 2 adhesions in 15.1% , grade 3 in 15.1% cases, and grade 4 in 38.4% cases.[23]

Polymerase chain reaction: PCR is a rapid, sensitive and specific molecular biological method for detecting mycobacterial DNA in both pulmonary and extra-pulmonary samples from suspected TB patients. It can detect less than 10 bacilli per ml of the specimen and the results are available within 1-2 days . It provides a very useful role in early confirmation of diagnosis in paucibacillary extrapulmonary forms of tuberculosis There has been a genuine concern of false positivity due to contamination occurring in clinics and laboratories. The problem of false positivity can be substantially reduced by proper lab design, strict discipline about collection and processing the sample, handling of reagents and by the use of certain blocking agents. In case of false negative results several strategies like immunogenic beads and capture resins have been used with which the sensitivity of PCR assays can be significantly improved.[16]

A variety of PCR methods have been developed for detection of specific sequences of Mycobacterium tuberculosis and other mycobacteria. These PCR assays may either target DNA or rRna and these could be based on conventional DNA base

PCR, nested PCR and RT-PCR. Targets include insertion and repetitive elements, various protein encoding genes or rRNA. PCR assays targeting the IS*6110* element and the *mpt64* gene have abbreviated the turnaround time for definitive mycobacteriological detection in the laboratory to 1–2 days, besides being more sensitive than conventional methods. [24]

Techniques like real time PCR have markedly decreased the incidence of false positive cases because amplification and detection takes place in the same reaction tube. This is known as mycoreal PCR and this method has been adopted by many laboratories recently. It has sensitivity of 90-94% and specificity of 70-78%.[16]

Nested PCR involves the use of two pairs of PCR primers for a single locus. After the first pair amplifies the locus, the second pair of primers (nested primers) binds within the first PCR product and produce a second PCR product that will be shorter than the first one. The logic behind this strategy is that if the wrong locus were amplified by mistake, the probability is very low that it would also be amplified a second time by a second pair of primers. The repetitive nature of the gene increases the sensitivity to almost 100% as mentioned in few literatures. The specificity of the assay is 96 to 99%, with the lower limit of detection of 10 TB bacilli per ml of sample.[25]

Genital tuberculosis is a paucibacillary disease and if detected in early stage and treated can improve conception rate significantly. PCR represents rapid and sensitive method for detection of mycobacterium DNA in early female genital TB and may be a useful adjunct to diagnostic modalities in genital TB.[16]

METHODOLOGY

Source of data: Infertile women undergoing hysterolaparoscopy as a part of their infertility workup at ARC of KLES PRABHAKAR KORE Hospital, Belgaum.

Study Design: Cross sectional study

Duration : 1 year

Setting: Assisted Reproduction Centre of KLES PRABHAKAR KORE Hospital, Belgaum.

Inclusion criteria: All infertile women subjected to hysterolaparoscopy as a part of infertility workup.

Exclusion Criteria: Women not giving consent for hysterolaparoscopy.

Method : Hysterolaparoscopy was performed in the pre ovulatory period usually on day 6^{th}-10^{th} of the cycle for evaluation of infertility.

Diagnostic Laparoscopy was performed using a 7mm Karl Storz laparoscope with a 30 degree deflection angle telescope powered with a fibreoptic cable for light source and a careful evaluation of the uterine cavity,fallopian tubes,ovaries,pelvic peritoneum,pouch of douglas and peritoneal cavity was done. Features suggestive of genital tuberculosis were looked for by noting the presence of

- miliary tubercles on the uterus and tubes
- nodular salphingitis

- caseosalphinx
- hydrosalphinx
- presence of peritubal , periovarian, omental and bowel adhesions
- free fluid in the pouch of douglas

Biopsy from suspicious areas was taken in selected cases.

Following this hysteroscopy using normal saline as the distention media was done to identify features suggestive of tuberculosis that included

- presence of tubercles
- microcaseation
- distorted ostia
- calcifications
- synechie(grade 1 to 3)[26]

Grade 1- less than $1/4^{th\ of}$ uterine cavity involvement

Grade 2- $1/4^{th}$ to $3/4^{th}$ of uterine cavity involved;ostia and fundus partly involved

Grade 3-more than $3/4^{th}$ of uterine cavity involved;ostia and upper cavity occluded.

Chromopertubation was done and any delayed or absent spillage of dye was noted. At the end of the procedure endometrial curetting was taken and sent for histopathology in formalin and for nested TB PCR in normal saline.

On histopathogy of endometrial curretings the features suggestive of tuberculosis were; the presence of tubercle bacilli, caseous necrosis,giant cells,epithelial cell clusters and lymphocytic infiltration.

TB PCR of the endometrial curettings was done using the nested TB PCR in which there is double amplification of the IS6110 gene locus .

Video recording of all the cases was done.

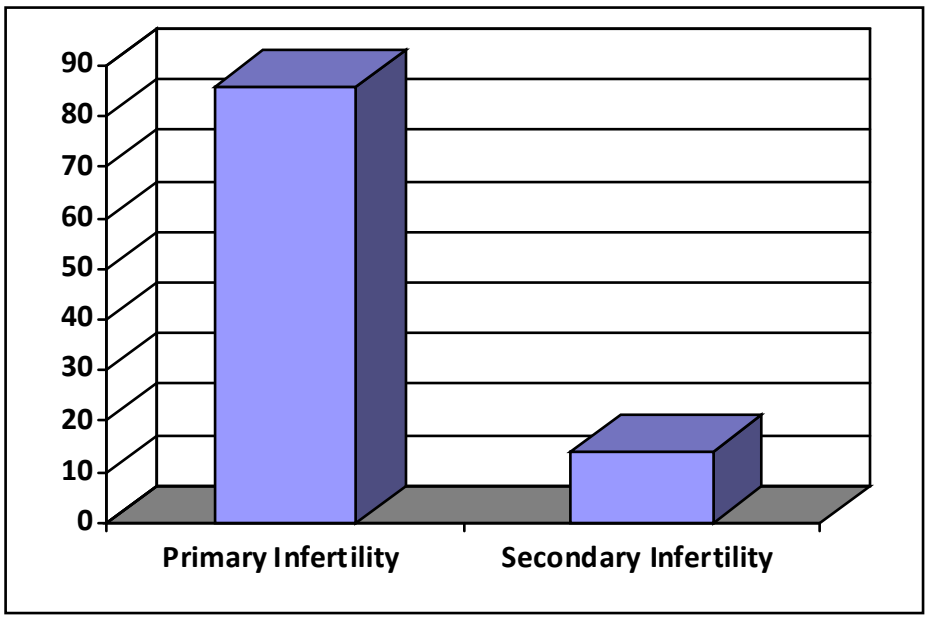
In cases found to be positive for genital tuberculosis by TB PCR and/or histopathology of endometrial curettings the clinical findings were analyzed and video recordings of the hysterolaparoscopic findings were carefully reviewed for the presence of features suggestive of genital tuberculosis.

RESULTS

A total of 96 cases were enrolled in the study over a period of 1 year from Nov 2007-Nov 2008 and underwent diagnostic hysterolaparoscopy as a part of their infertility workup.

The diagnosis of genital tuberculosis was confirmed in 21 of the 96 enrolled cases (21.8%) by either TB PCR and/ or histopathology of endometrial curettings. The clinical and hysterolaparoscopy findings were analyzed in cases found to be positive for genital tuberculosis.

In 20 cases (95.3%) TB PCR of endometrial curettings came positive while histopathology of endometrial curettings came positive in only one case .The mean age group of the women was 27.5 years. 86% of the positive cases presented with primary infertility while 14% manifested with secondary infertility.



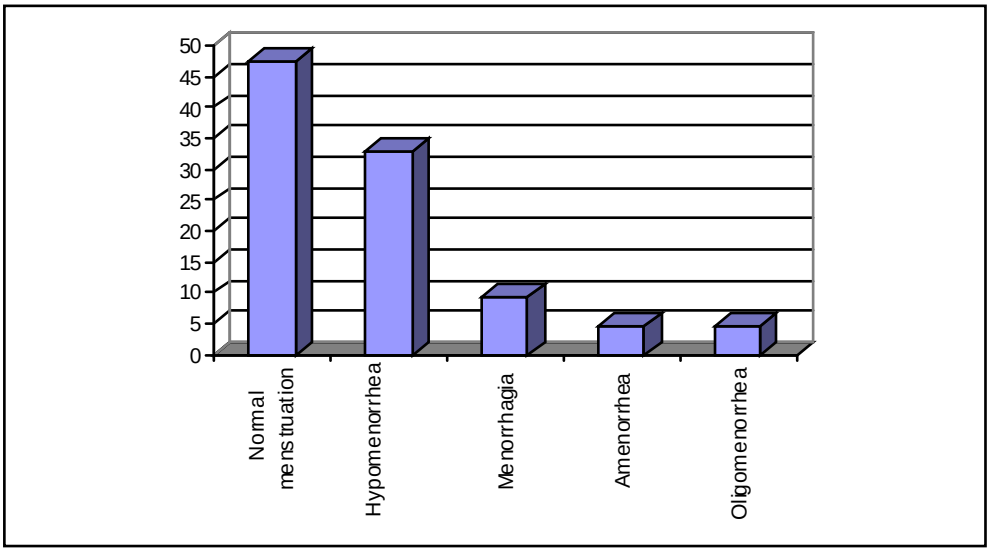
Graph 1:Percentage frequency of infertility pattern in patients of GTB

Another finding was the presence of genital tuberculosis in 11% cases of unexplained infertility. Amongst the 21 positive cases of genital tuberculosis history of recurrent pregnancy loss was present in 2 cases (9.5%).

Majority of the women had normal menstrual function (47.6%), but the most common menstrual complaint was hypomenorrhea (33%).Menorrhagia was seen in 9.5% cases while oligomenorrhea and amenorrhea was present in 4.7% cases.

Table 1: Different menstrual patterns seen in GTB

Menstrual abnormality	**No of Cases**	**Percentage**
Normal menstruation	10	47.6
Hypomenorrhea	07	33
Menorrhagia	02	9.5
Amenorrhea (primary)	-	-
(secondary)	01	4.7
Oligomenorrhea	01	4.7



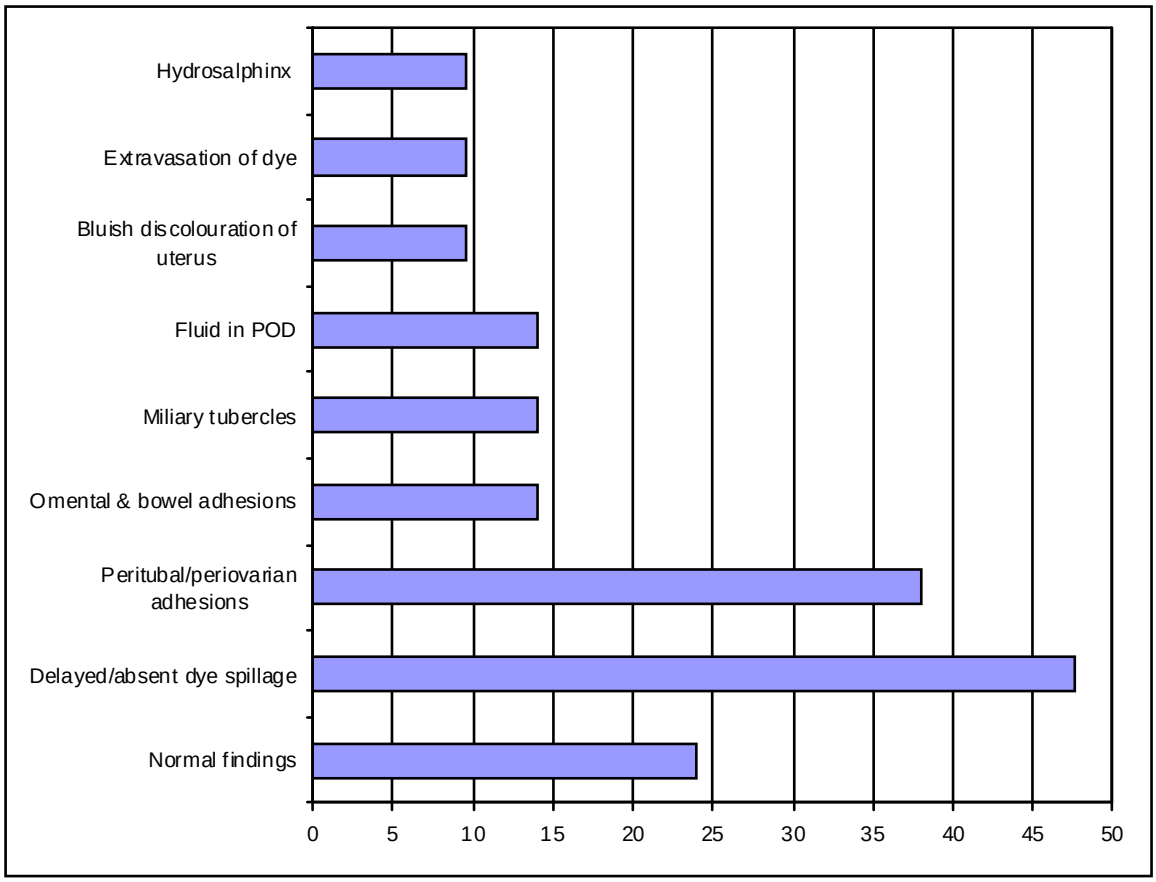
Graph 2: Percentage frequency of different menstrual patterns seen in GTB

On undergoing laparoscopy 24% of the patients had normal findings while the most common abnormality was the presence of delayed or absent spillage of the dye seen in 47.6% cases. Peritubal and periovarian adhesions were found in 38% cases, while omental and bowel adhesions were seen in 14% cases. Miliary tubercles over the surface of the uterus, tubes and ovaries were visualized in 14% cases. Presence of fluid in pouch of douglas was also noted in 14% cases. Bluish disolouration of the uterus and extravasation of the dye was visualized in 9.5% of the cases.

Table 2 : Laparoscopic findings in patients of GTB

Laparoscopy findings	**No of cases**	**Percentage**
Normal findings	5	24
Delayed/absent dye spillage	10	47.6
Peritubal/periovarian adhesions	8	38
Omental & bowel adhesions	3	14
Miliary tubercles	3	14
Fluid in POD	3	14
Bluish discolouration of uterus	2	9.5
Extravasation of dye	2	9.5
Hydrosalphinx	2	9.5

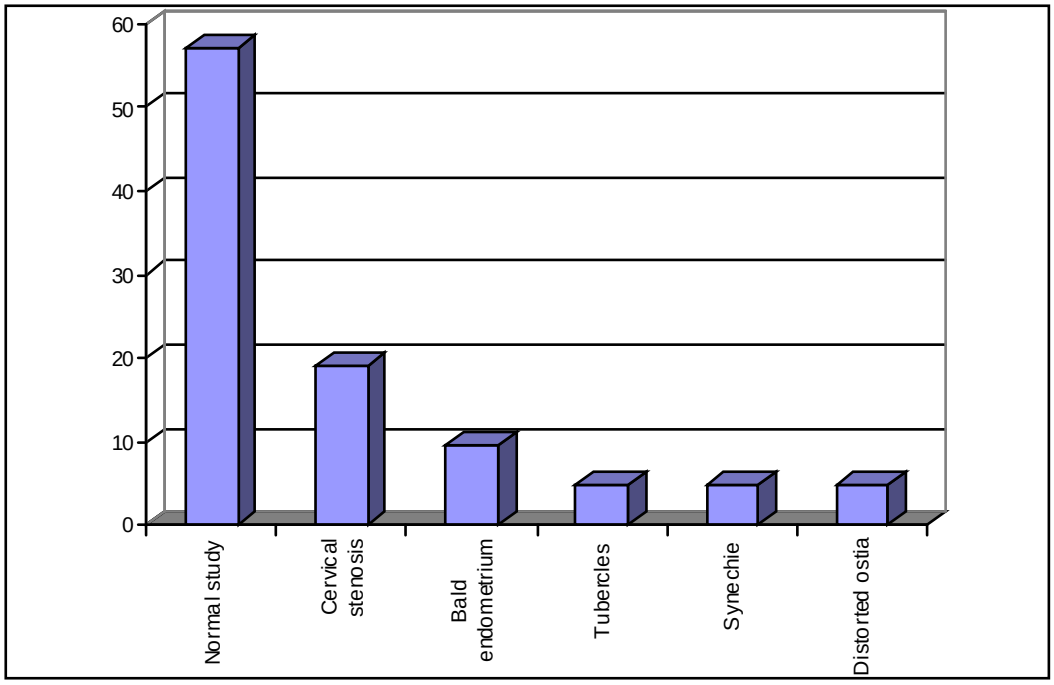
On hysteroscopy, 57% of the positive patients had normal findings, cervical stenosis was seen in 19% cases, pale or bald appearance of the endometrium was observed in 9.5% cases while synechie and distorted ostia were seen in 4.7% cases. Presence of tubercles studded in the uterine cavity was visualized in 4.7% cases.



Graph 3: Percentage frequency of laparoscopic findings in patients of GTB

Table 3 : Hysteroscopy findings in patients of GTB

Hysteroscopy findings	No of cases	Percentage
Normal study	12	57
Cervical stenosis	4	19
Bald endometrium	2	9.5
Tubercles	1	4.7
Synechie	1	4.7
Distorted ostia	1	4.7

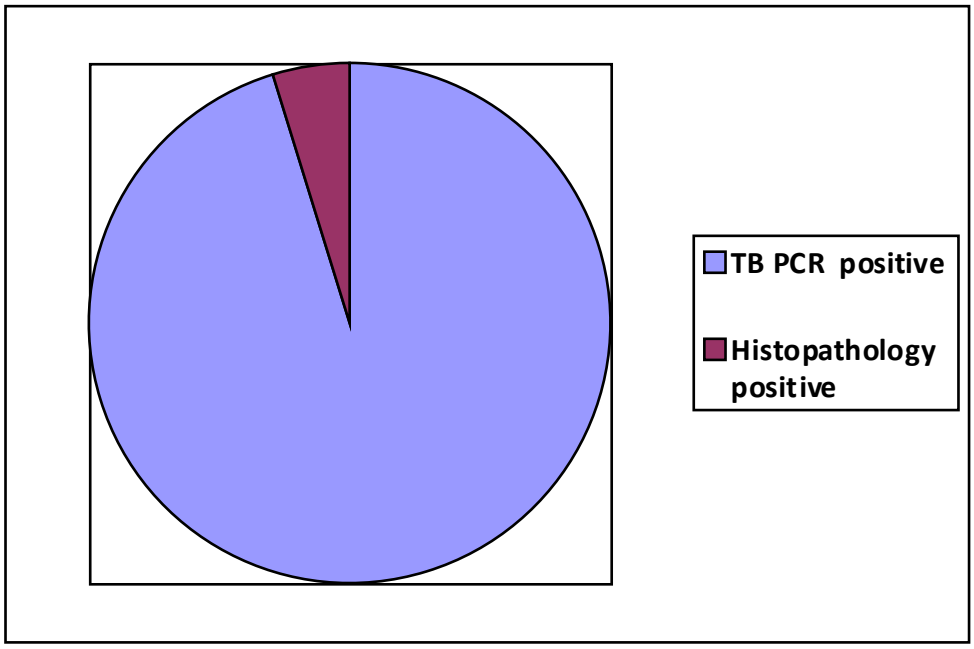


Graph 4: Percentage frequency of hysteroscopy findings in patients of GTB

Normal hysterolaparoscopic findings were observed in 14% of the cases found to be positive for genital tuberculosis.

All patients who underwent hysterolaparoscopy had samples of endometrial curetting sent for histopathology and TB PCR. Out of 96 samples sent 21 cases (21.8%) were found to be positive for genital tuberculosis. In 20 cases (95.2%) TB PCR of endometrial curetting was positive while histopathology of endometrial curetting was positive only in 1 case (4.7%). In 1 case PCR and histopathology of endometrial curetting showed negative results but biopsy of the tubercle studded over the peritoneum had histopathological features of tuberculosis.

	TB PCR positive	Histopathology positive
Endometrial curettings	20(95.3%)	1(4.7%)



Graph 5: Pie diagram comparing the two diagnostic modalities used

DISCUSSION

The reported prevalence of genital tuberculosis varies widely world over [3,4.]This is due to the differences in the population group studied, sensitivity and specificity of tests used for its diagnosis and the timing of the sample with respect to the menstrual cycle. Multiple and repeat sampling is also known to enhance the sensitivity of the diagnostic tools used.[8]

In our study the criteria for diagnosis of GTB was positive TB PCR and granulomatous lesion on histopathology ,however several other studies have quoted the incidence of GTB using combined diagnostic tests including hysteroscopy and laparoscopy findings which may be quite nonspecific.[16]

In the present study the incidence of genital tuberculosis was 21%.A study done in South Africa[27] on 109 infertile women, also reported an incidence of genital tuberculosis to be 21%.In this study the diagnosis of genital tuberculosis was made using positive culture of AFB from three samples of endometrial tissue, menstrual blood and peritoneal fluid from pouch of douglas.

In our study 95.3% of the cases of GTB were diagnosed using TB PCR while various other authors have reported a positivity of TB PCR in the range of 46-64 %.[16,28]This high pick up rate of GTB in our study could be due to the use of nested TB PCR which is known to increase the sensitivity of the test. However in the above mentioned study the authors have not described the type of PCR being used nor the gene locus being studied at their centre.

A study comparing AFB smear, culture, histopathology and TB PCR found that the detection rate amongst the suspected cases was highest with TB PCR 43.1% compared to 11% with histopathology,7.8% with culture and 5% with AFB staining.[29]

The detection of genital tuberculosis by histopathology in our study was 4.7% as against 11.5% observed by other authors.[29] A low pick up rate in our study is probably due to the fact that endometrial biopsy was done in the post menstrual phase during the evaluation by hysterolaparoscopy.

Another feature observed was the detection of genital tuberculosis in 11% of the cases in whom infertility was presumably thought to be unexplained. This is due to the fact that many cases of genital TB have asymptomatic presentation and the disease could be in an early stage before the manifest of classical features of GTB. Similar finding has also been reported from a hospital based South African study where genital tuberculosis was present in 10-15% of cases presenting with 'idiopathic' infertility.[27]

Majority of the cases had a normal menstrual cycle (47.6%) while hypomenorrhea was the most common menstrual abnormality present in 33% cases. Similar findings were reported in an Indian study where normal menstrual pattern was seen in 57.6% of cases and hypomenorrhea in 30.1% cases.[28]

Laparoscopy demonstrated peritubal and periovarian adhesions in 38% cases & omental and bowel adhesions in 14% cases. This is corroborating well with results obtained by other authors who have quoted it to be present in 59% cases.[20] On chromopertubation ,delayed and absent spillage of the dye was seen in 47.6% cases which is correlating well with the figures obtained from other studies which have

reported abnormal chromopertubation results in 50%cases.[20]This is due to the fact that fallopian tubes are the initial and most frequently affected site in pelvic mycobacterial infection. Bluish disolouration of the uterus and extravasation of the dye was visualized in 9.5% of the cases which is an indicator of endometrial involvement. Presence of encysted ascites and fluid in pouch of douglas was detected in 9.5% and 4.7% cases respectively while other authors have reported in 8.45% cases.[21] Normal laparoscopic findings were observed in 24% cases which could be due to the fact that it generally detects macroscopic changes that are seen in chronic stages.

In our study hysteroscopy revealed normal findings in 67% cases thereby implicating less than 50%endometrial involvement as has been reported by most authors.[11]Intrauterine synechie and distorted ostia were visualized in only 4.7% cases while authors have noticed abnormal hysteroscopy findings in 6.5% cases of genital tuberculosis.[16]

Another finding noted in our study was the presence of cervical stenosis in 19% of cases which could be due to adhesion in the cervical canal .This finding will be of special clinical significance in infertile women. Various studies have reported cervical involvement in 5-10% of the cases in the form of ulcerative lesions which has a different clinical presentation and implication .[11]

A combined normal hysteroscopy and laparoscopy finding was observed in only 14% cases thereby suggesting a strong correlation between genital tuberculosis and hysterolaparoscopy findings.

CONCLUSION

- Genital tuberculosis remains an important under diagnosed cause of infertility.
- The alarming high incidence of genital TB underlines the importance of investigating and treating this condition in all infertile women.
- Since genital tuberculosis is a paucibacillary disease and majority of the women have an asymptomatic presentation, PCR appears to be a rapid, reliable and a highly sensitive diagnostic modality.

SUMMARY

A cross sectional study was done over a period of one year to evaluate the diagnostic value of PCR in detection of genital tuberculosis in infertile women and to correlate the clinical and hysterolaparoscopic findings in cases found positive for genital tuberculosis by TB PCR or/and histopathology of endometrial curettings.

Hysterolaparoscopy was performed in the pre ovulatory period usually on day 6^{th}-10^{th} of the cycle for evaluation of infertility At the end of the procedure endometrial curettings were taken and sent for histopathology in formalin and for nested TBPCR in normal saline.

In cases found to be positive for genital tuberculosis by TB PCR and/or histopathology of endometrial curettings the clinical findings were analyzed and video recordings of the hysterolaparoscopic findings were carefully reviewed for the presence of features suggestive of genital tuberculosis.

The diagnosis of genital tuberculosis was confirmed in 21 of the 96 enrolled cases (21.8%) by either TB PCR and/ or histopathology of endometrial curettings. In majority of the cases (95.3%) TB PCR of endometrial curettings came positive while histopathology of endometrial curettings came positive in only one case for which TB PCR was also positive.

Since genital tuberculosis is a paucibacillary disease and majority of the women have an asymptomatic presentation. PCR appears to be a rapid, reliable and a highly sensitive diagnostic modality.

A combined normal hysteroscopy and laparoscopy finding was observed in only 14% cases thereby suggesting a strong correlation between genital tuberculosis and hysterolaparoscopy findings.

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CONSENT FORM

Sr. No. : _____

Patients name Mrs. :

We hereby request you to participate in "DIAGNOSTIC VALUE OF PCR FOR DETECTION OF GENITAL TUBERCULOSIS IN EVALUATION OF FEMALE INFERTILITY"

The study is designed to assess the diagnostic value of TB PCR in comparison with histopathology and to analyze the clinical and hysterolaparoscopic findings in cases found positive for genital tuberculosis infertile females attending ARC KLES Prabhakar Kore Hospital, Belgaum.

This study is being done under the direct supervision of Dr. Shobhana Patted. Only if you agree to undergo hysterolaparoscopy for your infertility workup will you be a part of this study. Your hysterolaparoscopic, TB PCR and histopathological findings will be analyzed. All information collected about you during the course of this study will be kept confidential.

There are no financial incentives promised to you for being a part of this study. Your participation in this study is entirely voluntary and you may withdraw from the study at any time and you will be treated according to the existing protocol.

If you have any questions about the study you may please contact chief investigator Dr. Shobhana Patted, ARC, KLES Prabhakar Kore Hospital and MRC, Ph. No. 9845273929, Dr. Jasneet PG Department of Obstetrics and Gynaecology, JNMC, Belgaum Ph No. 9964104491 and regarding participant rights you may contact Chairman

of ethical committee Dr. V. D. Patil, Principal, J. N. M.C., Belgaum Tel No. 958312473777.

I have been explained in my vernacular language regarding the proposed procedure and the risk and benefits associated with it and I undersigned give my consent for the same.

Signature of the Participant or legally authorized representative

Participants Name

Signature

Address

Telephone NO.

Experimenters / Witness name

Signature

Date

PROFORMA

"DIAGNOSTIC VALUE OF PCR FOR DETECTION OF GENITAL TUBERCULOSIS IN EVALUATION OF FEMALE INFERTILITY"

Sr. No._____ Date_____

Patient's name: _____Age _____

Husbands Name:_____ Age _____

Address:_____

_____

Married life: _____

Duration of infertility_____

Primary :_____

Secondary: _____ Parity: _____

Abortions: _____

Living:_____

Ectopic pregnancy:_____

Last Delivery: _____

Last abortion:_____

Menstrual history

LMP :_____

Menstrual flow	**Yes**	**No**
Normal	☐	☐

Hypomenorrhea (Scanty menstruation last in for less than2 days)	☐	☐
Oligomenorrhea (bleeding where cycle length exceeds 35 days)	☐	☐
Menorrhagia (bleeding excessive in amount > 80ml or duration > 7 days)	☐	☐
Any other findings		
	Yes	**No**
Symptoms suggestive of pulmonary tuberculosis		
Fever	☐	☐
Cough	☐	☐
Hemoptysis	☐	☐
Loss of weight or appetite	☐	☐
History of treatment with ATT	☐	☐
Duration of treatment	_____	(in months)
History of contact with Tuberculosis	☐	☐
Examination findings		
Lymphadenopathy	☐	☐
Respiratory findings	☐	☐
Abdominal examination		
Palpable lump	☐	☐

Genital examination

P/S.

P/V

Ultra sound findings (D2-D5 of cycle)

Uterine measurements

Endometrial thickness

Left adnexa

Right adnexa

Any other relevant observation

Laparoscopy findings

Dilated, tortuous tubes

Military tubercles on uterus and tubes

Nodular salphingitis

Hydrosalphinx

Fluid in pouch of Douglas

Presence of peritubal, periovarian, bowel or omental adhesions

Hysteroscopy findings

Calcifications

Microcaseation

Presence of synechie

	Positive	Negative
Distorted ostium	☐	☐
Fluffy endometrium	☐	☐
Tubercles	☐	☐
TB PCR :	☐	☐
Histopathology of endometrial currettings	☐	☐

KEY TO MASTER CHART

HPR	: Histopathology report
Hystero	: Hysteroscopy
Lap	: Laparoscopy
Menst abn	: Menstrual abnormality
N	: Negative
P	: Positive
PCR	: Polymerase chain reaction
Prim	: Primary
Sec	: Secondary

Figure No. 1 : Lowenstein Jensen media

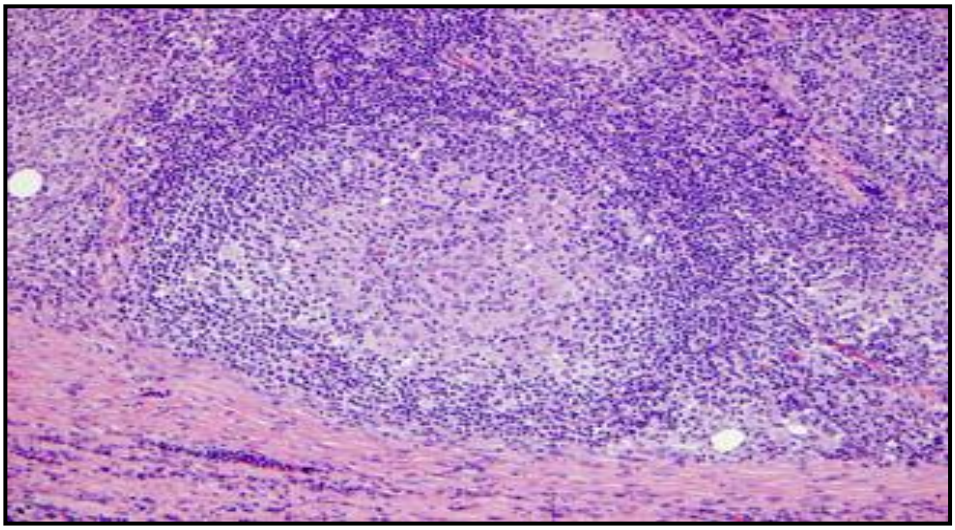

Figure No. 2 : Tubercular granuloma

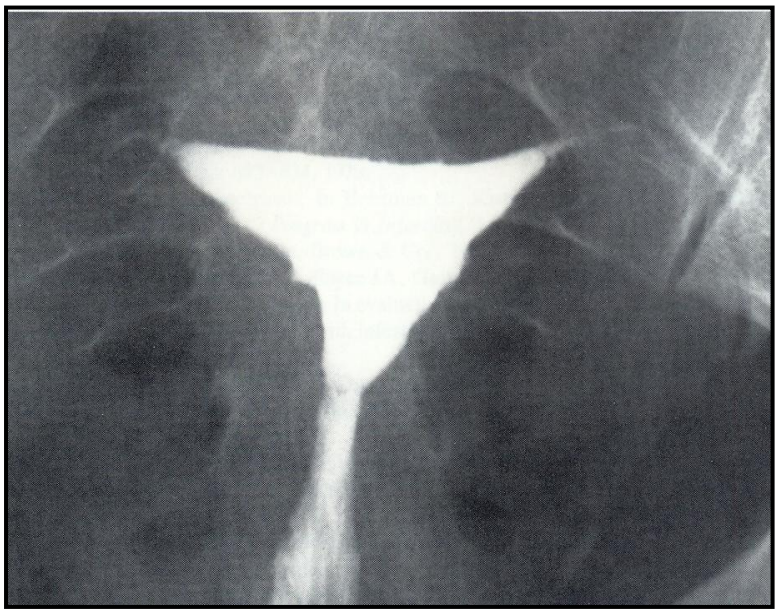

Figure No. 3 : Bilateral Cornual block

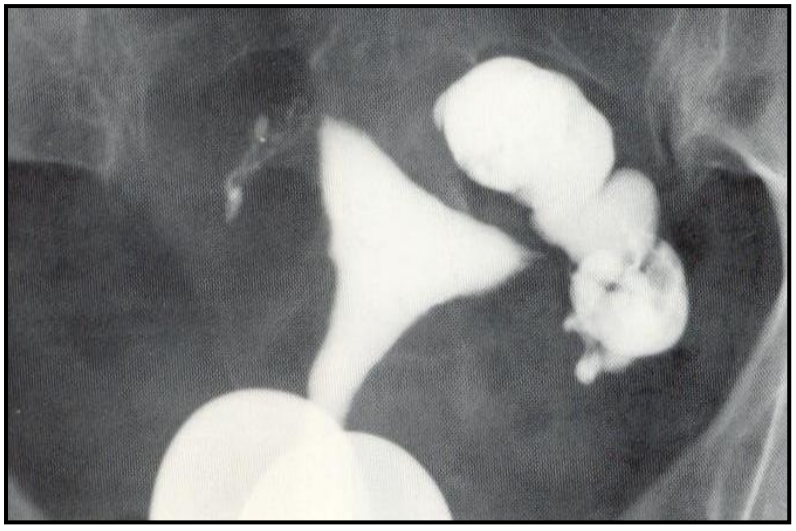

Figure No. 4 : Lt. sided Hydrosalpinx & Rt. sided tubal block

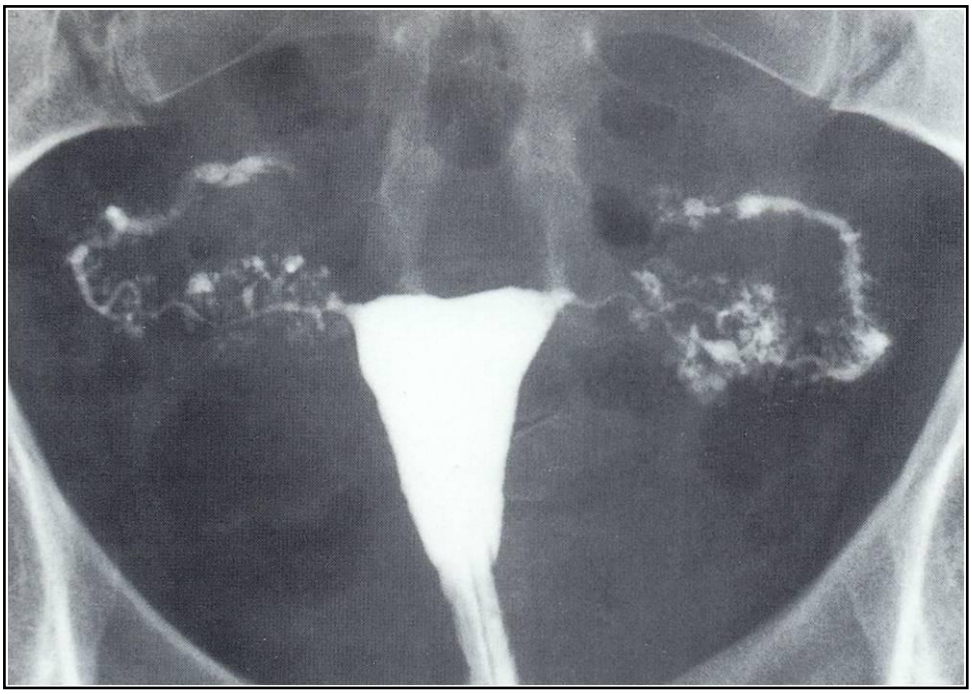

Figure No. 5 : Bilateral salphingitis isthmica nodosa

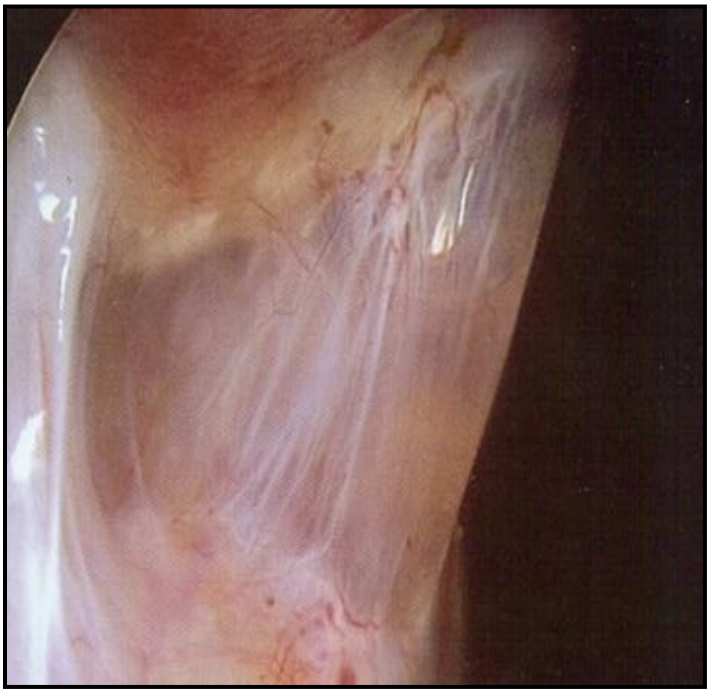

Figure No. 6 : Flimsy avascular adhesions

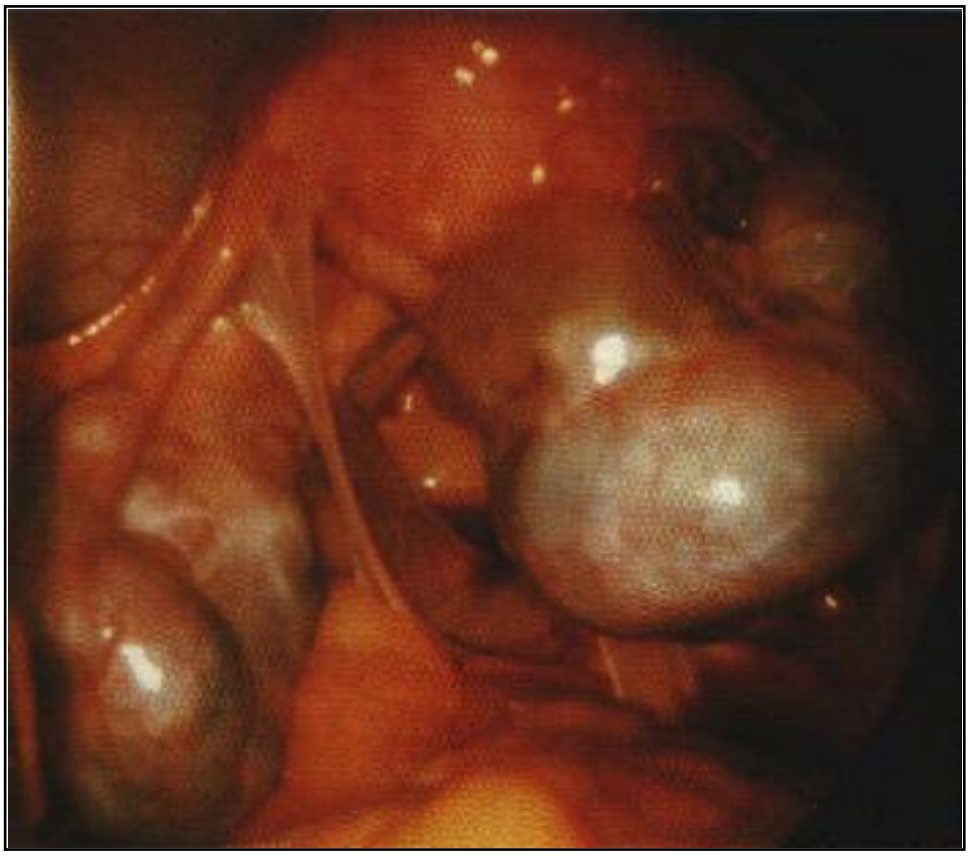

Figure No. 7 : Hydrosalphinx

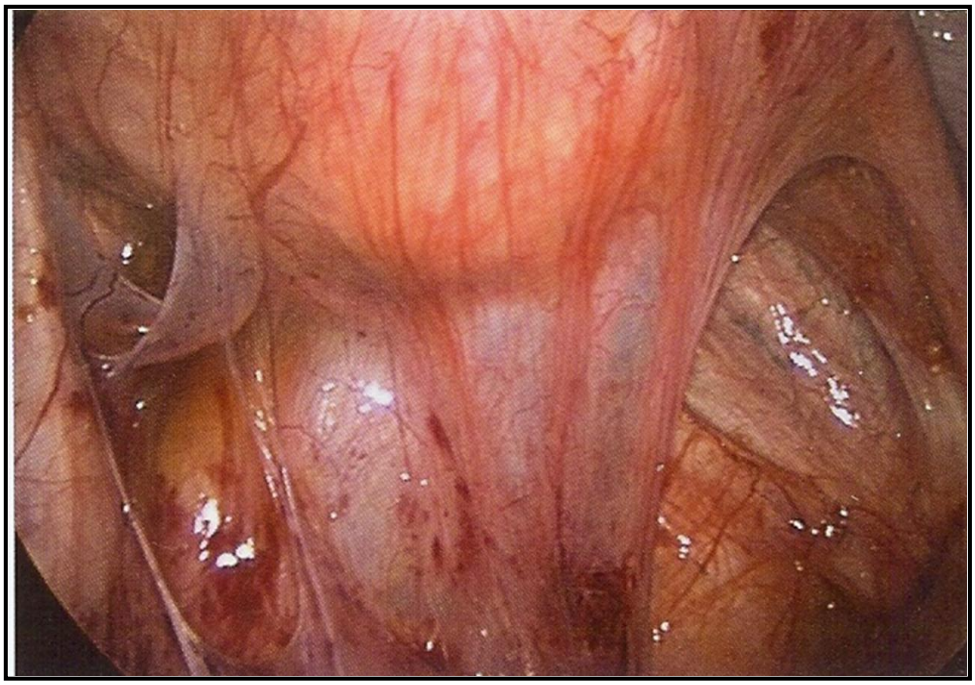

Figure No. 8 : Flimsy vascular adhesions

Sl. No.	Name	Age	TB PCR	HPR	Infertility	Menst abn	Examn	Lap findings	Hysterofindings
1	pratibha gudas	21	p	n	prim	hypo/oligo		fluid in pod	
2	hampamma	29	p	n	prim	normal	cervical stenosis	peritubal/periovarian adhesions/nodular tubes/delayed spillage	distrorted ostia
3	gouri nashipudi	32	p	n	prim	normal	cervical stenosis	peritubal/periovarian adhesions	pale endometrium
4	roopa havaldar	26	p	n	prim	hypo		b/l spillage absent	
5	dilshad jore	31	p	n	prim	menorrhgia			
6	sulochana desai	34	p	n	sec	normal	cervical stenosis		
7	gangawwa	30	p	p	prim	2 amenor		adhesions bw uterus and omentum	
8	gayatri nadegar	31	p	n	sec	oligo			bald endometrium
9	chanamma uppar	30	p	p	prim	menorrhgia		uterus bluish discolouration	
10	bharti guddas	29	p	n	sec	normal		b/l spillage absent	
11	khushi b	32	p	n	prim	normal		extravasation of dye	synechie
12	jyoti kallyamath	28	p	n	prim	hypo		fluid in pod	polypoidal
13	asha kochhikar	38	p	n	sec	hypo	cervical stenosis	tubercles/adhesions/cpt delayed spillage	
14	pushpa servi	23	p	n	prim	normal		adhesions/fluid in pod	
15	vijaylaxmi k	27	p	n	prim	normal		extravasation of dye/delayed spillage	
16	raksha kulkarni	28	p	n	prim	normal			
17	savita	29	p	n	prim	hypo		tubercles/adhesions/cpt delayed spillage	
18	sneha murgod	28	p	n	prim	normal		uterus bluish discolouration/delayed spillage	
19	seema sanikop	24	p	n	prim	normal		tubercles/adhesions/cpt delayed spillage/hydrosalphinx/fluid in pod	tubercles
20	geeta mutagi	28	p	n	prim	hypo		peritubal/periovarian adhesions/bowel adhesionsdelayed spillage	
21	vijaylaxmi kodivam	29	p	n	prim	hypo		hydrosalphinx/delayed spillage	
22	mamta	32	n	n	prim			normal study	
23	shahista m	30	n	n	prim			normal study	
24	mahadevi bainaik	28	n	n	prim			delayed spillage of dye	
25	malavva gangar	28	n	n	prim			b/l absent spillage	
26	shehnaz	26	n	n	prim			normal study	
27	akshya nak	35	n	n	prim			peritubal/periovarian adhesions/endometriotic spot	
28	kavita	32	n	n	prim			normal study	
29	vijaylaxmi r	28	n	n	prim			peritubal /periovarian adhesion/choclate cyst/endo spot	
30	renuka c	27	n	n	prim			peritubal /periovarian adhesion/omental adhesions	
31	kanchan b	38	n	n	prim			peritubal/periovarian adhesions/absent spillage	
32	vidya h	23	n	n	prim			delayed spillage	
33	shashikala	33	n	n	prim			normal study	
34	rashmi p	29	n	n	prim			peritubal /perivarian adhesions/hydrosalphinx	distorted ostia
35	sharda u	32	n	n	prim			subserous fibroid/paraovarian cyst	
36	soumya r	25	n	n	sec			normal study	
37	sumangala	26	n	n	prim			normal study	
38	sunita punoshi	30	n	n	prim			fimbrial cyst	
39	rajani n	29	n	n	prim			normal study	
40	veena hattikal	30	n	n	prim			delayed spillage	
41	charu kataria	27	n	n	prim			tube/ovary absent/adhesions b/w uterus and lat pelvic wall	
42	mahadevi pujari	30	n	n	prim			pertubal /periovarian adhesions/hydrosalphinx	distorted ostia
43	sarita s	32	n	n	prim			intramural fibroid/hydrosalphnx/delayed spillage	
44	shobha nirwani	22	n	n	prim			pritubal/periovarian adhsions/cystic ovary	
45	shabana m	25	n	n	sec			peritubal /perivarian adhesions/no spillage	
46	hadisha k	38	n	n	prim			no spillage rt tube	adhesions
47	laxmibai	35	n	n	prim			normal study	
48	geeta h	23	n	n	prim			normal study	
49	sangeeta katti	24	n	n	prim			b/l spillage absent/pcos picture	

Sl. No.	Name	Age	TB PCR	HPR	Infertility	Menst abn	Examn	Lap findings	Hysterofindings
50	asha jainar	27	n	n	prim			pcos picture/arcuate uterus	
51	chandrakala	29	n	n	prim			ovarian cyst/cornual fibroid	
52	seema	24	n	n	sec			normal study	
53	vijaylaxmi a	29	n	n	prim			normal study	
54	rekha m	32	n	n	prim			peritubal/periovarian adhesions	
55	anjali mogale	31	n	n	prim			periovarian/peritubal adhesions/endometriotic spot	
56	shilpa	25	n	n	prim			normal study	
57	medha patil	29	n	n	prim			pcos picture	
58	bhavani galgali	34	n	n	prim			extravasation of dye present	
59	geeta	37	n	n	sec			normal study	
60	mahadevi	32	n	n	prim			normal study	
61	geeta mane	29	n	n	prim			subserous fibroid	
62	sunanda patl	31	n	n	prim			normal study	
63	bhagyamma	27	n	n	sec			normal study	cervical stenosis
64	gayatri	32	n	n	prim			pcos	
65	rohini	39	n	n	prim			normal study	
66	kamlawwa y	28	n	n	sec			peritubal/periovarian adhesions/absent spillage	
67	aarti s	34	n	n	sec			normal study	
68	shobha s	32	n	n	prim			subserous fibroid	
69	resma	34	n	n	prim			normal study	
70	sulochana	29	n	n	prim			pcos picture	
71	sushma	34	n	n	prim			normal study	
72	mahadevi s	33	n	n	prim			normal study	
73	ahwini	38	n	n	prim			normal study	
74	bharti	27	n	n	prim			normal study	
75	laxmi b	32	n	n	sec			hydrosalphinx	
76	sarita	28	n	n	prim			pcos picture	
77	kamla	25	n	n	prim			normal study	
78	anita s	27	n	n	prim			peritubal/periovarian adhesions/ovarian cyst	
79	bhavana m	30	n	n	prim			normal study	
80	prema b	26	n	n	prim			normal study	
81	seemA	37	N	N	sec			normal study	
82	hema	30	n	n	prim			pcos picture	
83	jaya	28	n	n	prim			normal study	
84	bibijaan	34	n	n	prim			normal study	
85	vijaya	28	n	n	prim			ovarian cyst	
86	jyoti	38	n	n	prim			endometriotic spots on peritoneun	
87	yallawa	29	n	n	prim			normal study	
88	afreen	26	n	n	prim			pcos picture	
89	vaihali	34	n	n	prim			normal study	
90	priya	30	n	n	prim			delayed spillage	
91	bismillah	29	n	n	prim			peritubal/periovarian adhesions	
92	ganga	34	n	n	prim			normal study	
93	asma	28	n	n	prim			normal study	
94	anupama	27	n	n	prim			choclate cyst	
95	smita	24	n	n	sec			normal study	
96	gangubai	28	n	n	prim			normal study	