Role of GeneXpert in endometrial biopsy for diagnosis of genital tuberculosis in women presenting with infertility

Aafreen Naaz¹, Vikram Sarbhai², Vinita Sarbhai¹*  

¹Department of Obstetrics and Gynecology, Kasturba Hospital, Daryaganj, New Delhi, India  
²Senior Consultant, Pulmonology, Critical Care and Sleep Medicine, National Heart Institute, East of Kailash, New Delhi, India  
³Department of Obstetrics and Gynaecology, Kasturba Hospital, Daryaganj, New Delhi, India  

Received: 03 January 2021  
Revised: 08 April 2021  
Accepted: 09 April 2021  

*Correspondence:  
Dr. Vinita Sarbhai,  
E-mail: vinitasarbhai@gmail.com  

ABSTRACT  

Background: The diagnosis of female genital TB is challenging due to low sensitivity of conventional diagnostic modalities. The new GeneXpert Test on endometrial biopsy is studied for its role in diagnosis of female genital TB.  
Methods: This is an observational cross-sectional study on 50 infertile women. Premenstrual endometrial biopsy was performed and sent for AFB Smear, M. TB (LJ) medium culture, CB-NAAT by GeneXpert, and histopathology. The results were compared for diagnosis of FGTB.  
Results: Endometrial Biopsy (EB) could confirm diagnosis of genital TB in 4 cases (8%) out of 50 infertile women. Amongst these 4 cases, GeneXpert was positive in two, AFB were detected on smear in three while one showed both AFB on smear and positive GeneXpert. GeneXpert adds additional value but surely cannot replace any of the other gold standard investigations.  
Conclusions: GeneXpert is a rapid diagnostic method which is accurate, feasible and affordable. It is useful adjunct to the existing armamentarium in diagnosis of female genital tuberculosis.  
Keywords: Acid fast bacilli, Endometrial Biopsy, Female genital TB, GeneXpert test, Infertility, Tuberculosis

INTRODUCTION  

Genital tuberculosis, caused by Mycobacterium tuberculosis (M. TB) is a well-recognized etiology in causing infertility amongst women in developing countries like India. As per the WHO Global TB report 2019, the estimated incidence of tuberculosis (TB) in India is approximately 27% of the world’s TB cases.¹ The prevalence of female genital TB is as high as 10-19% amongst Indian women as compared to less than 1% in developed countries.²,³ It is even more likely that the actual incidence of female genital TB be under-reported since in most cases, the disease is asymptomatic or presents with a few symptoms among which infertility is the most common (11%) besides paucity of definitive investigations.⁴ Female genital TB occurs most commonly in the reproductive age (15-45 years), causing infertility in 44-74% of the affected women.⁵  

Female genital TB is paucibacillary disease and is mostly secondary infection acquired by hematogenous spread from an extra-genital source such as pulmonary or abdominal tuberculosis.⁶ The fallopian tubes are affected in almost all cases (90-100%) followed by the endometrium (50-60%), ovaries (20-30%), cervix (5-15%), vulva and vagina (1%).⁷ High index of suspicion, elaborate clinical evaluation, number of investigations to detect presence of M. tuberculosis as well as imaging
methodologies for characteristic structural changes in the female reproductive organs are essential for establishing diagnosis of female genital TB.\textsuperscript{7} Demonstration of Mycobacterium tuberculosis bacilli on either smear or Lowenstein Jensen (LJ) medium culture, are the most specific tests for diagnosis of TB and remains the gold standard. Other diagnostic gold standard investigation is detection of characteristic caseating granulomas on histopathology.

Confirmation of diagnosis of TB still offers hurdles due to limitations in smear microscopy, long time required in culturing tubercule bacilli and variable sensitivity of molecular tests. Hence, diagnosis of female genital TB and early institution of treatment is always challenging. There are no accepted guidelines for diagnosing female genital TB in view of the low sensitivity of bacteriological tests and the poor specificity of most immunological and serological investigations.

Diligent and perseverant attempts have to be made for conclusive diagnosis of female genital TB, considering high endemicity and its strong association with infertility in view of limitations of available diagnostic tests.

Advanced molecular methods such as Polymerase Chain Reaction (PCR) and Cartridge Based Nucleic Acid Amplification Test (CB-NAAT) or GeneXpert, have shown very promising results for early and rapid diagnosis of Pulmonary TB. GeneXpert has emerged as a highly reliable diagnostic investigation for TB as it simultaneously detects Mycobacterium tuberculosis complex (MTBC) and resistance to Rifampicin (RIF) in less than 2 hours.\textsuperscript{8} However, their role in diagnosing extrapulmonary tuberculosis is still being explored.

Our present study has incorporated GeneXpert for TB to assess its utility in diagnosis of female genital TB in infertile women, besides conventional investigations such as histopathology, acid fast bacilli (AFB) Smear and AFB Culture on surgically obtained endometrial samples.

**METHODS**

This is an observational cross-sectional study conducted after approval from ethical committee between January 2019 and January 2020. The study included 50 women with complaints of infertility who presented at the Outpatient Infertility Clinic of the Department of Obstetrics and Gynecology, Kasturba Hospital, New Delhi, India. Female Genital TB was considered as one of the possible reasons for their infertility and therefore mandated confirmation and appropriate management. Infertility due to male factors or due to ovulation factors or active pelvic inflammatory disease (PID) or those who took anti-TB treatment (ATT) in the past were not considered for this study.

These patients underwent a thorough clinical evaluation besides routine blood and radiological investigations.

These patients thereafter underwent premenstrual endometrial biopsy between 24\textsuperscript{th}-28\textsuperscript{th} day of cycle. Each of the endometrial samples were subjected to 4 investigations i.e. AFB Smear microscopy, M. TB (LJ) medium culture, CB-NAAT by GeneXpert and histopathology.

It must be reemphasized that positivity of anyone of the following is considered gold standard for the diagnosis of female genital tuberculosis i.e. detection of AFB in a smear or M. TB on culture or positive GeneXpert for TB or histopathology showing caseating granulomas in the endometrial samples. Data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Quantitative variables were compared using unpaired t-test/Mann-Whitney test. Qualitative variables were compared using chi-square test/Fisher’s exact test.

**RESULTS**

The present study included female patients presenting with Infertility, between the age group 20-37 years with the mean age being 28.28±4.02 years. Their infertility durations ranged from 2-14 years, with the mean duration being 4.88±3.24 years. 58% of patients presented with primary infertility while 42% with secondary infertility. Amongst this group of patients, other reported complaints were menstrual abnormalities (34%), chronic pelvic pain (14%), discharge per vagina (10%) and features of recurrent pelvic inflammatory disease (10%). The commonest menstrual abnormality reported was hypomenorrhea (22%) while others had oligomenorrhea (14%), amenorrhea (6%) and menorrhagia (2%).

The investigative workup reported positive Mantoux test in 11 cases (22%) and raised ESR in 14 (28%). Chest X-rays revealed feature suggestive of old TB only amongst 5 patients (10%). Ultrasonography in majority (74%) of the patients was normal. However, in rest of the patients, ultrasound detected tubo-ovarian masses (4 cases), hydrosalpinx (4 cases), uterine abnormalities (4 cases), ovarian cyst (3 cases), and fluid in the Pouch of Douglas (POD) (1 case).

**Table 1: Results of endometrial biopsy sample.**

<table>
<thead>
<tr>
<th>Endometrial biopsy</th>
<th>Total</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFB stain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>47</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td><strong>AFB/MTB culture LJMed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic Endometritis</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>NAD</td>
<td>48</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td><strong>CBNAAT/ GeneXpert</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>48</td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>
Endometrial Biopsy (EB) conducted on these 50 cases could confirm diagnosis of genital TB in 4 cases (Table 1 and Figure 1). Amongst these 4 cases, GeneXpert was positive in two. AFB were detected on smear in three while one showed both AFB on smear and positive GeneXpert. Additionally, GeneXpert could detect both the cases as rifampicin sensitive. However, two EB cases that were AFB Positive were missed by GeneXpert. None of the endometrial biopsy samples grew M. TB in LJ medium culture. On histopathology, only 2 endometrial biopsy cases showed features of chronic endometritis.

Typical tuberculous granulomas with epithelioid cells, caseating necrosis and giant cells were not seen in any of the cases. The presence of chronic endometritis may be suggestive but not diagnostic of tuberculosis. All the 4 cases that showed bacteriological evidence on endometrial sample were started on ATT under RNTCP using DOTS Strategy.

![Figure 1: Distribution of positive patients on endometrial biopsy.](Image)

**DISCUSSION**

Tuberculosis is endemic in developing countries. In India, female genital TB is a common cause of infertility related to chronic infections. Amongst infertile women, FGTB because of its subtle presentation, often poses complex diagnostic challenge for clinicians.

Diagnosis of FGTB is crucial, because definitive Anti TB therapy can be initiated with confidence both for the treatment of the patient and improving chances for conception. Expectedly, proper anti TB treatment might salvage functional damage of the female reproductive organs such as fallopian tubes and uterus. Cornerstone for the diagnosis of FGTB have always been identification of AFB either through smears (stain) or isolation of M. tuberculosis bacilli on LJ medium culture or the detection of classical TB granulomas on histopathology. These respective investigations must essentially have a critical number of M.

TB bacilli for their positivity. Microscopic smear examination for detection AFB requires presence of at least 10,000 organisms/ml in a sample. Culture on LJ Medium of M. TB is more sensitive and requires a lesser number i.e. 1000 organisms/ml but as a downside takes 4-5 weeks for its processing.9

Direct smear microscopy of AFB and LJ medium culture of M. TB are great success in diagnosis of pulmonary TB, it is of lesser value in genital TB.10 In spite of inoculation into multiple media only 5-6% of samples yield microbiological proof of mycobacteria by culture, the reason being the paucibacillary nature of the disease, bacteriologically dormand genital lesions, presence of bacteriostatic substance in endometrium and cyclic shedding of endometrium which leads to inadequate granuloma formation in each cycle.11 Therefore, even though desired, a negative bacteriological report from endometrial sampling does not exclude the diagnosis of genital tuberculosis.

GeneXpert is a new technology, using automated nucleic acid amplification, is a rapid diagnostic method which is accurate, feasible and affordable with the additional advantage of detecting M. TB in the diagnostic armamentarium for TB. It further detects M. TB sensitivity to Rifampicin.

It has evolved as an important breakthrough in the diagnosis of pulmonary tuberculosis yet its role in extrapulmonary tuberculosis is still under investigation. Few studies have been done using GeneXpert for diagnosis of female genital TB. The present study incorporates GeneXpert for its additional utility in the diagnosis of female genital TB. However, not much data is available to compare our results.

In this study of 50 infertile women who underwent endometrial biopsy procedures as part of their diagnostic evaluation only four cases (8%) were confirmed to have FGTB. Of these four cases AFB Smear alone was positive in two (4%); one case had CB-NAAT or GeneXpert positive while one case had both AFB Smear and GeneXpert positivity.

Therefore, GeneXpert was positive in 4% (2 cases) of this study cases but also missed TB diagnosis in 2 cases (i.e. 50% of diagnosed cases) which were AFB smear positive. None of the endometrial sample showed growth of Mycobacterium bacilli on LJ medium culture. Our results were not much different than the earlier reported studies that used GeneXpert.

Sharma et al, reported 2.9% GeneXpert positivity in endometrial samples (with 46.6% sensitivity and 100% specificity) in confirmed cases of female genital TB on the basis of positive M.TB culture and histopathology on endometrial biopsy.12 They proposed the limitations of the GeneXpert are its sensitivity to high temperature and
humid conditions which are quite prevalent in countries like India with heavy TB burden.

They also suggested that Blood contamination in endometrial biopsy can also inhibit GeneXpert making it negative. Hence, by sending endometrial biopsy, endometrium and not endometrial blood should be sent for GeneXpert testing for better results.

Farhana et al included 87 cases of suspected genital tuberculosis, who were also subjected to Ziehl Neilson (ZN) smear staining, culture on LJ media and GeneXpert assay. None of their samples were positive in ZN staining. However, 4.6% and 8.05% positivity were observed in culture and GeneXpert respectively. Two samples that were positive on culture were found negative on GeneXpert which could be because of blood contamination of endometrial biopsy sample as quoted by the authors.

However, due to higher rate of detection and minimal technical expertise GeneXpert was recommended by the authors for rapid diagnosis and detection of drug resistant tuberculosis in genital tuberculosis.

Kashyap B, reported a higher incidence of 8% out of 62 cases who tested positive for Mycobacterium tuberculosis by GeneXpert which were all sensitive to Rifampicin, as they included all the cases suspected of tuberculosis. They have recommended GeneXpert as a useful adjunct in diagnosis of genital tuberculosis.

Saxena et al concluded that conventional methods i.e. liquid-solid culture have their specific place for the diagnosis of FGTB. Along with the clinical diagnosis, conventional gold standard culture should be collaborated with the rapid, appropriate and cost-effective test like GeneXpert for tuberculosis diagnosis.

Therefore, one can deduce that GeneXpert if also included in investigational workup for Female Genital TB adds additional value but surely cannot replace any of the other gold standard investigations in surgically obtained samples neither is fully dependable because of the dynamic nature of the female reproductive organ involved.

**CONCLUSION**

Therefore, to conclude, GeneXpert adds further assistance in confirming FGTB besides conventional diagnostic Tests for TB. It is a rapid diagnostic method which is accurate, feasible and affordable. It is useful adjunct to the existing armamentarium in diagnosis of female genital tuberculosis.

It has evolved as an important breakthrough in the diagnosis of pulmonary tuberculosis but its role in extrapulmonary tuberculosis is still under the scanner. More studies preferably multicentric with large sample size on GeneXpert as a diagnostic modality in female genital tuberculosis will truly bring out its utility and position. A multi-pronged approach increases the chances of FGTB diagnosis and initiating ATT with more realistic and confidant management outcomes.

**ACKNOWLEDGMENTS**

The authors are deeply indebted to all patients in this study. The resources used in the research were totally provided by hospital and Municipal Corporation of Delhi.

**Funding:** No funding sources  
**Conflict of interest:** None declared  
**Ethical approval:** The study was approved by the Institutional Ethics Committee

**REFERENCES**


