
**COMPARING THE DIAGNOSTIC EFFICACY OF TUBERCULOSIS
(TB) POLYMERASE CHAIN REACTION (PCR) WITH CULTURE
OF TB BACILLI USING RADIOMETRIC ASSAY IN DETECTION
OF GENITAL TUBERCULOSIS (GTB) IN INFERTILE WOMEN**

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DISSERTATION

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ABBREVIATIONS

AFB	: Acid Fast Bacillus
ARC	: Assisted Reproduction Centre
CT	: Computerized Tomography
ESR	: Erythrocyte Sedimentation Rate
GTB	: Genital Tuberculosis
HSG	: Hysterosalpingography
LJ media	: Lowenstein Jensen media
MRI	: Magnetic Resonance Imaging
PCR	: Polymerase Chain Reaction
POD	: Pouch of Douglas
DNA	: Deoxyribonucleic Acid
rRNA	: Recombinant Ribonucleic Acid
RT-PCR	: Reverse Transcriptase Polymerase Chain Reaction

ABSTRACT

"Comparing the diagnostic efficacy of tuberculosis (TB) Polymerase Chain Reaction (PCR) with culture of TB bacilli using Radiometric Assay in detection of Genital Tuberculosis (GTB) in infertile women".

Objective: Assessment of the diagnostic value of Radiometric Assay (BACTEC) in comparison with PCR in diagnosis of genital tuberculosis and correlation of the clinical and hysteroscopic findings in cases found positive for genital tuberculosis by TB PCR and/or BACTEC.

Methodology: This study was carried out at Assisted Reproductive Centre of KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum. Hysteroscopy was done in the preovulatory period between the 6th-10th days of the cycle. Endometrial curettings were sent in normal saline for both TB PCR and BACTEC and HPR in formalin. Clinical and hysteroscopic findings were analyzed in cases found to be positive for genital tuberculosis by TB PCR and/or BACTEC.

Results: The diagnosis of genital tuberculosis was confirmed in 14 of the 61 enrolled cases (22.95%) by TB PCR and/or TB BACTEC and/or histopathology of endometrial curettings. Of the 14 positive cases, 13 cases (92.85%) were positive by PCR, two cases (14.28%) were positive by BACTEC, of which one case (7.14%) was positive by both PCR and BACTEC. None of the cases had histopathological evidence of mycobacterial infection. Out of 14 positive cases, eight (57.14%) PCR positive cases had positive correlation with hysteroscopy. None of the BACTEC positive cases had hysteroscopic features suggestive of GTB.

Conclusion: Though culture is the gold standard for diagnosis of genital tuberculosis, further research is needed definitively to conclude the diagnosis of genital tuberculosis as it is paucibacillary.

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INTRODUCTION

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

One third of the world's 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 two deaths occurring every three minutes. 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. The 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. Genital tuberculosis is seen in 12% of all patients with pulmonary tuberculosis. It accounts for 5-10% of all pelvic infections. It is an important cause of infertility, being an etiological factor in 1-8% of the cases. ^{2, 3, 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 (95-100%),

followed by the endometrium (50%), ovaries (20%), cervix (5%), vagina and vulva (<1%).⁴

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 and hence a definitive modality is required to diagnose this condition.⁵

A high ESR and a positive Mantoux test are usually nonspecific.⁶

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.²

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 hydrosalpinx, peritubal adhesions, rigid pipe appearance and irregularity of the tube.²

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

Hysteroscopy is a well recognized procedure for the diagnosis of genital tuberculosis and detects macroscopic changes like tubercles, peritubal, periovarian adhesions, hydrosalpinx, and tuboovarian mass though it may miss subtle changes found in the early stages of the disease.⁷

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. It also has low detection rates and limitations as GTB is paucibacillary.^{8,9}

Rapid nucleic acid amplification techniques such as PCR of mycobacteria tuberculosis is reported to be 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 has its own limitations. It cannot distinguish between live and dead bacilli. Its role in monitoring the treatment is less clear and also it does not provide antibiotic susceptibility data. False negative results are due to contamination of the sample with heparin. There is genuine concern about false positivity with PCR which could be because of cross contamination and inadequate lab standardization.^{5,6}

Culture for TB bacilli is the gold standard for diagnosis of genital TB. However traditional AFB culture by LJ medium has a low detection rate. Colonies are seen if the bacillary count is more than 1000 bacilli and it also takes a longer time to give positive results.^{5,6}

The introduction of broth based growth systems, has significantly reduced the time to detection and increased the total number of positive cultures. The first such system developed was the BACTEC 460 TB system by M/S Becton Dickinson (Figure 1), Cockins Ville, Mary Land USA. BACTEC uses palmitic acid as the substrate for the growth of AFB. The carbon atoms in this substrate are radio-labeled and thus the carbon dioxide (CO₂) released is also radio-labeled and this is measured in the form of growth index. A growth index of more than 10 gives a strong suspicion of TB. BACTEC gives rapid results and is highly sensitive and the time needed for culture and drug sensitivity testing is about 2 to 3 wks. Colonies are seen even if the bacillary count is 100.^{5,10}

BACTEC has a sensitivity of 80-90% whereas LJ medium has sensitivity as 30-40%. Whether cultured by LJ media or BACTEC, the detection of a positive culture depends on:

- 1) Number of organisms in the specimen - heavy smear positive specimens may turn positive as early as 48 hours but if bacterial load is low it takes longer time to grow the bacilli.
- 2) Treatment status of the patient.⁶

In the present study a comparison is made between diagnostic efficacy of TB PCR with culture of TB bacilli using radiometric assay in detection of GTB and correlation

between the positive cases of genital tuberculosis either by PCR and/or BACTEC and/or HPR and/or hysterolaparoscopy findings.

Figure 1: BACTEC 460 System



AIMS AND OBJECTIVES

PRIMARY OBJECTIVE: To evaluate the diagnostic efficacy of TB PCR with culture of TB bacilli using radiometric assay in detection of genital tuberculosis in infertile women.

SECONDARY OBJECTIVE: Correlation of hysteroscopic findings in cases found positive for genital tuberculosis either by PCR and/or BACTEC and/or 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 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 asymptomatic 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. It is found in 0.75 to 1% of all Gynaecological admissions in India. ^{3, 4}

The disease is responsible for 5% of all female pelvic infections and occurs in 13% cases of pulmonary tuberculosis. ⁴

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 amenorrhoea associated with infertility and an adnexal lump alone or associated with infertility. ^{2, 11}

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%, leucorrhoea 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%). 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 and 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. ^{11, 12}

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 GTB is mainly by hematogenous, 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. ¹¹

Pelvic tuberculosis may present in three clinical forms - tuberculous salpingitis, tubercular peritonitis and endometrial tuberculosis.

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. ¹¹

A. 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 hematogenous 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

B. 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 50-70% 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. ¹¹

C. 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

D. 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 albuginea. 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.¹³

E. Cervical Involvement: In cervix, the tuberculous lesion can be ulcerative or proliferative. In ulcerative form, the ulcers have serpiginous 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. ^{11,13}

F. Vaginal or Vulval Involvement: It is uncommon to have tuberculosis involving the vagina or the vulva. It is seen in less than 1% of the cases with genital tuberculosis. The gross appearance may be ulcerative or hypertrophic with the presence of multiple sinuses. ^{4,11}

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 curettings can only provide the diagnosis of disease with certainty. ⁴

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

Histopathology of the premenstrual tissue can provide the diagnosis of this condition with certainty. Histopathology demonstrates the typical caseous granulomatous lesion with giant epithelioid cells with or without Langerhans's giant cells. Caseating necrosis is rare in specimens from the genital tract. The minimum mycobacterial concentration at which histological evidence appears is 10,000 bacilli/ml. The lesion is highly suggestive of but not diagnostic of genital TB, as it appears in fungal infections and sarcoidosis. 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. ²

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 inoculum 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.^{2, 6, 16}

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 and MRI findings of abdominal tuberculosis may mimic diffuse peritoneal malignancy. These features include ascites, omental and mesenteric infiltration and smooth thickening of the parietal peritoneum.¹⁷

Hysterosalpingography: 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. (Figure 2, 3, 4)

Other features are:

- Calcified lymph nodes or small irregular calcification in the adnexal area.
- Obstruction of the fallopian tube in the transitional zone between the isthmus and the ampulla.
- Multiple constrictions along the course of the fallopian tubes, or beaded appearance, or salpingitis isthmica nodosa.
- Jagged and ragged tubal contour with small lumen defects and fistulous tracts.
- Stem pipe like configuration of the tube due to straight rigid contour of the lumen.
- Completely filled tube with rigid and irregular outline, known as Maltese cross appearance.

- Golf club appearance in which only isthmus and proximal ampulla are visualized with isthmus having rigid stove pipe appearance.
- Rosette type in which the distal part of the tube is filled with dye giving a rosette type image
- Leopard skin like speckled appearance of the ampulla due to the tube partially filled with dye.
- Tobacco pouch appearance due to hydrosalpinx and blockage of tube at the distal end resembling pouch of tobacco.

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 hydrosalpinx in 16% cases, synechiae and intrauterine adhesions in 16% of women and venous and lymphatic intravasation of dye in 27% of women.^{6, 18}

Laparoscopy: It 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 hyperemic areas in the tubes and uterus in acute stages. In chronic stages the tubes show nodular salpingitis, patchy salpingitis, hydrosalpinx, caseosalpinx or adhesions.¹⁹ (Figure 5, 6, 7) On laparoscopy, Tripathy observed adhesions, tubercles and hyperemia in 59.6% cases as well as adhesions in the POD in 11.3% cases.¹⁹

Hysteroscopy: It should be combined with laparoscopy to exclude/confirm endometrial involvement. Tubercles, microcaseation, distorted ostium, caseous materials 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, 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.^{20, 21}

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 extra pulmonary forms of tuberculosis.^{6,7}

There has been a genuine concern of false positivity due to:

1. Contamination of the sample with heparin which is a known PCR inhibitor.
2. High concentration of salt in the specimen which interferes with the PCR results.
3. Lack of laboratory standards.
4. Cannot distinguish between live and hiked bacilli.

The problem of false positivity can be substantially reduced by proper laboratory design, strict discipline about collection and processing of 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.^{5, 6,}

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 based PCR, nested PCR and RT-PCR. Targets include insertion and repetitive elements, various protein encoding genes or rRNA. PCR assays targeting the IS6110 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.^{6,7}

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%.⁶

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. ⁶

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. ⁶

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 is the most accurate diagnostic method. Traditionally used Lowenstein-Jensen Culture media requires only 100 organisms per milliliter. However, culture may take up to eight weeks to grow on LJ medium. ^{5,6} (Figure 8)

BACTEC has a sensitivity of 80-90% compared to Lowenstein-Jensen medium, which has sensitivity of 30-40%. 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.^{5,6}

A study on diagnostic value of endometrial TB PCR in detection of genital tuberculosis in infertility women was conducted in KLE University during 2007-2008, in which 96 women who underwent hysterolaparoscopy as a part of infertility work up were subjected to endometrial curettage and the material was sent for TB PCR and HPR. Out of 96 women 21 (21.9%) cases were detected positive by TB PCR and HPR was positive in only one case (4%). But it was noted that HPR positive case was PCR negative.

Considering the high endemicity of Genital TB, its strong association with infertility and limitations of available diagnostic tests, constant attempts have been made to develop definitive diagnostic criteria to make a conclusive diagnosis of Genital TB.

To the best of our knowledge there is no study comparing the diagnostic value of BACTEC and PCR in detection of genital tuberculosis in infertile women and hence is the need for the study.

Figure 2: Tubercular Granuloma

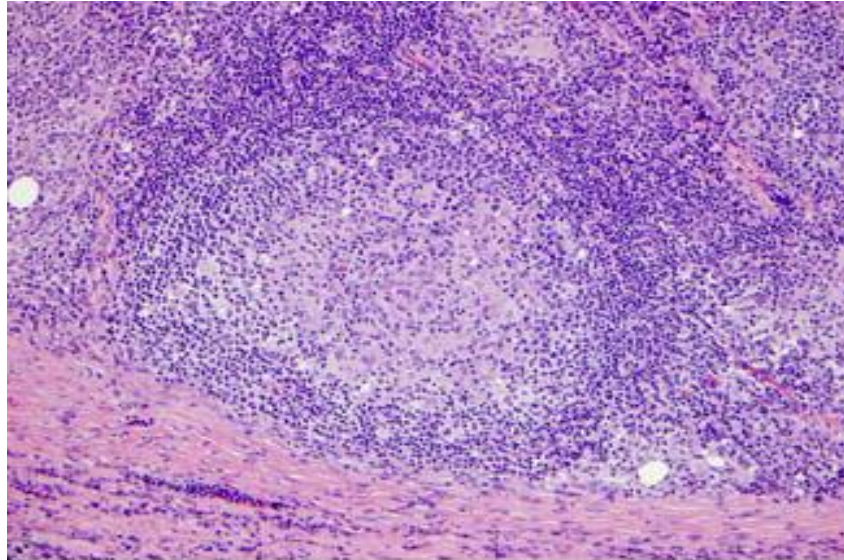


Figure 3: Bilateral Corneal Block

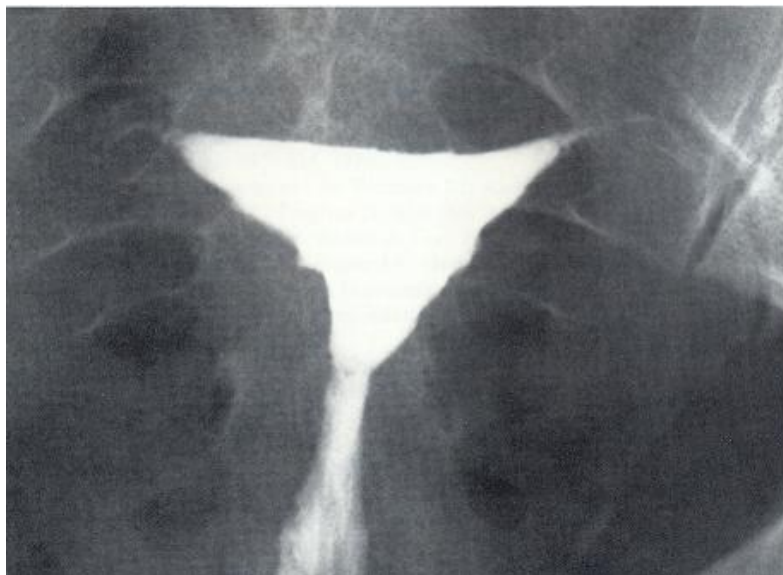


Figure 4: Lt. Sided Hydrosalpinx & Rt. Sided Tubal Block

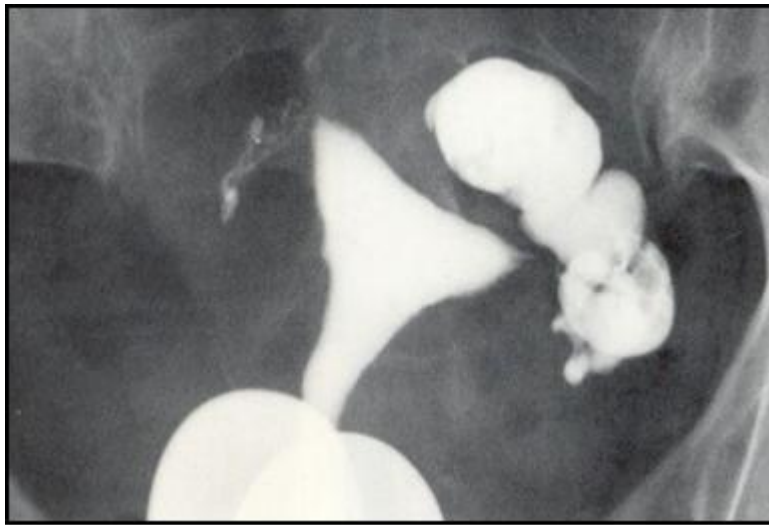


Figure 5: Bilateral Salpingitis Isthmica Nodosa

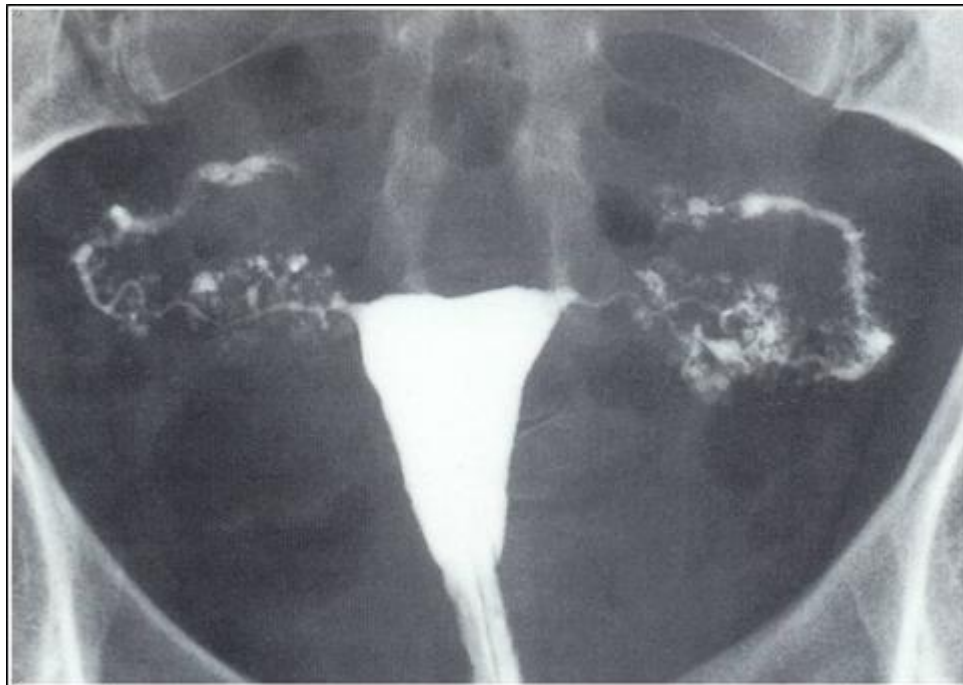


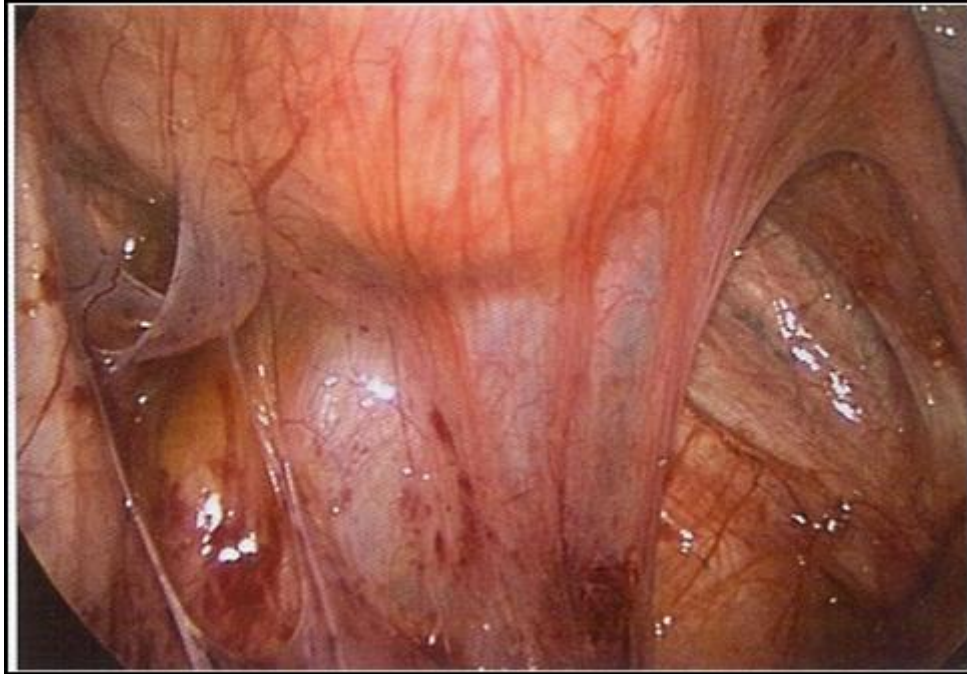
Figure 6: Flimsy Avascular Adhesions



Figure 7: Hydrosalpinx



Figure 8: Flimsy Vascular Adhesions



METHODOLOGY

Source of Data: Infertile women undergoing hysterolaparoscopy as a part of their infertility workup at KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

Study Design: Cross sectional study

Sample Size: $p = \text{Sensitivity} = 90\%$

$$q = 100 - p$$

$$\text{Error} = 15\%$$

$$Z\alpha = \text{value of } Z \text{ for } 95\% \text{ confidence is } 1.96$$

$$n = 4Z\alpha^2 pq/d^2 = 4 \times 1.96^2 \times 90 \times 10 / 0.15^2$$

$$= 61$$

Setting: Assisted Reproduction Centre at KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

Inclusion Criteria: Infertile women subjected to hysterolaparoscopy as a part of infertility workup.

Exclusion Criteria: Acute pelvic inflammatory disease.

Method: Hysterolaparoscopy was performed in the pre ovulatory period between days 6-10 of the cycle for infertility evaluation.

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. A careful evaluation of the 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 salpingitis
- Caseosalpinx
- Hydrosalpinx
- Presence of peritubal, periovarian, omental and bowel adhesions
- Free fluid in the pouch of Douglas

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

- Synechiae²⁶
 - a. Grade 1 - less than 1/4th of uterine cavity involved
 - b. Grade 2 - 1/4th to 3/4th of uterine cavity involved; ostia and fundus partly involved
 - c. Grade 3 - more than 3/4th 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 curettings were taken and sent for histopathology in formalin and for nested TB PCR and BACTEC in normal saline.

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

Video recording of all the cases was done.

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

RESULTS

A total of 61 cases were enrolled in the study and underwent diagnostic hysterolaparoscopy as a part of their infertility workup.

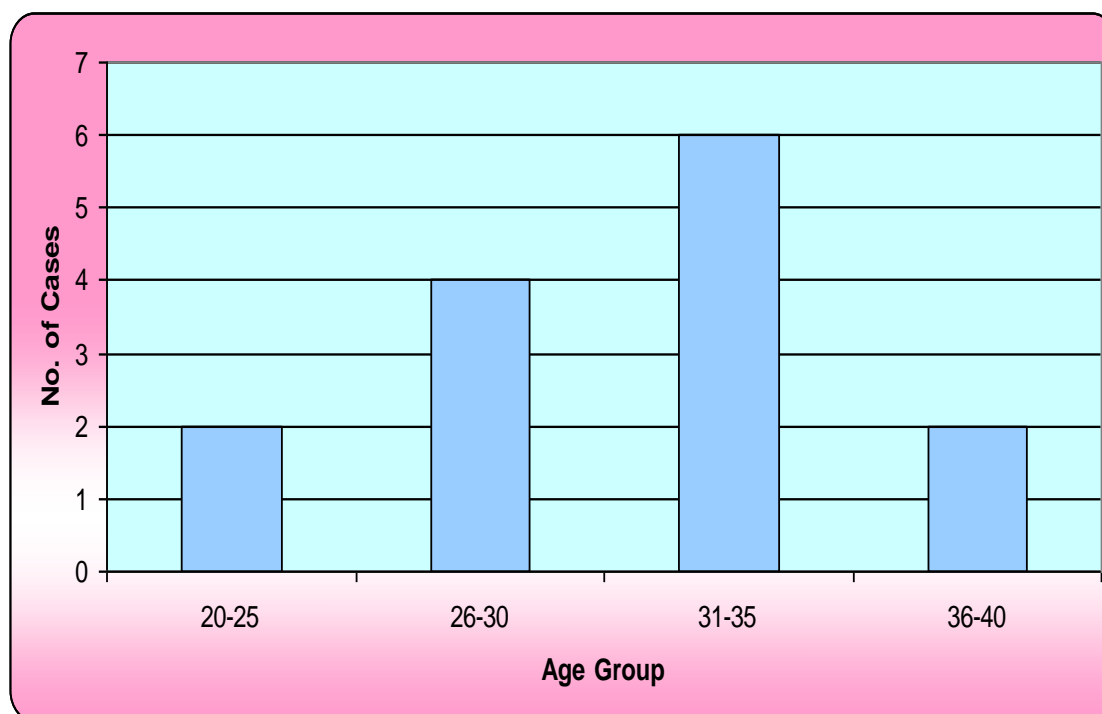
The diagnosis of genital tuberculosis was confirmed in 14 of the 61 enrolled cases (22.95%) by TB PCR and/or TB BACTEC and/or histopathology of endometrial curettings. The clinical and hysterolaparoscopy findings were analyzed in cases found to be positive for genital tuberculosis.

Of the 14 positive cases, 13 cases (92.85%) were positive by PCR, two cases (14.28%) were positive by BACTEC, of which one case (7.14%) was positive by both PCR and BACTEC. None of the cases had histopathological evidence of mycobacterial infection.

Out of 14 positive cases, eight (57.14%) PCR positive cases had positive correlation with hysterolaparoscopy. None of the BACTEC positive cases had hysterolaparoscopic features suggestive of GTB.

Table 1: Age Distribution Pattern in GTB

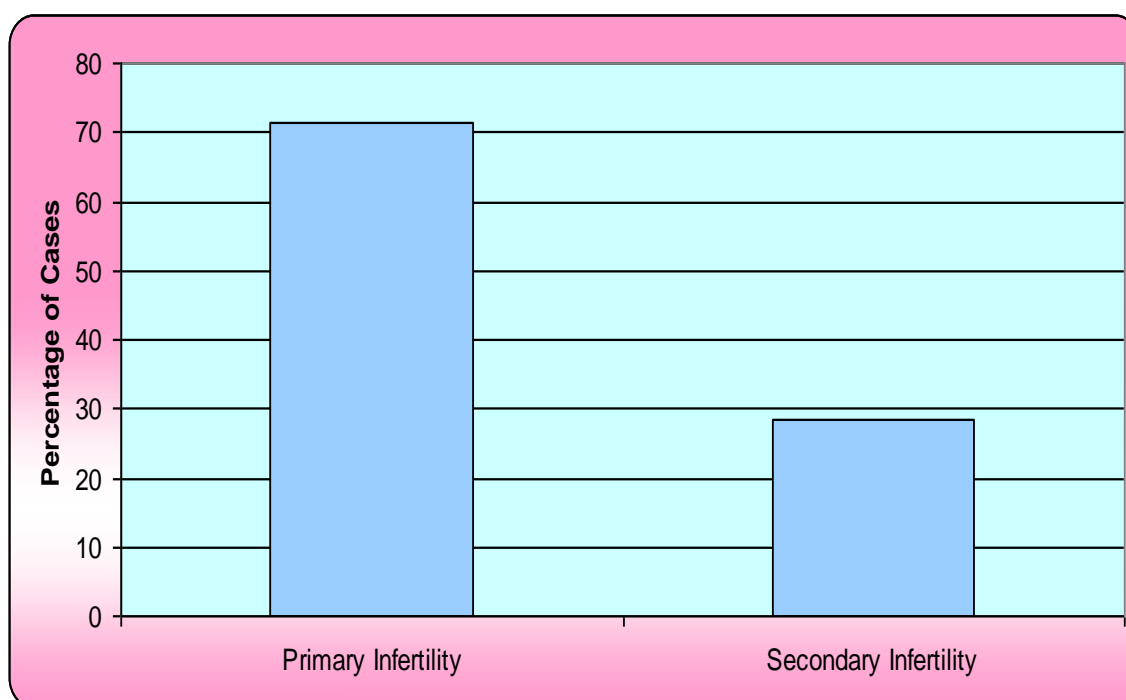
Age Group	No. of Cases
20-25	2
26-30	4
31-35	6
36-40	2

Graph 1: Age Distribution Pattern in GTB

The mean age group of the women was 29.78 years.

Table 2: Infertility Pattern in GTB

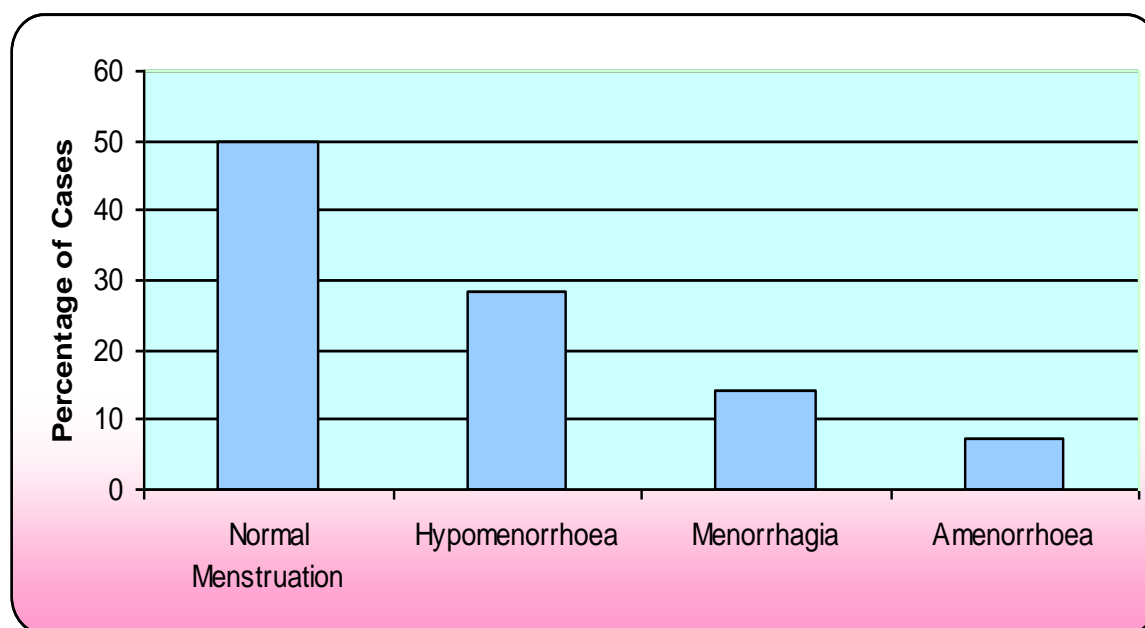
	Primary Infertility	Secondary Infertility
No. of Cases	10	4
Percentage of Cases	71.42	28.58

Graph 2: Infertility Pattern in GTB

Majority of the positive cases presented with primary infertility which accounts for 71.42% (10 cases) while 28.58% cases (4 cases) presented with secondary infertility.

Table 3: Different Menstrual Patterns in GTB

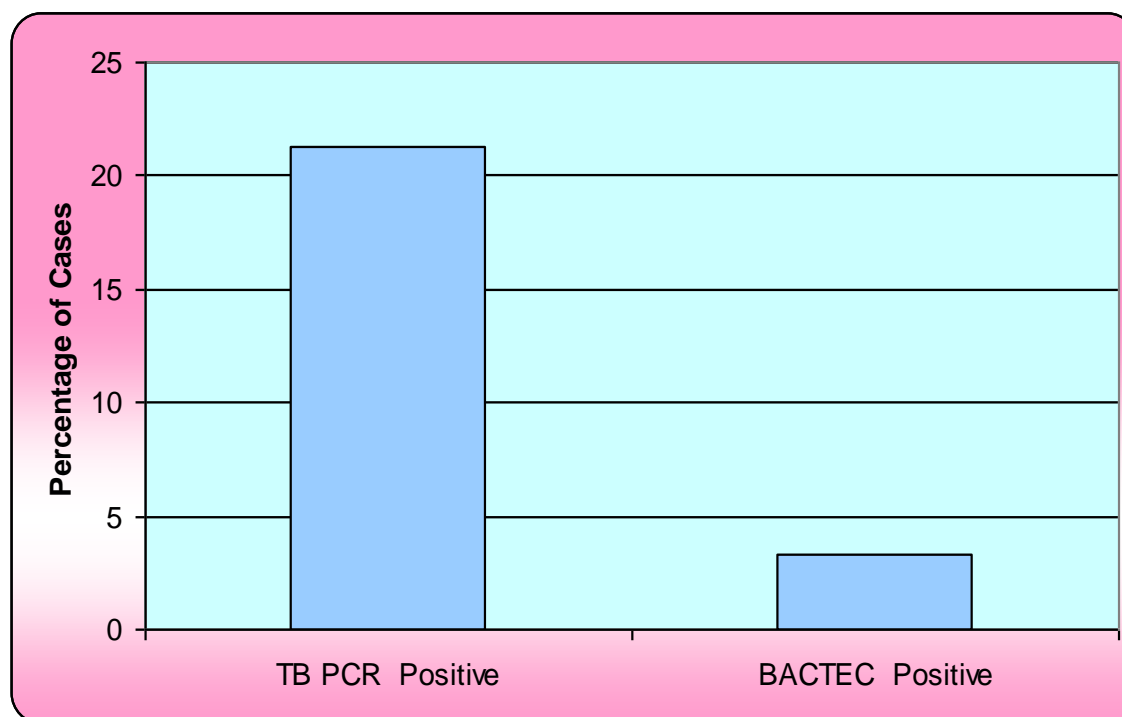
Menstrual Abnormality	No. of Cases	Percentage
Normal Menstruation	7	50.00
Hypomenorrhoea	4	28.57
Menorrhagia	2	14.29
Amenorrhoea	1	7.14

Graph 3: Different Menstrual Patterns in GTB

Majority of the women had normal menstrual function (50.00%), but the most common menstrual complaint was hypomenorrhoea (28.57%). Menorrhagia was seen in 14.29% cases while amenorrhoea in 7.14 % cases.

Table 4: Comparison of Two Diagnostic Modalities in GTB

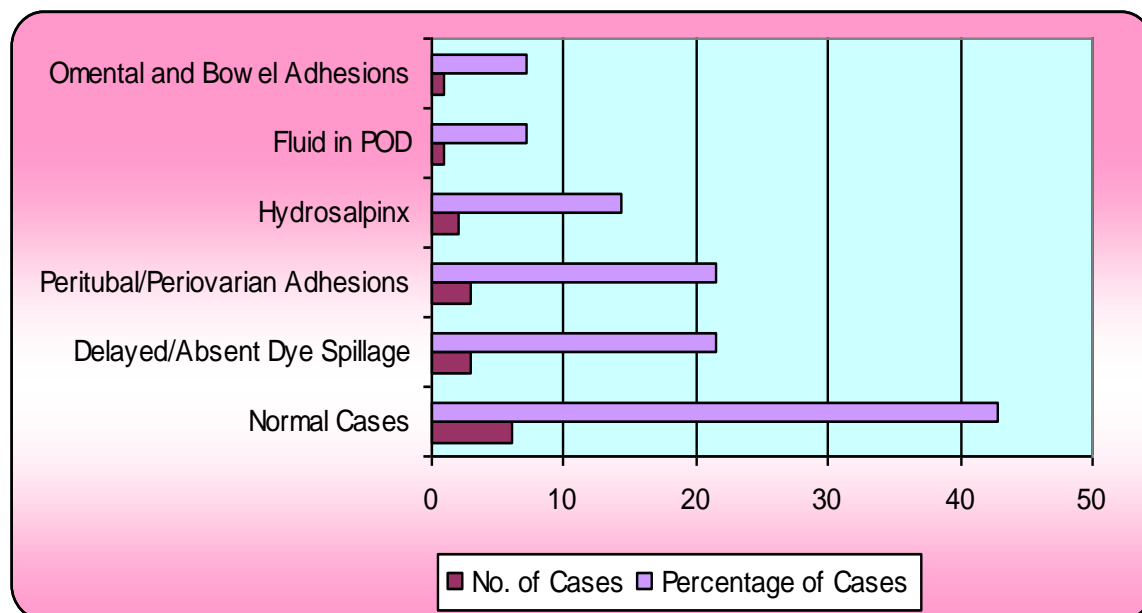
	TB PCR Positive	BACTEC Positive
Endometrial Curettings n=61	13 (21.31%)	2 (3.28%)

Graph 4: Comparison of Two Diagnostic Modalities in GTB

All patients who underwent hysterolaparoscopy had endometrial curettings sent for TB BACTEC, TB PCR and HPR. Out of 61 samples sent, 14 cases were found to be positive for genital tuberculosis. Of these, TB PCR was positive in 13 cases (21.31%), TB BACTEC was positive in two cases (3.28%) and none of the cases had histopathological evidence of GTB. In one case (1.64%), both TB PCR and TB BACTEC were found to be positive.

Table 5: Laparoscopic Findings in GTB

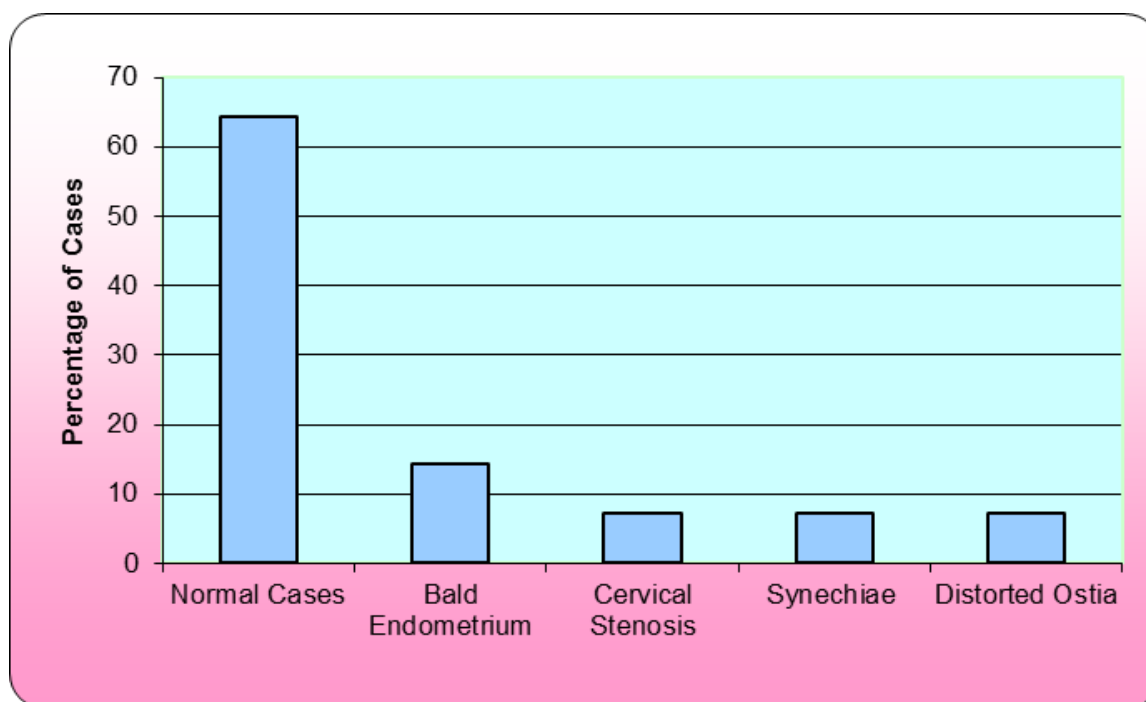
Laparoscopy Findings	No. of Cases	Percentage
Normal Cases	6	42.86
Delayed/Absent Dye Spillage	3	21.43
Peritubal/Periovarian Adhesions	3	21.43
Hydrosalpinx	2	14.29
Fluid in POD	1	7.14
Omental and Bowel Adhesions	1	7.14

Graph 5: Laparoscopic Findings in GTB

Eight cases (57.14%) had laparoscopic features suggestive of GTB. On laparoscopy, six cases (42.86%) of the patients had normal findings while three cases (21.43%) had presence of peritubal and periovarian adhesions as well as delayed or absent spillage of the dye. Hydrosalpinx was noted in two cases (14.29%). Omental and bowel adhesions and presence of fluid in pouch of Douglas were noted in one case (7.14%).

Table 6: Hysteroscopy Findings in GTB

Hysteroscopy Findings	No. of Cases	Percentage
Normal Cases	9	64.29
Bald Endometrium	2	14.29
Cervical Stenosis	1	7.14
Synechiae	1	7.14
Distorted Ostia	1	7.14

Graph 6: Hysteroscopy Findings in GTB

On hysteroscopy, nine cases (64.29%) of the patients with GTB had normal findings and five cases (35.71%) had hysteroscopic features suggestive of GTB. Bald endometrium is seen in 2 cases (14.29%) and cervical stenosis, synechiae and distorted ostia were seen in one case each (7.14%).

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.^{3, 7, 11}

In our study the criteria for diagnosis of GTB was positive TB BACTEC and/or TB PCR and/or granulomatous lesion on histopathology. Hysterolaparoscopy findings in the cases found to be positive for GTB were carefully reviewed for structural lesions suggestive of GTB.

Several other studies have quoted the incidence of GTB using combined diagnostic tests including hysteroscopy and laparoscopy findings which may be quite nonspecific.⁶

In the present study, the incidence of genital tuberculosis was 22.95%. A study done in South Africa on 109 infertile women where 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, the incidence of GTB was found to be 21%.²²

In our study, 92.85% (13 cases) of the cases of GTB were diagnosed by TB PCR, 14.28% (2 cases) of the cases of GTB were diagnosed using TB BACTEC (culture) and none by HPR. A study comparing AFB smear, culture by Lowenstein Jensen media,

histopathology and TB PCR found that the detection rate amongst the suspected cases was highest with TB PCR, 43.1% compared to 11.05% with histopathology, 7.8% with culture and 5.2% with AFB staining.²³

This high pick up rate of GTB in our study by TB PCR (92.85%) as compared to various other studies which is in the range of 46.67% 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.^{6, 21}

Out of two BACTEC positive cases, one was PCR positive and one was PCR negative. PCR negative, BACTEC positive could be due to contamination or inadequate lab standards giving a false negative result. PCR positive and BACTEC (culture) negative cases could be explained as PCR can detect even very few bacilli and even dead bacilli hence such report may warrant therapy.

The detection of genital tuberculosis by histopathology in our study was nil as against 11.5% observed by other authors. A low pick up rate in our study is probably due to the fact that endometrial biopsy was done in the post menstrual phase.²³

Majority of the cases had a normal menstrual cycle (50.0%) while hypomenorrhoea was the most common menstrual abnormality present in 28.57% cases. Similar findings were reported in an Indian study where normal menstrual pattern was seen in 57.6% of cases and hypomenorrhoea in 30.1% cases.²⁴

Laparoscopy demonstrated peritubal and periovarian adhesions in 21.43% cases & omental and bowel adhesions in 7.14% cases. This is corroborating well with results obtained by other authors who have quoted it to be present in 20.11% cases and 1.72% respectively.²⁵

On chromopertubation, delayed and absent spillage of the dye was seen in 21.43% cases which is correlating well with the figures obtained from other studies which have reported abnormal chromopertubation results in 50% cases. This is due to the fact that fallopian tubes are the initial and most frequently affected site in pelvic mycobacterial infection. Fluid in pouch of Douglas was detected in 7.14% cases while other authors have reported in 8.45% cases. Normal laparoscopic findings were observed in 42.86% cases which could be due to the fact that it generally detects macroscopic changes that are seen in chronic stages.²²

In our study hysteroscopy revealed normal findings in 64.29% cases thereby implicating less than 50% endometrial involvement as has been reported by most authors. Intrauterine synechie and distorted ostia were visualized in only 7.14% .This is corroborating well with results obtained by other authors who have quoted 6.5% cases of genital tuberculosis.^{6,11}

Another finding noted in our study was the presence of cervical stenosis in 7.14% 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

Six patients found to be positive by TB PCR and/or BACTEC had no endoscopic abnormalities. This observation further emphasizes the part that GTB would be more frequently diagnosed if a high index of suspicion is considered in evaluation of every infertile patient especially in areas where TB is endemic.

This study gives scope for further prospectively assessing pregnancy outcome after ant tubercular treatment in women tested positive by both TB PCR and/or BACTEC as culture methods which have been considered gold standard in proving GTB may also fail to exclude mycobacterial infection.

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.
- Though culture is considered as gold standard for diagnosis of genital tuberculosis, since GTB is paucibacillary there is an urgent need for more research to come to conclusion whether culture is still gold standard.
- Further research is required to detect the most sensitive method for diagnosis GTB and therefore the diagnostic dilemma continues.

SUMMARY

A cross sectional study was done over a period of one year to evaluate the diagnostic value of culture for TB bacilli using Radiometric Assay and Polymerase Chain Reaction in detection of genital tuberculosis in infertile women and to correlate the clinical and hysteroscopic findings in cases found positive for genital tuberculosis by radiometric assay or/and TB PCR and/or HPR of endometrial curettings.

Hysteroscopy was performed in the pre ovulatory period usually on day 6th-10th of the cycle for evaluation of infertility. At the end of the procedure endometrial curettings were taken and sent for both BACTEC and for nested TB PCR in normal saline and for histopathology in formalin.

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

The diagnosis of genital tuberculosis was confirmed in 14 (22.95%) of the enrolled cases by either TB BACTEC and/or TB PCR of endometrial curettings. Of these, TB PCR of endometrial curettings was positive in 13 cases (21.31%) while TB BACTEC of endometrial curettings was positive in two cases (3.28%). One (1.64%) case is both TB PCR and BACTEC positive. HPR was negative in all 61 cases.

Out of 14 positive cases, eight cases (57.14%) had positive correlation with hysteroscopy and all eight cases were positive by PCR. That is, out of 13 PCR

Summary

positive cases, eight (61.53%) had positive findings suggestive of GTB by hysterolaparoscopy. None of the BACTEC positive cases had positive findings suggestive of GTB by hysterolaparoscopy.

This silent invader of the genital tract which is paucibacillary tend to create a diagnostic dilemma due to varied clinical presentations, diverse results on endoscopy and limitations of histopathologic, serologic, bacteriologic and culture methods. Hence, there is an urgent need to develop a definitive diagnostic modality.

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ANNEXURE I: Participants Information and Consent Form

Sl. No.: _____

Patient's Name: Mrs. _____

We hereby request you to participate in “DIAGNOSTIC EFFICACY OF TUBERCULOSIS PCR WITH CULTURE OF TB BACILLI USING RADIOMETRIC ASSAY IN DETECTION OF GTB IN INFERTILE WOMEN”

The study is designed to assess the diagnostic value of TB BACTEC in comparison with TB PCR and to analyze the clinical and hysterosalpingoscopic findings in cases found positive for genital tuberculosis in infertile females attending ARC, KLES PRABHAKAR KORE HOSPITAL.

This study is being done under the direct supervision of Dr. Bhavana Sherigar. Only if you agree to undergo hysterosalpingoscopy for your infertility workup, will you be a part of this study. Routinely, endometrial curetting will be done at the end of hysterosalpingoscopy which will be sent for BACTEC, PCR AND HPR. Your hysterosalpingoscopic, BACTEC and TB PCR findings will be analyzed. All information collected about you during the course of this study will be kept confidential. Even if you disagree for the consent, there will be no change in the treatment given to you.

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 according to the existing protocol.

Annexure I: Participants Information and Consent Form

If you have any questions about the study, you may please contact Chief Investigator, Dr. Bhavana Sherigar, KLES Dr. PRABHAKAR KORE HOSPITAL AND MRC Ph. No. 9886649339, Dr. V. Vishnupriyanka, P.G., Department of Obstetrics and Gynaecology, J.N.M.C. Belgaum, Ph. No. 9986097120. Regarding participants rights, you may please contact Dr. V.D. Patil, Principal and Chairman of Ethical Committee, JNMC, Belgaum, Telephone No. 958312473777.

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

Signature of the Participant or Legally Authorized Representative

Participant's Name: _____

Signature or Left Thumb Print: _____

Address: _____

Telephone Number: _____

Experimenters/Witness Name: _____

Signature: _____

Date: _____

ANNEXURE II: Proforma

“DIAGNOSTIC EFFICACY OF TB PCR WITH CULTURE OF TB BACILLI USING
RADIOMETRIC ASSAY IN DETECTION OF GTB IN INFERTILE WOMEN”

Sl. 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

Normal

Yes

No

Hypomenorrhoea (Scanty menstruation last
in for less than 2 days)

Annexure II: Proforma

Oligomenorrhoea (bleeding where cycle length exceeds 35 days)

Menorrhagia (bleeding excessive in amount > 80ml or duration > 7 days)

Any other findings

Symptoms suggestive of Pulmonary Tuberculosis

Yes

No

Fever

Cough

Hemoptysis

Loss of weight or appetite

History of treatment with ATT

Duration of treatment _____ (in mths)

History of contact with Tuberculosis

Examination Findings

Lymphadenopathy

Respiratory findings

Abdominal Examination

Palpable lump

Genital Examination

P/S:

P/V:

Ultrasound Findings (D2-D5 of Cycle)

Uterine measurements

Endometrial thickness

Left adnexa

Right adnexa

Any other relevant observation

Laparoscopy Findings

Yes

No

Dilated, tortuous tubes

Military tubercles on uterus and tubes

Nodular salpingitis

Hydrosalpinx

Fluid in pouch of Douglas

Presence of peritubal, periovarian, bowel or omental adhesions

Hysteroscopy Findings

Calcifications

Microcaseation

Presence of synechie

Distorted ostium

Fluffy endometrium

Tubercles

Annexure II: Proforma

	Positive	Negative
TB BACTEC :	<input type="checkbox"/>	<input type="checkbox"/>
	Positive	Negative
TB PCR :	<input type="checkbox"/>	<input type="checkbox"/>
	Positive	Negative
HPR:	<input type="checkbox"/>	<input type="checkbox"/>

ANNEXURE III: Master Chart

ANNEXURE IV: 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

Sl. No.	Name	Age	Bactec	TB PCR	HPR	Infertility	Menst. Abn.	Examination	Lap. Findings	Hystero. Findings
1	Susheela Pande	37	N	N	N	Sec.	Normal		Normal study	Normal study
2	Gangawwa Benni	24	N	N	N	Prim.	Normal		Normal study	Normal study
3	Priya Pramod	35	N	N	N	Prim.	Normal		Normal study	Normal study
4	Roopa M	25	N	P	N	Prim.	Normal		Peritubular/periovarian adhesions	Bald endometrium
5	Sharada Yaragudri	31	N	N	N	Prim.	Oligo		Normal study	Normal study
6	Geeta Kutte	34	N	N	N	Prim.	Normal		Normal study	Normal study
7	Poornima Ganiger	25	N	N	N	Prim.	Oligo		Normal study	Polypoidal endometrium
8	Pooja Panari	26	N	N	N	Prim.	Normal		Lt. ovarian cyst	Normal study
9	Savita Karpoor	29	N	N	N	Sec.	Oligo		Hydrosalpinx	Polypoidal endometrium
10	Kavya Bagurappa	26	N	N	N	Sec.	Normal		Normal study	Normal study
11	Rangare Sharadha	28	N	N	N	Prim.	Normal		Normal study	Normal study
12	Kaveri Hosurkar	22	N	N	N	Prim.	Normal		Unicornuate	Normal study
13	Meenaxi Mungojji	31	N	P	N	Prim.	Hypo		Hydrosalpinx	Distorted ostium
14	Manju Sharma	35	N	P	N	Sec.	Normal		Normal study	Normal study
15	Rekha Ghaste	28	N	N	N	Prim.	Normal		Normal study	Normal study
16	Savitri Basaprabhu	31	P	N	N	Prim.	Normal		Normal study	Normal study
17	Ganiger Netravati	20	N	N	N	Prim.	Normal		Normal study	Normal study
18	Rajashree Kulkarni	31	N	N	N	Prim.	Normal		Normal study	Normal study
19	Swapna Mruthanjay	27	N	N	N	Prim.	Normal		Normal study	Normal study
20	Girija Basavaraj	28	N	N	N	Prim.	Normal		Ovarian cyst	Normal study
21	Jabben Mulla	26	N	N	N	Prim.	Normal		Normal study	Normal study
22	Damayanti Patil	36	N	N	N	Sec.	Normal		Flimsy adhesions	Normal study
23	Lalita Vijaykumar	35	N	N	N	Sec.	Oligo		Normal study	Fluffy endometrium
24	Sayeda Shabona	36	N	N	N	Prim.	Normal		Adhesions on the posterior surface of uterus	Normal study
25	Amruta Avaradi	26	N	N	N	Prim.	Normal		Hydrosalpinx, flimsy adhesions	Normal study
26	Saroja Hurkadli	35	N	N	N	Prim.	Normal		Delayed spillage	Normal study
27	Pratima Das	22	N	N	N	Prim.	Normal		Peritubal/periovarian adhesions	Normal study
28	Salahalli Shanta	30	N	N	N	Sec.	Normal		Peritubular and periovarian adhesions	Normal study
29	Vijaylakshmi Nirwani	36	N	N	N	Sec.	Normal		Multiple fibroids	Normal study
30	Lalitha	27	N	N	N	Prim.	Oligo		Bilateral PCOS	Normal study
31	Pratima Parampur	33	N	N	N	Prim.	Normal		Peritubal/periovarian adhesions/endometriotic cyst	Normal study
32	Savitri Hulageri	32	N	P	N	Sec.	Hypo		Normal study	Normal study
33	Vijaylaxmi Kamble	29	N	N	N	Sec.	Oligo		Normal study	Normal study

Sl. No.	Name	Age	Bactec	TB PCR	HPR	Infertility	Menst. Abn.	Examination	Lap. Findings	Hystero. Findings
34	Sharada Shusheel	29	N	N	N	Prim.	Normal		Peritubular and periovarian adhesions	Normal study
35	Pranili Killeker	26	N	P	N	Prim.	Normal		Fluid in pod, peritubular/periovarian adhesions	Synechiaae
36	Radha Basakard	32	N	N	N	Prim.	Menorrhagia		Normal study	Polypoidal endometrium
37	Pushpa Charles	19	N	N	N	Prim.	Oligo		Normal study	PCOS
38	Mahefroz Patel	36	N	P	N	Prim.	Normal		No spillage	Cervical stenosis
39	Geeta Patil	28	N	N	N	Prim.	Oligo	Cervical stenosis	Cystic ovaries	Normal study
40	Smita Dilip	30	N	N	N	Prim.	Normal		Flimsy omental adhesions	Normal study
41	Savita Siddapa	27	N	N	N	Prim.	Normal		Fluid in pod	Normal study
42	Mungawwa	38	N	P	N	Prim.	Normal		Hydrosalpinx	Bald endometrium
43	Gangawwa	20	N	P	N	Prim.	Menorrhagia		Normal study	Normal study
44	Shobhana Munnoli	36	N	N	N	Prim.	Normal		Normal study	Normal study
45	Reshma Desai	29	N	N	N	Sec.	Normal		Multiple fibroids and both ovaries normal	Normal study
46	Poonam Pathani	30	N	N	N	Prim.	Normal		Normal study	Normal study
47	Sunanda Hatti	24	N	N	N	Prim.	Oligo		No spillage on right side, right ovary simple cyst	Both ostia small polyp
48	Swati Yadev	23	N	N	N	Prim.	Normal		Right ovarian simple cyst	Normal study
49	Swapna Desai	31	N	P	N	Sec.	Hypo		Peritubular/periovarian adhesions/endometriotic spot	Normal study
50	Seema Hubli	23	N	N	N	Prim.	Normal		Flimsy adhesions, periovarian adhesions	Normal study
51	Sridevi Raghu	26	N	N	N	Prim.	Normal		Normal study	Normal study
52	Sanjeevini	28	N	N	N	Prim.	Normal		Normal study	Normal study
53	Shana Banu	26	N	N	N	Prim.	Menorrhagia		PCOS	Normal study
54	Fouzia	28	N	N	N	Prim.	Normal		PCOS	Septum present
55	Alka Patil	32	N	N	N	Prim.	Normal		Endometriotic spots on peritoneun	Normal study
56	Ratna Wadhia	28	N	P	N	Prim.	Hypo		No spillage on left side	Normal study
57	Smita Maruti	32	N	N	N	Prim.	Oligo		Omental and bowel adhesion, lt. tube and ovary burried densely under adhesions	Normal study
58	Geeta Mallesh	26	N	P	N	Prim.	Amenorrhoea		Omental adhesions, delayed spillage from rt. tube	Normal study
59	Wahidabanu	28	P	P	N	Sec.	Menorrhagia		Fibroid	Normal study
60	Swetha Shet	26	N	P	N	Prim.	Normal		Normal study	Normal study
61	Rajeshwari Swamy	26	N	N	N	Prim.	Normal		Normal study	Normal study