

# Role of Gene Xpert in the Detection of Genital Tuberculosis in Endometrial Tissue among Women with Infertility

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ABSTRACT

**Objective:** The objective of the study is to evaluate the clinical utility of Gene Xpert compared with other available diagnostic modalities in prompt diagnosis of female genital tuberculosis (TB) causing infertility. **Material and Methods:** This was a prospective, cross-sectional analytical study. Premenstrual endometrial biopsy specimens were collected from 176 infertile women of reproductive age group suspected of having genital TB. Samples were processed for acid-fast bacilli, culture, histopathology, polymerase chain reaction (PCR), and Gene Xpert. Patients detected positive on Gene Xpert and PCR were subjected to laparoscopy to look for affirmative findings of genital TB. The results were analyzed using composite gold standard consisting of patients positive with culture, histopathology, and laparoscopy. **Results:** A total of 18 patients were found positive using composite gold standard. Laparoscopy was positive in 15 patients, whereas culture and histopathology were positive in three and two patients, respectively. Gene Xpert was positive in two patients. None of them was detected with rifampicin resistance. The sensitivity of Gene Xpert was 11.11% whereas the specificity was 100%. **Conclusion:** Since genital TB is a paucibacillary disease, multiple diagnostic modalities are needed for diagnosis. Gene Xpert appears to be a useful modality in diagnosis of genital TB, owing to its high specificity, and can be recommended in conditions where microscopy, culture, and histopathology are negative; however, further randomized studies are required to support our hypothesis.

**KEYWORDS:** Endometrium, Gene Xpert, genital tuberculosis, infertility

## INTRODUCTION

Female genital tuberculosis (FGTB) continues to be a major gynecological morbidity in Indian women. In India, 15%–20% of TB cases are estimated to be cases of extrapulmonary TB.<sup>[1]</sup>

TB is a major public health problem throughout the world affecting about 10.4 million people of which 3.5 million cases are from India and causing about 1.4 million deaths annually.<sup>[2]</sup> The exact prevalence of FGTB in India is not known but varies from 1% to 19% among gynecological patients, and it is responsible for 10% cases of infertility.<sup>[3,4,5]</sup>

Its presentation is very vague and may present as infertility, uterine adhesions, pelvic pain, abdominal mass, menstrual dysfunction (oligomenorrhea, hypomenorrhea, menorrhagia, and amenorrhea), vaginal discharge, and poor general health.<sup>[3,6,7]</sup>

The gold standard for diagnosis of TB is culture, but it requires a minimum of 10–100 bacilli/ml for diagnosis and a long incubation period causing delay in diagnosis and treatment.<sup>[5]</sup> Microscopy offers lower sensitivity in the detection of genital TB as it requires 10<sup>4</sup> bacilli/ml

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and genital TB being a paucibacillary disease, the yield is very low.<sup>[5]</sup> Laparoscopy is also considered gold standard for the diagnosis of genital TB, but there are chances of increased risk of complications and flaring up of infection.

In the present scenario, to establish the diagnosis of genital TB, a combination of clinical, endometrial sampling and use of laparoscopy and hysteroscopy provides the best available modalities in infertile women.<sup>[3,8]</sup> Hysterosalpingography and positron emission tomography can help in cases of tubo-ovarian masses by demonstrating increased glucose uptake and in differentiation from ovarian cancer as FGTB may masquerade like ovarian cancer.<sup>[9,10]</sup>

In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity.<sup>[11]</sup> Gene Xpert is DNA based and is capable of detecting 80–130 *Mycobacterium tuberculosis*/ml in the infected material and also detects rifampicin resistance within 2 h.<sup>[12,13]</sup> Its sensitivity was well compared to culture.<sup>[14]</sup> Most of the studies available in the literature are on the sputum samples of pulmonary TB patients and has shown high sensitivity up to 98% and 90% in smear-positive and smear-negative patients, respectively.<sup>[15,16]</sup>

Therefore, the purpose of this study was to diagnose FGTB by Gene Xpert, to compare the Gene Xpert with existing diagnostic modalities, and to detect rifampicin resistance in confirmed cases.

## MATERIALS AND METHODS

This was a prospective analytical cross-sectional study conducted at fertility and IVF clinic and gynecology outpatient department. Permission was granted by the institutional ethical committee. A sample size of 176 infertile patients aged between 20 and 40 years was selected according to the inclusion and exclusion criteria. Patients were excluded if any gynecological malignancy is detected, tested positive for HIV, and/or previous history of antitubercular treatment intake was present.

The primary outcome measure of the study was to find out the role of Gene Xpert in the detection of FGTB and the secondary outcomes were to detect the rifampicin resistance in confirmed FGTB and to compare the role of Gene Xpert with other existing diagnostic modalities.

A written informed consent was taken from all study participants. A detailed history comprising duration of infertility, presence or absence of constitutional symptoms such as chest pain, fever, and cough with sputum, loss of appetite, weight loss, and detailed menstrual history was noted. Past history of

pulmonary/extrapulmonary TB, history of pulmonary/extrapulmonary TB in the family members, or history of contact with known TB cases was also noted, and a detailed clinical examination was done in all the patients.

Chest radiography posteroanterior view and hysterosalpingography were done in all women for any evidence of TB.

Premenstrual endometrial biopsies were taken from all women.

The endometrial samples were sent for processing in a four-point scale for acid-fast bacilli (AFB) microscopy, culture, TB-polymerase chain reaction (PCR), Gene Xpert (1 ml each in normal saline), and histopathological examination (HPE, in formalin).

TB-PCR was done for the  $\beta$ -globin gene to assess the quality of extracted DNA and its suitability for amplification. The DNA was extracted using Qiagen DNA extraction kit (Qiagen, GmbH, 40724 Hilden, Germany) following the protocol as proposed by the manufacturer. PCR was carried out to amplify the 165 bp region of the 65 kDa HSP gene of *M. tuberculosis*. Electrophoresis was done on the amplified products on 1.5% agarose gel and stained with ethidium bromide to look for the presence of specified bands.<sup>[17]</sup>

Gene Xpert purifies *M. tuberculosis* bacilli from sample, isolates genomic material from the captured bacteria by sonication, and subsequently amplifies the genomic DNA by PCR. It utilizes a real-time hemi-nested automated PCR and gives results within 2 h. Besides, it identifies all the clinically relevant rifampicin resistance-inducing mutations in the RNA polymerase beta (*rpoB*) gene in the *M. tuberculosis* genome in a real-time format using fluorescent probes called molecular beacons.

TB-PCR assay requires sample manipulation which inhibits amplification reaction of specimens by decreasing proteins and enzymes and takes more amount of time as compared to the Xpert MTB/RIF assay. All these can lead to unrecognized errors such as cross-contamination and sample dilution.<sup>[18]</sup>

All women who tested positive with Gene Xpert and TB-PCR further underwent thorough laparohysteroscopic evaluation to support the diagnosis.

Findings observed under direct visualization through laparoscope were subdivided into two headings:

- a. Affirmative findings for TB such as the presence of tubercles, Grade II adhesions, tubo-ovarian masses, beaded tubes, and cornual blockage<sup>[5]</sup>
- b. Suspicious findings for TB such as Grade I adhesions, hydrosalpinx, sacculated tubes, signs of

chronic inflammation, pelvic inflammatory disease, and obliterated pouch of Douglas.<sup>[5]</sup>

The affirmative findings on laparoscopy were taken as confirmatory for FGTB.

Grading of adhesions covering adnexa was done as follows:

- Grade I - Localized covering one-third of the adnexa
- Grade II - Moderate, covering one-third to two-thirds of the adnexa.

A composite gold standard was created comprising AFB culture, histopathology, and laparoscopy. A case was labeled as TB positive if either culture or histopathology tested positive or there were affirmative findings of TB on laparoscopy in patients positive with TB-PCR and Gene Xpert.

All cases having evidence of genital TB were put on anti-TB treatment according to the Revised National Tuberculosis Control Programme guidelines-Category I regimen, which comprised daily 2 (HRZE) for a period of 2 months followed by 4 (HR)<sub>3</sub> thrice weekly for 4 months.

Statistical analysis of differences between the two groups was done using Stata 13.0 (Stata Corp LLC, Texas, and USA). Quantitative variables were determined by unpaired *t*-test or Mann-Whitney U-test. Qualitative variables were determined by Chi-square test or Fisher's exact test. Level of significance ( $P \leq 0.05$ ) was used.

## RESULTS

Demographic profiles showed the mean age of women being  $28.95 \pm 4.7$  years. Most of the women (54.6%) were in the age group of 20–30 years. The mean body mass index was calculated as  $25.72 \pm 4.61$ . Majority of the women were Hindu by religion (134, 76.1%) followed by Muslim women (42, 23.9%).

Majority of the women were vaccinated with BCG vaccine (92.6%) in their childhood. Constitutional symptoms such as fever in 18 (10.2%), cough in 3 (1.7%), chest pain in 1 (0.6%), anorexia in 11 (6.2%), and weight loss in 21 (11.9%) women were present.

81.2% of women were diagnosed as primary infertility and 18.8% had secondary infertility. The mean duration of infertility was  $7.3 \pm 3.6$  years.

Irregular cycles were present in 123 (69.9%) women. Oligomenorrhea was found to be common, which is present in 76 women (43.2%). Menorrhagia was seen in 35 (19.9%) women whereas hypomenorrhea was present in 10 (5.7%) women. Two (1.1%) women were amenorrhic for the past 6 months. Dysmenorrhea was

seen in 99 (56.2%) women, dyspareunia in 138 (78.4%) women, and history of chronic pelvic pain in 21 (11.9%) women.

On local examination, vaginal discharge was present in 69 out of 176 women (39.2%). Forniceal fullness, adnexal masses, bulky uterus, and restricted mobility of uterus were found in 19 (10.8%) women.

Anemia was present in 20 (11.4%) women. Mantoux test ( $>10$  mm) was seen in 104 (59.1%) women. Erythrocyte sedimentation rate (ESR) ( $>20$ ) was raised in 126 (71.6%) women. Both Mantoux and ESR were raised in 77 women. Positive findings of old pulmonary TB were present in 11 (6.2%) women on chest X-ray.

### Composite gold standard

Composite gold standard was positive in 18 (10.23%) out of 176 women.

Microscopy was positive in four patients, but one of the women on culture was found to have nontubercular *Mycobacterium* so was excluded from the TB-positive group; rest all the three patients were culture positive for *M. tuberculosis*. Culture was positive in 3 (1.7%) women. Histopathology was positive in two women (1.14%), out of which one was also culture positive and the other was positive with Gene Xpert.

Gene Xpert was positive only in 2 (1.14%) women. Out of the two positive cases, one was positive with HPE and other was positive with TB-PCR and both had affirmative findings on laparoscopy. No woman was detected with rifampicin resistance in our study group. TB-PCR was positive in 15 (8.52%) women.

Laparoscopy was performed in all 16 women who tested positive with Gene Xpert and TB-PCR. Out of 16 women, affirmative findings suggestive of TB were seen in 15 (93.5%) women such as the presence of tubercles, caseations, and Grade II adhesions in the pelvis. Fallopian tubes were involved in all 15 women and included findings such as beaded tubes, cornual block, tubercles, and hydrosalpinx.

One woman who was positive for TB-PCR had normal laparoscopic findings. Hence, a total of 18 women were diagnosed with genital TB in our study. There is a statistically significant correlation between the diagnostic ability of Composite Gold Standard (CGS) and TB-PCR and Gene Xpert ( $P < 0.001$ ) [Table 1].

The sensitivity of Gene Xpert was low, but its specificity was 100%. The sensitivity and specificity of other diagnostic modalities are shown in Table 2.

**Table 1: Comparison of tuberculosis-polymerase chain reaction and Gene Xpert with composite gold standard (N1 + N2=176)**

Parameters (N1 + N2=176)	Composite gold standard normal, N1 (%)	Composite gold standard abnormal, N2 (%)	P
TB-PCR			
Normal	157 (99.4)	4 (22.22)	<0.001
Abnormal	1 (0.63)	14 (77.78)	
Gene Xpert			
Normal	158 (100)	16 (88.89)	<0.001
Abnormal	0 (0.00)	2 (11.11)	

TB-PCR=Tuberculosis-polymerase chain reaction

**Table 2: Sensitivity and specificity of acid-fast bacilli microscopy, tuberculosis-polymerase chain reaction, Gene Xpert, acid-fast bacilli culture, and histopathological examination in endometrial biopsy samples of study population**

Parameters	Sensitivity (%)	Specificity (%)
Microscopy	16.67	100.00
TB-PCR	77.78	99.37
Gene Xpert	11.11	100.00
AFB culture	16.67	100.00
HPE	11.11	100.00

AFB=Acid-fast bacilli, HPE=Histopathological examination, TB-PCR=Tuberculosis-polymerase chain reaction

## DISCUSSION

Genital TB poses a major diagnostic challenge as it is a paucibacillary disease. Diagnosis is often delayed and is made presumptively on the basis of clinical features, radiological investigations, endometrial biopsy sample evaluation, and laparoscopic evaluation. None of the test is 100% sensitive. Prognosis is directly related to time delay in starting treatment. Mortality further increases in patients having multidrug-resistant (MDR)-TB. Thus, an early diagnosis, information regarding multidrug resistance and institution of appropriate antitubercular treatment is critical for better outcome.<sup>[2]</sup>

The present study has evaluated the role of Gene Xpert in the detection of FG TB and found the sensitivity of 11.11% and specificity of the test of 100%, whereas Sharma *et al.* in their study found the sensitivity and specificity of the test of 46.6% and 100%, respectively.<sup>[19]</sup> Both tested positive patients in our study were having affirmative findings on laparoscopy/hysteroscopy, such as presence of tubercles, Grade II adhesions in the pelvis, and bilateral cornual block, and on hysteroscopy such as intrauterine synechiae, suggesting the importance of running the test in cases where advanced stage of the disease is suspected. Sharma *et al.* had found the role of Gene Xpert in the detection of rifampicin resistance, especially in MDR-TB in high-risk patients, and were able to detect it in five patients; however, in our study,

none of the patients were detected with rifampicin resistance.<sup>[20]</sup> Garg *et al.* were not able to detect any patient positive with Gene Xpert among 81 infertile women suspected of having genital TB as a cause of infertility.<sup>[21]</sup>

Radhika *et al.* had shown the higher sensitivity of TB-PCR in the detection of genital TB similar to our study, but authors had raised concern regarding the high false-positive rate of TB-PCR.<sup>[22]</sup> Similar results have also been given by Sethi *et al.* and Prasad *et al.*<sup>[5,23]</sup>

Jindal *et al.* had shown that both TB-PCR and laparoscopy are required for the detection of genital TB, since there are many cases that may give false-negative result of TB-PCR due to destroyed endometrium, but laparoscopy can pick up these cases.<sup>[24]</sup> Our study had also shown one case in which laparoscopy was positive and TB-PCR was negative, but Gene Xpert was positive.

Gene Xpert can be preferred over TB-PCR as it offers advantage in terms of detection of both *M. tuberculosis* and rifampicin resistance within 2 h of starting the test with minimal hands-on technical time, and also, TB-PCR has high false-positive as well as false-negative results.

Gene Xpert is also unique because sample processing, PCR amplification, and detection are all integrated into a single self-enclosed test unit, the Gene Xpert cartridge, however; it has few limitations such as the limited shelf-life of the diagnostic cartridges, requirement for continuous electricity supply, unknown durability, and the need for annual servicing and calibration of each machine.<sup>[25]</sup> The lower sensitivity of the test could be attributed to high operating temperature and humidity restrictions, which are quite prevalent in India, paucibacillary nature of disease, and blood acting as an inhibitor in the endometrial tissue.

The limitation of the study includes small sample size due to time constraints, no treatment response was monitored due to cross-sectional nature of the study, and laparoscopic-guided biopsy was not performed. Although biopsy results would have yielded better

accuracy, we did not perform owing to the affirmative findings seen in our cases as reported by other authors to be the diagnostics of genital TB.<sup>[5,19,24]</sup>

## CONCLUSION

Gene Xpert appears to be a useful modality in diagnosis of genital TB owing to its high specificity and can be recommended in conditions where microscopy, culture, and histopathology are negative; however, further randomized studies are required to support our hypothesis.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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