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# LATENT GENITAL TUBERCULOSIS: A COMPARATIVE STUDY OF THE DIAGNOSTIC MODALITIES

Obstetrics & Gynaecology

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## ABSTRACT

GTB, or genital tuberculosis, is a primary cause of severe tuberculosis-related infertility. For instance, when it comes to pulmonary tuberculosis (TB), the majority of the time the disease presents itself clinically with no symptoms, making the clinical diagnosis of GTB difficult for medical specialists. Infertility in India is caused by approximately 10% of cases of extra-pulmonary tuberculosis (EPTB), a clinical subtype known as female genital tuberculosis (FGTB). Due to changes in uterine endometrial receptivity and fallopian tube obstruction, both latent genital tuberculosis (LGTB) and FGTB can result in infertility. This study compared the methods of acid-fast bacilli (AFB) culture, polymerase chain reaction (PCR) methodology, and AFB staining. **Materials And Methods:** This study includes women who visited in vitro fertilization centers. 227 endometrial tissue samples that were collected aseptically were treated in total. Standard operating procedures were followed when doing AFB staining, AFB culture, and PCR. **Result:** AFB smear microscopy, culture, or PCR were used to determine the status of 133 out of 227 patients who were suspected of having GTB. Two (1.5%) of the 133 samples were found to be positive by microscopy, culture, and PCR; 11 (4.8%) of the samples were found to be positive by both PCR and culture; and 126 (86%) of the samples were found to be positive exclusively by PCR. Seven cases that tested positive using the standard culture approach were missed by the PCR. **Conclusion:** This study shown that, in comparison to PCR, traditional diagnostic techniques such as microscopy and culture are less sensitive. PCR aided in the early detection of infection as well. Simultaneously, erroneous negative results constituted a significant methodological constraint. Positive results were obtained from PCR-negative material using culture techniques. Due to erroneous positive or negative results, deoxyribose nucleic acid PCR is unreliable for diagnosing tuberculosis. Therefore, we recommend both culture and PCR as significant diagnostic techniques for the detection of GTB based on our observations from this investigation. PCR and culture selection are useful for diagnosis.

## KEYWORDS

Polymerase chain reaction, acid fast bacilli culture, genital tuberculosis, acid fast staining.

## INTRODUCTION

One prominent kind of extrapulmonary tuberculosis (TB) that affects women and can lead to menstruation problems, infertility, ectopic pregnancy, and tuboovarian mass is female genital tuberculosis (FGTB).I–four Infertile women with FGTB prevalence ranges from 7% to 15% in impoverished nations to 26% in tertiary referral hospitals and up to 48% in cases of tubal factor infertility.[3,5]

The infection spreads to genital organs normally by hematogenous route with the frequency of involvement of fallopian tubes (90%), endometrium (50–80%), ovaries (20–30%), cervix (5–10%), and rarely vulva and vagina.[1] FGTB is an important cause of intrauterine adhesions (Asherman's syndrome), pelvic adhesions, and perihepatic adhesions (Fitz Hugh–Curtis syndrome).[6,7] Female genital TB is a form of extra-pulmonary TB affecting the female genital organs, with fallopian tubes being affected most commonly (90 %), followed by the endometrium (50 %) and the ovaries (10-30 %).[8,9]

Conventional methods for the diagnosis of TB include microscopy and culture. Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB) requires $10^4$ -$10^6$ bacilli/ml of tissue or fluid specimens to give a positive result.[10,11]

Nucleic acid amplification (NAA) tests represent a major advance in the diagnosis of TB.[12] Nucleic acid sequences specific to MTB can be directly identified in clinical specimens using amplification techniques, which provides faster and more accurate results than microscopy. Because of its detection limit of one to ten bacilli in a variety of clinical samples, advanced molecular techniques like the polymerase chain reaction (PCR), a form of NAA system, have demonstrated highly promising results for early and rapid diagnosis of the disease. [13]

It is nearly invariably a side effect of another tubercular lesion in the body. The exact cause of this disease remains not known, as the majority of the cases remain undiagnosed due to asymptomatic presentation of genital TB and paucity of investigations specially in developing countries. A diagnostic method with less time-consuming and at the same time has high sensitivity and specificity is therefore desirable.

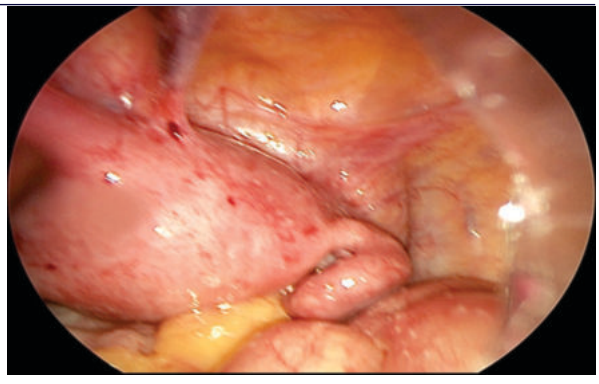

**Figure 1: Laparoscopic findings showing tubercles on ovary before**

Antitubercular therapy in one of the cases. Few pelvic adhesions are also seen.

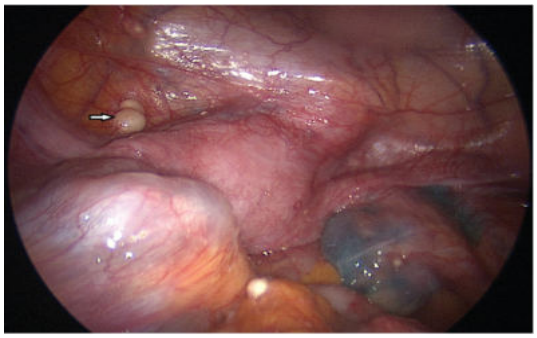

**Figure 2: Laparoscopic findings showing caseous nodule in a case of female genital tuberculosis.**

## MATERIALS AND METHODS

### Study design and settings

In a teaching tertiary care hospital, the current prospective study was carried out in the microbiology department.

**Sample size**
Approximately 227 endometrial tissue samples were obtained from all women who underwent testing for genital tuberculosis (GTB) and reported with reproductive concerns.

**Collection of samples**
At the time of laparotomy, a tubal biopsy was performed, and endometrial tissue was harvested by curettage and stored in normal saline. Prior to processing, each sample was stored at 4°C. The fragile tissues measured 3–4 mm in length. The microbiology department handled the processing of every sample.

**Inclusion criteria**
In this study included those women who visited IVF center with disorder of Infertility and given the Endometrial sample.

**Exclusion criteria**
All Clinical and Histopathological finding were excluded.

## METHODS
Following the ZN staining of both direct and concentrated smears (N-acetyl-L-cystine-NaOH concentration method) (LYFECTOL Tulip Diagnostics), all electron microscopy (EM) tissue samples were inspected under a microscope. Concurrently, the concentrated samples were inoculated on Lowenstein-Jensen media and cultivated aerobically at 37°C in a biochemical oxygen demand incubator.14 The infected media were checked after 48 hours, 24 hours, and then once a week for eight weeks. The MPT64Ag card test was used to identify the species in the culture samples that tested positive (SD Bioline Standard Diagnostics Inc.).

The Qiagen QUamp DNA micro kit was used to extract deoxyribose nucleic acid (DNA). After homogenizing all of the EM tissue with a pestle and mortar, the material was centrifuged for 10 minutes at 6,000 rpm. After discarding the supernatant, 3 ml of tris-buffer was added to the resulting pellet. To reduce the possibility of contamination, every step of the PCR process was carried out in an isolated room.

Following another centrifugation of the homogenized tissues for 10 minutes at 6,000 rpm, 250 µl of lysis buffer I and 20 µl of proteinase K were added to the pellet. All the samples were then mixed by vortexing, maintained in a dry bath at 90°C for 20 to 25 minutes, then centrifuged for 10 minutes at 10,000 rpm. In a 1.5 ml eppendorf tube, 200 µl of lysis buffer II (including internal control at a concentration of 10 µl/ml) was added to 200 µl of supernatant, and the mixture was incubated at 70°C for 10 minutes. Next, 200 µl of 96–100% ethanol was added and vortexed to combine. After adding this combination to a spin column that was put within a 2-ml collecting tube, the mixture was centrifuged at 6000 rpm for three minutes. To guarantee that all of the wash buffer was removed, the spin column was placed in a fresh 2-ml collection tube and cleaned twice using the wash buffer that came with the kit. A final centrifugation was carried out for two minutes at 14,000 rpm. Next, the spin columns were placed in a 1.5-ml tube, and 100 µl of the kit's preheated (at 50°C) elution buffer was added. It was centrifuged at 10,000 rpm for two minutes to extract the DNA after it had been incubated for five minutes at room temperature. The DNA specimens were maintained at -20°C until subsequent examination.[15]

Amplified DNA underwent electrophoresis using 1.5% agarose gel at 120 volts for 1 h and the resultant bands were interpreted by ultraviolet trans-illumination. A product of 123 bp was indicative of infection with MTB and an amplified product of 340 bp was used as an internal control.[15] Each PCR run is controlled by adding negative (without templates) and positive controls (with templates) to maintain false positive and negative results.

## RESULTS
Out of 227 patients suspected from GTB, 132 were found to be positive by either of the three diagnostic methods i.e., AFB smear microscopy, culture and PCR. Out of 132 positive samples, 2 (1.5%) samples were found to be positive by all the three methods, i.e. microscopy, culture and PCR, 11 (4.9%) were found to be positive by both PCR and culture. A total of 125 samples were found to be positive only by PCR, whereas six samples were found to be positive only by culture method. Not a single sample were found to be positive by microscopic examination. Two samples that were positive by microscopic examination were also positive by PCR. Out of 10 culture positive samples, eight were MTB and 2 samples were positive for *Mycobacterium* other than tuberculosis (MOTT). PCR positive samples were all detected as MTB. Overall positive predictive value of PCR was 125 (almost 86%) and culture was 18 (12%) Table-1

Percentage positivity of the various EM samples detected by various diagnostic methods:

| | | |
|---|---|---|
| Total Samples= 227 | Total Positive Samples=132 [57.4%] | Microscopy+ Culture-PCR- 0 [0%]<br>Microscopy-Culture+PCR-07 [5.3%]<br>Microscopy-Culture-PCR+116 [87.1%]<br>Microscopy+ Culture+PCR-0 [0%]<br>Microscopy+ Culture-PCR-0 [0%]<br>Microscopy+ Culture-PCR+12 [9.2%]<br>Microscopy+ Culture+PCR+2 [1.5%] |

EM=Electron microscopy, PCR=Polymerase chain reaction

## DISCUSSION
After the lungs, genitourinary tract is the second most common site for tuberculous infection after the lungs. GTB is usually secondary to renal tuberculous infection.[16]

In present time there are many communities where TB is still a major health problem, it is important to anticipate the possibility of GTB in an infertility patient.[17] Most probably there are chances of lack of awareness and diagnostic modalities which are prone to false positive as well as false negative results.

The presence of tuberculosis in the histopathological diagnosis of tuberculosis (TB) can be confused with a number of other illnesses, including sarcoidosis, syphilis, leprosy, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, and pneumoconiosis.[17] Consequently, tissue Mycobacterium culture or acid-fast staining must be carried out to verify the TB diagnosis. The paucibacillary tissue samples used in both of these assays contribute to their low sensitivity. Moreover, the process of cultivating culture is extremely time-consuming and laborious. Modern molecular methods, like PCR, are highly sensitive in diagnosing tuberculosis.[18–19]

We investigated the efficacy of several diagnostic techniques for GTB in this prospective investigation, and PCR had the highest sensitivity when compared to other techniques. We were able to identify MTB in 116 cases using the PCR test, even though the culture approach had yielded negative results. According to prior research, the PCR test identified MTB in less than 24 hours, as opposed to the typical 24 days needed for identification by traditional methods.[20]

With the sequence (IS6110) originating from endometrial tissue, PCR demonstrated nearly 81% sensitivity in this investigation. This finding is in good agreement with the research conducted by Cheng et al.[10]

The current study has demonstrated successful PCR outcomes. It has been observed that when PCR was carried out carefully, in a clean, and uncontaminated environment, true positive results were obtained. Conversely, when TB is suspected, PCR results should be correlated with culture reports if the test yields negative results. In our investigation, seven samples that were positive in culture for MTB were not detected by PCR. Rozati and colleagues also made similar observations.[21] There's a chance that some of the specimen used for the PCR processing didn't have AFB. Potential reasons for these PCR false-negative results include the specimen including blood or PCR inhibitors, or the possibility that the human bacterial genome ratio is affecting the mycobacterial DNA amplification.

Although we were able to distinguish between MTB and MOTT using the culture approach, the PCR result might be negative since it was unable to identify the species of Mycobacterium.

This complex method is further constrained by the high test cost and requirement for an appropriate infrastructure. In conclusion, PCR-based molecular diagnosis of tuberculosis offers a significant potential to enhance the capacity for GTB diagnosis. For the early diagnosis of GTB, PCR is a quick, accurate, and targeted diagnostic. Even though culture is a laborious and unreliable procedure, early PCR can help the consultant diagnose GTB and begin treatment. Based on our research, we can conclude that there is a high level of clinical suspicion, a negative smear result, and the presence of both.

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