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Research Article

Diagnosis of genital tuberculosis: correlation between polymerase chain reaction positivity and laparoscopic findings

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ABSTRACT

Background: Female genital tuberculosis (GTB) leads to infertility in young women as it involves the fallopian tube and the endometrium. Since it does not produce any dramatic symptoms, disease remains untreated until the female fertility is totally compromised. Diagnostic hystero-laparoscopy is an integral part of management of infertile women. Visual manifestations of pelvic TB are well described, but subtle signs of subclinical disease are not identified nor correlated with laboratory tests. PCR has been described as a rapid, highly sensitive and specific test for detecting DNA of MTB. Hence it's worth studying whether positive PCR test will help make early diagnosis of GTB. This study correlates laparoscopic visual inspection (LVI) findings with endometrial TB-PCR positivity to diagnose GTB. **Methods:** A prospective observational study was done in which infertile women who had clinical and HSG findings

suggestive of GTB underwent endometrial TB-PCR and hysterolaparoscopy. Among them 69 endometrial TB-PCR positive cases were included in the study. Clinical and hysterolaparoscopy findings in these cases were reviewed. **Results:** GTB is the disease of young women and most commonly present with primary infertility (65.2%). On

laparoscopy 60% of cases showed positive correlation with endometrial TB- PCR and tubal involvement was seen in majority of cases.

Conclusions: Even though PCR is a very valuable tool in diagnosing genital TB, by itself it can neither confirm nor exclude genital TB. The routine application of endometrial TB-PCR assays in addition to clinical and laparoscopic evaluation carries a great potential in improving diagnosis of genital TB.

Keywords: Genital tuberculosis, Endometrial TB-PCR, Hysterolaparoscopy

INTRODUCTION

Tuberculosis (TB), one of the oldest diseases known to affect humans, is caused by bacteria belonging to the Mycobacterium tuberculosis complex. Although tuberculosis usually presents with pulmonary disease, extra-pulmonary tuberculosis (EPTB) is increasingly encountered. Female genital tuberculosis (GTB) is an uncommon type of EPTB that can lead to infertility in young women as it mostly involves the fallopian tubes (90%) and the endometrium (50%).

Genital tuberculosis almost always occurs secondary to pulmonary tuberculosis (commonest) or extrapulmonary tuberculosis such as gastrointestinal tract, kidneys, skeletal system, meninges and miliary tuberculosis.² Primary genital tuberculosis has also been reported in women whose male partners have acute genitourinary tuberculosis by transmission through infected semen.³

The spread of tuberculosis from lungs and other sites is normally haematogenous or by lymphatic. Contiguous spread from nearby abdominal organs like intestines or abdominal lymph nodes can cause genital tuberculosis although less common.10-15% of women suffering from genital tuberculosis is asymptomatic. Symptomatic patients present with menstrual dysfunction (especially oligomenorrhoea or amenorrhoea), primary or secondary infertility, lower abdominal pain, chronic pelvic pain, and/or a pelvic mass.

The global prevalence of GTB is estimated to be 8 to 10 million cases and the disease is showing a rise in incidence in developing countries due to increasing population and overcrowding. It is increasing in industrialized countries also as a result of its association with HIV infection and increasing immigrant population from under developed countries. Incidence of genital tuberculosis (GTB) in females is found in 0.75 to 1% of gynecological cases in India.⁴ The disease is responsible for 5% of all female pelvic infections and occurs in 10% cases of pulmonary tuberculosis.⁵ Most of the affected cases belong to reproductive age-group. Hence genital tuberculosis is a common cause of infertility in females. The prevalence of GTB in infertile women is reported to vary from 7-15% in developing countries to 1-2% in industrialized countries.⁶ Actual incidence of genital tuberculosis may be more because a large proportion of cases go unreported due to low grade symptomatology and lack of sensitive and specific investigations till now.

Since GTB does not produce any dramatic symptoms most of the times disease remains untreated until the female fertility is totally compromised. And the diagnosis of genital TB has profound implications for the asymptomatic women seeking fertility because the multidrug therapy required for more than 6 months is not very promising in terms of pregnancy outcome and the social stigma associated with TB in our country.

Moreover this silent invader of genital tract tends to create diagnostic dilemma because of varied clinical presentation, the diverse results on the imaging, laparoscopy, histopathology and a mixed bag of bacteriological and serological tests, each of which has its limitation in diagnostic sensitivity and specificity. Hence diagnosis of GTB for early institution of treatment remains a clinical challenge.

The gold standard for diagnosis of Tuberculosis is mycobacterial detection and isolation. While direct microscopy in AFB smear and culture in Lowenstein-Jensen medium (LJ) are great success in pulmonary TB, it is of lesser value in genital TB. The reason being these methods have very low sensitivity as in the genital tissue the bacterial load is not much to be detected easily. And bacteria take 4-5 weeks to show growth on LJ media. Nevertheless, even the liquid culture methods such as BACTEC system or more recent introduction of BacTAlert 3D neither reduce the turnaround time below 12days nor improves the rate of positivity beyond a limit.

The conventional Mantoux test for tuberculin sensitivity is positive in over 40% of normal healthy individuals in India and has a sensitivity of 55% and specificity of 80%. Hence Mantoux test is unhelpful in the diagnosis.

Morphological imaging on hysterosalpingography (HSG) and ultrasonography have low sensitivity and specificity for diagnosis of GTB. ^{2,8}

In the recent years, the molecular diagnostic methods hold the key to the future of the better and efficient diagnosis of genital tuberculosis. Polymerase chain reaction (PCR) has been described as a rapid, highly sensitive and specific molecular biological method for detecting DNA of Mycobacterium tuberculosis in both pulmonary and extra pulmonary samples from suspected tuberculosis patients. 9,10,12 PCR is an efficient technique with the theoretical possibility of amplifying one DNA sequence of interest to 106 copies in the course of one working day. PCR enables detection of less than 10 pg of DNA that corresponds to about three genome equivalents, thus making it a highly sensitive tool. Endometrial TB-PCR assays targets various gene segments, including a 65 kDa protein-encoding gene (Noel b et al), the IS6110 element (Eisenach et al, Kolk et al) and the mpt 64 gene (Manjunath et al, Seth et al, Dar et al). False PCR positivity due to contamination occurring in clinics and laboratories is one of genuine concern. This can be reduced by strict laboratory design and discipline in collection and processing of specimens, handling of reagents and use of blocking reagents. In case of false negative results, there are individual problems of appropriate sample collection, extraction and assays. False negatives may be caused by inadequate number of organisms and inhibitors in specimen.

Both diagnostic and operative laparoscopy and hysteroscopy are an integral aspect of management of infertile women. Visual manifestations of abdominal and pelvic TB at laparotomy and laparoscopy in established cases are well described. However, subtle signs of subclinical disease have not been identified nor have they correlated with laboratory tests.

Hence because of obvious demand for a reliable and rapid means of diagnosing genital TB for public health reasons it would be worthwhile to study whether positive PCR test is likely to help in making an early diagnosis and treatment of genital TB. This study describes correlates and compares laparoscopic visual inspection (LVI) findings with endometrial TB-PCR positivity to diagnose GTB.

METHODS

Cross sectional, prospective observational study was done for one and a half years. Infertile women undergoing hystero-laparoscopy as a part of infertility workup at a tertiary care hospital were taken as study data.

Inclusion criteria

All infertile women suspected of genital tuberculosis, who undergo hystero-laparoscopy as a part of infertility work up and show positive endometrial TB PCR Result.

Exclusion criteria

- Women not giving consent for hystero-laparoscopy.
- Women who show negative endometrial TB-PCR Result.

Method

- Explanation of procedure to all women participating in the study.
- Consent from every women participating in this study.
- All women had: detailed history, general examination, abdominal examination, local examination, Mantoux test, CBC, pelvic scan, hysterosalpingogram. In relevant cases hysterolaparoscopy was performed in the pre-ovulatory period usually on day 6th to 10th of the cycle and pipelle endometrial sample was taken for TB-PCR.

Endometrial TB-PCR

Endometrial sample was taken with the help of pipelle's sampling curette. The sample taken is sent for DNA TB-PCR. TB-PCR of the endometrial sample was done using the Real time TB-PCR in which there is amplification and detection of the IS6110 gene locus.

Hystero-laparoscopy

Diagnostic laparoscopy was performed using a 5mm/10mm stryker 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: suprahepatic adhesions, pelvic adhesions, pelvic congestion, bowel/omental adhesions, peritubal adhesions, cornual block, delayed spillage, fimbrial phimosis, hydrosalphynx, granulomas, short tubes, sacculations/beaded tubes, rigid tubes, TO mass, other incidental findings: fibroids, endometriosis, PCOS.

Following this hysteroscopy using normal saline as the distension media was done to identify features suggestive of tuberculosis that included: cervical stenosis, poor endometrium, bald endometrium, distorted ostia, tubercles, microcaceations, calcifications, and synechiae.

Chromopertubation was done and any delayed or absent spillage of dye was noted. Video recording of all the cases was done. In cases found to be positive for genital tuberculosis by TB PCR, the clinical findings were analyzed and video recordings of the hysterolaparoscopic findings were carefully reviewed for the presence of features suggestive of genital tuberculosis.

Laparoscopic criteria for diagnosis of GTB were grouped as either suggestive (type 1) or non-suggestive (type 2) of GTB

Type I

Suggestive of GTB

- Confirmatory: Clear evidence of GTB in the form of multiple granulomas, exudates, loculated fluid, adhesions, hydrosalpinx, Rigid and blocked tubes.
- Suspicious: Subtle signs of chronic inflammation, e.g., patent tubes with pelvic congestion, only a few scattered small granulomas, fimbrial agglutination and phimosis, tubal sacculation, mild adhesions and straw-coloured fluid in of Douglas and peri-hepatic adhesions.

Type II

Non- suggestive of GTB: Normal findings.

Results of Positive PCR for endometrial TB were then collaborated with hystero-laparoscopic findings.

Statistical methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean±SD (min-max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance.

The following assumptions on data are made

- Dependent variables should be normally distributed
- Samples drawn from the population should be random, and Cases of the samples should be independent.

Statistical software

The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

A prospective observational study was done in which infertile women who had clinical and HSG findings suggestive of GTB underwent endometrial TB-PCR and hysterolaparoscopy for further evaluation. Among them 69 endometrial TB-PCR positive cases were included in the study. Clinical and hysterolaparoscopy findings in these cases were carefully reviewed.

Table 1: Age distribution.

Age in years	No of patients (n=69)	%
20-30	43	62.3
31-40	26	37.7

Mean age observed in our study was (Mean \pm SD): 29.89 \pm 4.23 years.

Table 2: Nature of infertility

Nature of IF	No of patients (n=69)	%
Primary infertility	45	65.2
Secondary infertility	24	34.8

In our study 65.2% of patients presented with primary infertility and 34.8% patients presented with secondary infertility. In our study majority of the patients (76.8%) had regular menstrual cycle and rest 24.2% patients presented with menstrual abnormality. Hypomenorrhoea was the most common menstrual abnormality present in 8.7% patients followed by oligomenorrhoea in 5.8% of patients, menorrhagia in 5.8% of patients, amenorrhea in 2.9% of patients.

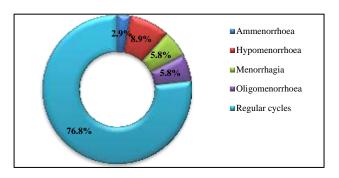


Figure 1: Menstrual pattern.

Table 3: Previous history of PTB/EPTB.

Prev. H/o. of TB	No. of patients (n=69)	%
Pulmonary	4	5.9
Genital	0	0.0
Abdomen/intestinal	2	2.8
Bone/joint	1	1.4
Nil	62	89.9

Among the 69 patients in the study, previous history of pulmonary TB was seen in 5.9% of patients, abdominal TB was seen in 2.8% of patients, 1.4% of patients had history of TB of bones. In our study, among 69 cases of positive endometrial TB-PCR none of the cases shown positive results on TB BACTEC (culture) or by ZN-staining.

Indications for doing endometrial TB-PCR and laparoscopy

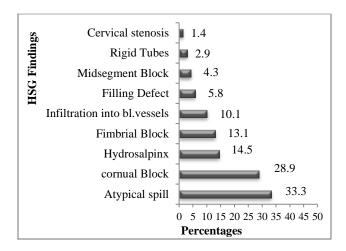


Figure 2: HSG findings.

Table 4: Culture and AFB findings in endometrial TB-PCR positive cases.

Pipelle	No. of patients (n=69)	%
PCR	69	100.0
Culture (bact)	0	0.0
AFB smear	0	0.0

Table 5: Laparoscopic findings in endometrial TB-PCR positive cases.

Laparoscopy findings	No. of patients (n=69)	%
Suprahepatic adhesion	13	18.8
Pelvic adhesion	24	34.8
Pelvic congestion	4	5.8
Bowel/omental adhesions	11	15.9
Peritubal adhesions	19	27.5
Cornual block	15	21.7
Delayed spillage	18	26.0
Fimbrial phimosis	6	8.7
Hydrosalpinx	11	15.9
Granulomas	3	4.3
Shortened tubes	3	4.3
Sacculations/beaded tubes	14	20.2
Rigid tubes	3	4.3
TO mass	4	5.7
Other incidental findings(Endometriosis)	9	13.1

In our study 40% of patients had no evidence of GTB on laparoscopy. In rest 60% of patient on laparoscopy had: suprahepatic adhesions in 13 patients (18.8%), pelvic adhesions in 24 patients (34.8%), pelvic congestion in 4 patients (5.8%), peritubal adhesions in 19 patients (27.5%), omental and bowel adhesions in 11 patients

(15.9%) and TO-mass in 4 patients (5.7%). On chromopertubation, delayed spillage of the dye was seen in 18 patients (26%), cornual block in 15 patients (21.7%), sacculations in 13 patients (20.2%), hydrosalpinx in 11 patients (15.9%), shortened tubes in 3 patients (4.3%),rigid tubes in 3 patients (4.3%) and fimbrial phimosis in 6 patients (8.7%). Other incidental finding was endometrioma (in the form of endometriotic spots) was seen in 9 patients (13.1%).

Based on the laparoscopic visual inspection (LVI) Findings: 41 patients (60%) belong to type 1 (features suggestive of GTB) and 28 patients (40%) belong to type 2 group (features not suggestive of GTB/normal laparoscopic findings).

Type 1 group was further divided into: Type 1A-confirmatory findings of GTB (31 patients) and Type 1B-suspicious findings of GTB (10 patients).

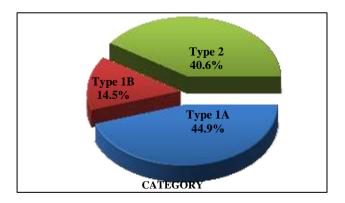


Figure 3: Laparoscopic visual inspection findings.

Table 6: Hysteroscopic findings.

Hysteroscopy	No. of patients (n=69)	%
Normal findings	51	73.9
Abnormal findings	18	20.3
Cervical stenosis	1	1.4
Poor endomerium	8	11.6
Bald areas	4	5.8
Distorted Ostia	2	2.9
Synechiae	1	1.4
Fibrosis	2	2.9
Tubercles	0	0.0
Calcification	0	0.0
Others	4	5.8
Endometrial polyp	2	2.9
Polypoidal endomerium	2	2.9

In our study hysteroscopy revealed normal findings in 51 of patients (73.9%), 2 patients (2.9%) each had distorted ostia and fibrosis, poorly vascularised endometrium was seen in 8 patients (11.6%), bald areas was seen in 4 patients (5.8%), synechiae was seen in 1 patient (1.4%),

cervical stenosis in 1 patient (1.4%). None of the patients had tubercles or calcification.

DISCUSSION

With the advent of effective chemotherapy and the wider use of IVF, the outlook on infertile women suffering from GTB has improved significantly. But the vast majorities of patients are not diagnosed in the earlier stages and miss the benefits of early treatment. Hence there is a need for more reliable and rapid diagnostic criteria for early diagnosis of the disease.

A number of reports have described the importance of Endometrial TB-PCR for early diagnosis of GTB and other extra pulmonary TB. 9-12,24 In India, endometrial TB-PCR is also used as screening method for GTB but it demands more evaluation as it is debatable whether PCR positivity indicates infection or disease of the genital tract. By treating all asymptomatic TB-PCR-positive cases, are patients being over-treated? Do the high TB-PCR positive rates represent laboratory contamination and false-positivity, or do they confirm the diagnosis of early GTB? Unfortunately, it is generally not possible to substantiate the diagnosis by mycobacterial culture as GTB is a paucibacillary disease. Rigid quality control of PCR is therefore important. In the interests of early treatment, other questions are generally overlooked in favour of a clinical diagnosis of GTB on the basis of TB-PCR.

In our study infertile women who had clinical and HSG findings suggestive of GTB underwent endometrial TB-PCR and Hysterolaparoscopy for further evaluation. Among them 69 endometrial TB-PCR positive cases were included in the study. Clinical and hysterolaparoscopy findings in these cases were carefully reviewed.

Classically, female genital tuberculosis has been described as a disease of young women, with 80-90% of patients diagnosed between 20-40 years of age 25 and most of the women present with infertility. In our study the mean age of women with genital tuberculosis was 29 years and 65.2% patients present with primary infertility and rest 34.8% patients presented with secondary infertility.

Primary genital tuberculosis is extremely rare. In most series, a history of previous diagnosis of or treatment for extra genital tuberculosis is present in 25-50% of patients. ^{3,25} In our study 7 (11.1%) patients had a previous history of extra genital TB.

The menstrual cycle may be regular and undisturbed in many cases of genital TB. Abnormal uterine bleeding in genital TB has been reported in 10%-40% of patients. ^{26,27} In our study majority of the patients had a normal menstrual cycle (76.8%) and 24.2% patients presented with menstrual abnormality. Hypomenorrhoea was the

most common menstrual abnormality present in 8.7% patients followed by oligomenorrhoea in 5.8% of patients. Similar findings were reported in an Indian study where normal menstrual pattern was seen in 57.6% of patients and most common menstrual abnormality was hypomenorrhoea (30.1%) followed by oligomenorrhoea (3.5%). 28

In our study, among 69 cases of positive TB-PCR none of the cases shown positive results on TB BACTEC (culture) and by ZN-staining. In few other reports in which mycobacterial cultures were performed, positivity rates were between 10% and 15% of GTB patients diagnosed on clinical and morphological criteria and a positive TB-PCR. PCR positive and BACTEC (culture) and smear negative cases could be explained by the fact that even though smear and culture has remained a gold standard in diagnosis of pulmonary TB, the very low sensitivity for diagnosis of paucibacillary disease limits its practical utility in extra-pulmonary TB. Hence the use of PCR is suggested as an alternative test especially in culture-negative GTB.

Laparoscopy and hysteroscopy are part of the standardized protocol for evaluation of infertile women. Several reports have described findings suggestive of GTB. Several reports have described findings have described f

Normal laparoscopic findings were observed in 40% of positive TB PCR which could be due to the fact that laparoscopy generally detects macroscopic changes that are seen in chronic stages.³⁶ This is a very important observation which further emphasizes the fact that genital TB would be more frequently diagnosed if a high index of suspicion is considered in the evaluation of every infertile patient, especially in areas where TB is endemic.

An interesting observation of this study is that 13.1% of patients along with features suggestive of TB had associated endometriosis evidenced by endometriotic spots. In a study done by Asha B et al, endometriosis was associated with 10.7% of TB-PCR positive cases. There is no proven theory to justify the association of genital TB with endometriosis but perhaps the change in intrauterine milieu and vascularity might play some role in provoking retrograde spillage of endometrial cells resulting in endometriosis.³⁵

In our study hysteroscopy revealed normal findings in 51 cases (73.9%) there by implicating less than 50% endometrial involvement as also been reported by most authors. This can be explained by the fact that unlike the

fallopian tubes, the endometrium is comparatively immune to damage by *Tubercular bacilli*, because of the monthly shedding and repair cycle. Normal looking or non-specific findings of periosteal congestion, etc. can be highly subjective and misleading.

In some of very advanced cases in whom the endometrium was significantly destroyed and adequate endometrial tissue was not available, PCR was often negative. In such cases, EB TB-PCR was non-representative either because of inadequate samples or because this was old, healed disease. TB is well described as a significant underlying etiology in many cases of intrauterine synechiae.³⁷

Another finding noted in our study was the presence of cervical stenosis in 1.4% 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.⁹

Based on the laparoscopic visual inspection (LVI)

Findings

41 patients (60%) belong to type 1(features suggestive of GTB) and 28 patients (40%) belong to type 2 group (features not suggestive of GTB/ normal laparoscopic findings). Type 1 category was further divided into: type 1A- confirmatory findings of GTB (31 patients) and type 1B- suspicious findings of GTB (10 patients).

Hence 60% cases showed positive correlation with endometrial TB-PCR on laparoscopy which is a significant finding. Implying that endometrial TB-PCR and laparoscopy are complementary tests and the two together can effectively confirm an early clinical diagnosis of GTB.

CONCLUSION

Genital tuberculosis is the disease of young women and majority of them present with primary infertility.

Majority of the women have an asymptomatic presentation, hence a high degree of suspicion is needed in the evaluation of these patients.

Tubal disease appears to be the most common factor causing infertility in women with GTB.

Since GTB is paucibacillary disease, PCR appears to a rapid and highly sensitive diagnostic modality. But by itself it can neither confirm nor exclude genital TB.

Endometrial TB-PCR and Laparoscopy are complementary tests and together can effectively confirm an early clinical diagnosis of GTB.

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Ethical approval: The study was approved by the

Institutional Ethics Committee

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