

Limited role of GeneXpert in peritoneal fluid in the diagnosis of genital tuberculosis in infertile women

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ABSTRACT

Background and Objectives: Genital tuberculosis (TB) is an important cause of infertility in women that poses many challenges in diagnosis. The study is done to understand the utility of GeneXpert test in peritoneal fluid in the diagnosis of genital TB in infertile women. **Methods:** All infertile women in postmenstrual phase who were planned for laparoscopy in study period were included. Women who were already on anti-TB therapy were excluded. Peritoneal fluid/washings were retrieved during laparoscopy to test for *Mycobacterium tuberculosis* by GeneXpert. A note was made of laparoscopy evidence of TB. Endometrial sample was sent for microbiological testing of mycobacterium on smear and liquid culture. Histopathological test of endometrium was also done to look for granulomas. **Results:** In a total of 57 women, 8 (14.03%) women were diagnosed with TB on the basis of laparoscopy or microbiological or histopathological tests. Six women had caseating tubercles in pelvis, of them two women had presence of mycobacterium on smear, one woman also had positive liquid culture. In two women endometrial smear was positive. None of the women had a positive GeneXpert test in peritoneal fluid. **Conclusion:** Genital TB is a clinical problem in infertile women. Even in women with confirmed genital TB the peritoneal fluid/washings were negative for mycobacterium. GeneXpert did not pick *Mycobacterium* in peritoneal fluid in women with genital TB. Hence, it is not a sensitive and good tool for the diagnosis of female genital TB.

Keywords: CBNAAT, extrapulmonary tuberculosis, genital tuberculosis, molecular tests, PCR

Introduction

As per WHO report 2019, there were 10 million incident tuberculosis (TB) cases reported globally in 2018 and India alone contributed 27% to this scenario.^[1] In India about 80% of TB cases are pulmonary TB (PTB) cases and 20% are extrapulmonary TB (EPTB). Common sites of EPTB are lymph nodes, pleura, meninges, bone and joints, female urogenital organs, eye, etc.^[2-7] Urogenital TB constitutes around 15% of cases of EPTB.^[8] Incidences of genital TB ranges from 10% in India to less than

1% in US.^[9] Symptoms of genital TB may vary from menstrual disturbances, pelvic pain or failure to conceive. Sometimes, there are usually no constitutional symptoms associated and a high index of suspicion is needed. A total of 65-70% of women with genital TB develop infertility; it is therefore important that all infertile women are investigated for genital TB.^[10]

Conventional diagnostic tests including demonstration of acid-fast bacilli (AFB) on microscopy, growth of AFB on solid and liquid culture medium, presence of granulomas on histopathological examination in endometrium have been used so far.^[11] The sensitivity of these conventional tests is very poor as female genital TB (FGTB) is a paucibacillary disease with a poor pick up rate (1.8–18.5%).^[12-16] *Mycobacterium* DNA polymerase chain reaction (PCR) has been widely used with sensitivity of 22.2%-97.6%; however, it has high false positives and is not considered as the sole basis

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to diagnose TB.^[15] Also, these diagnostic techniques fail address the drug resistance pattern in EPTB samples.^[17] Diagnostic laparoscopy helps to visualize the fallopian tubes, uterus and take biopsy from suspicious areas. This test is nevertheless expensive, requires expertise and is invasive as well. Presence of tubercles, caseating deposits and beaded tubes on laparoscopy confirms the diagnosis. To confirm FGTB, composite reference standard has been taken as gold standard which includes evidence on either laparoscopy or microbiological or histopathological criteria.

GeneXpert (Cepheid, Sunnyvale, CA, USA) is a cartridge based fully automated nucleic acid amplification test, is 99% sensitive and 99.3% specific to diagnose PTB cases. A distinct advantage is this test is it takes less than 2 h and also identifies rifampicin sensitivity.^[18] A Cochrane review on use of Mtb/RIF (*Mycobacterium tuberculosis* DNA and resistance to rifampicin) assay for PTB specimen in 18 studies found a pooled sensitivity of 88% and specificity of 98%.^[19] GeneXpert is being made available to health care facilities in India through National Tuberculosis Elimination Programme (NTEP) erstwhile RNTCP (Revised National Tuberculosis Control Program) programme since 2015. Several researchers have tested the diagnostic test accuracy of GeneXpert in non-pulmonary specimens; however, the results depend on the type of specimen and bacillary load.^[20]

Genital TB is a paucibacillary disease and as of now, there are limited data to suggest utility of GeneXpert test in FGTB. If its sensitivity in diagnosing FGTB is found to be as high as in PTB, it may have far reaching implications in early diagnosis and management of FGTB. Genital TB has traditionally been described as presence of congestion, oedema, adhesions and fluid-filled pockets in pelvis. It is also known to involve peritoneum which may present as fluid in pouch of Douglas. Since endometrial tissue is blood stained and blood is considered a known DNA inhibitor, it is presumed to affect the sensitivity of PCR based tests; hence, it was decided to test for genital TB in peritoneal fluid by GeneXpert. Primary care physicians are usually first point of contact and this study will elucidate the utility of Genexpert in FGTB.

Materials and Methodology

The pilot study was conducted in the Department of Obstetrics and Gynaecology in collaboration with Department of Microbiology at King George Medical University Lucknow over a period of 6 months from August 2017 to January 2018. Approval of Institution Ethics Committee was taken (81st ECM II A/P 11, 5 August 2017).

All the infertile women attending the gynaecology OPD and planned for diagnostic laparoscopy and hysteroscopy were included in the study after they satisfied the inclusion and exclusion criteria. Informed consent was taken before recruiting in the study.

All infertile women in postmenstrual phase who were planned for laparoscopy (presence of bilateral tubal block on

hysterosalpingogram, women not conceiving despite multiple cycles of Artificial reproductive technology (ART)) were included in the study. Women who were already on anti-TB therapy were excluded. A detailed history and examination was done for all the women. A note was also made of history of any contact with TB. Human immunodeficiency virus (HIV) testing was offered to all women and the result was noted.

During laparoscopy, women were evaluated for the presence of straw-coloured fluid in pouch of Douglas, presence of any tubercles, adhesions or caseation. A note was also made of presence of any concomitant pathology like fibroids, endometriosis, pelvic inflammatory disease. Peritoneal fluid was aspirated and sent for GeneXpert test. If there was no peritoneal fluid, 10 cc of saline was instilled in pouch of Douglas and peritoneal washings were sent for GeneXpert test. One ml of peritoneal fluid/washings was mixed with double volume of sample reagent. After vigorous shaking and incubation of 15 min the suspension was loaded into cartridges. Test was performed by following the manufacturer's instructions (Cepheid) and standard protocol.^[21] Endometrial aspirate was taken in phosphate buffered saline for AFB smear, AFB culture and sensitivity to look for *Mtb* and 10% formalin for histopathology to rule out granulomas.

Woman was diagnosed as having TB as per composite reference standard (CRS). CRS was taken as presence of *Mycobacterium* bacilli on smear or culture or presence of granulomas on histopathological examination. Women were also diagnosed as TB if there were presence of tubercles, caseation or beaded tubes on laparoscopy. Result of GeneXpert was then compared with the other tests. Patient was given anti-TB therapy as per RNTCP protocol.

Statistical analysis

Descriptive statistics were computed for all the variables. Chi-square test of association was performed between demographic factors and TB. Mann-Whitney U test was performed to compare difference in age and duration of infertility across TB. *P* value < 0.05 was taken as significant. All the statistical analysis has been done using Statistical Packages for Social Sciences (SPSS) v20 Software.

Results

There were 57 infertile women enrolled in the pilot study. Table 1 shows demographic details of women enrolled. On laparoscopy, peritoneal fluid was present in 27 women and in rest 30 women, peritoneal washings were taken. Eight women of 57 (14.03%) with infertility had some evidence of genital TB. Six women had tubercles on uterine surface/tubes in laparoscopy, of them two women had positive smear for *Mtb* and one also had positive liquid culture. In the rest two women there was only laparoscopic evidence of TB with no supportive microbiological or histopathological results. Peritoneal fluid/washings were sent for GeneXpert testing and were negative for *Mtb* in all the

women. There was no evidence of any granulomas suggestive of TB in endometrial histopathology in any women. Details of diagnostic tests of women positive for genital TB are shown in Table 2. There was no association of demographic factors with presence of TB as shown in Table 3.

Discussion

There have been several studies in the past where study sample is endometrial tissue as well as tissue from other organs where the TB is not easily detected.^[22,23] The pick-up rates of *Mycobacterium* are poor (1-18%)^[12-16] probably due to monthly shedding of superficial layers of endometrium. A noninvasive sample like menstrual blood has also been studied in 123 infertile women and *Mycobacterium* was detected by liquid culture in only one woman (0.8%).^[23] The authors suggested that menstrual blood is not a potential alternative clinical sample for diagnosis of genital TB. Presence of blood acts as a potent DNA inhibitor and may interfere with amplification-based tests. Considering this, peritoneal fluid was sampled during laparoscopy to look for evidence of genital TB.

Peritoneal fluid or washings have also been sampled for presence of *Mtb* by DNA PCR by Thangappa *et al.* (2011) in 72 infertile

women. Laparoscopy was positive in 43 (59.7%) women, AFB smear was positive in 8.3%, culture positive in 5.6%, HPE positive in 6.9% and PCR was positive in 36.7% of cases. In seven women peritoneal fluid was also aspirated and sent for PCR and culture examination. Two of seven samples were positive by PCR and none was culture positive. It was proposed by authors that it could be due to paucibacillary nature of the specimen.^[13] Bhanu *et al.* (2005) also studied peritoneal fluid and showed that DNA PCR was positive in 16% women only. In another study by Rana *et al.* (2011) on 200 infertile women where various molecular and microbiological tests were done including DNA-PCR (in house PCR targeting *mpb64* gene) on endometrial/peritoneal fluid, it was seen that DNA PCR was positive from 9.57% in peritoneal fluid specimens to 44.85% in endometrial specimens. Seven women had positive DNA PCR in both endometrial and peritoneal fluid specimen; however, two women had only positive DNA PCR in peritoneal fluid specimen with negative endometrial specimen. It was suggested that there may be earlier involvement of tubes before endometrium and bacillary spill over from tubes may lead to early affection of peritoneum before fibrosis sets in which was seen as positive PCR in peritoneal fluid.^[16]

GeneXpert has emerged as an excellent diagnostic modality in confirmation of PTB with high sensitivity and specificity.^[18] Based on the review on use of GeneXpert in extrapulmonary specimens by Denkinger *et al.*^[24] World Health Organization (WHO) now recommends this test over conventional tests for the diagnosis of TB in lymph nodes and other tissues and as the preferred initial test for the diagnosis of TB meningitis.^[11] GeneXpert test was also studied in 533 extra pulmonary specimens and the study showed that it was 86–100% sensitive for synovial, pericardial and peritoneal fluid; 63–73% sensitive for tissues, lymph nodes, pleural fluid and 29% sensitive in CSF specimen.^[16] In a study by Sharma *et al.* (2015). GeneXpert was used to diagnose EPTB in 1292 specimens, wherein endometrial specimens' sensitivity was 33–50% and specificity was 100% for the diagnosis of FGTB.^[25] A recent Cochrane review in 2018 on EPTB showed pooled sensitivity and specificity of GeneXpert (95% CI) was 59.2% (45.2 to 73.5) and 97.9% (96.2 to 99.1), respectively (16 studies, 712 specimens).^[26] Recently in a study by Sharma *et al.* (2015) 240 endometrial samples were studied, GeneXpert was positive in seven (2.9%) women. All seven women also had positive AFB culture. GeneXpert showed 100% sensitivity; however, there was no advantage of its performance over other tests. The authors also postulated that blood contamination can inhibit GeneXpert testing making it

Table 1: Demographic characteristics of women enrolled in the study

Variables	Value
Age in years (mean±SD)	30.16±4.38
Area of residence (urban)	48/57; (84.2%)
Type of infertility (primary)	44/57; (77.2%)
Duration of infertility in years	6.19±3.07
Prior history of anti-tubercular treatment intake	13/57; (22.8%)

Table 2: Details of women detected positive for genital tuberculosis

Patient No.	Smear	Liquid culture	Histopathology	Peritoneal fluid for GeneXpert	Laparoscopic evidence
1	Absent	Absent	Absent	Absent	Present
2	Absent	Absent	Absent	Absent	Present
3	Absent	Absent	Absent	Absent	Present
4	Absent	Absent	Absent	Absent	Present
5	Present	Absent	Absent	Absent	Absent
6	Present	Absent	Absent	Absent	Absent
7	Present	Absent	Absent	Absent	Present
8	Present	Present	Absent	Absent	Present

Table 3: Association of presence of tuberculosis with demographic factors

Demographic details	Presence of tuberculosis (n=8)	Absence of tuberculosis (n=49)	P
Age (years)	32.00±4.07	29.86±4.39	0.192
Duration of infertility (years)	7.13±3.18	6.04±3.06	0.354
Residence Rural: Urban	1:7	8:41	0.876
Prior History of TB	2/8	11/49	0.873
Presence of peritoneal fluid	2/8	25/49	0.172
Tubal abnormality	3/8	12/49	0.697

negative.^[27] Another recent study was conducted in 16 medical centres in Iran where 838 extrapulmonary specimens were tested. The study found a variable sensitivity for different EPTB specimens ranging from 40% to 100%. Here also concern about presence of blood and PCR inhibitors affecting the test was cited as a possible reason for poor sensitivity in biopsy specimens and it was suggested to develop a special protocol/buffers for EPTB specimens.^[28] Use of Ultra technology, where two new PCR assays for IS6110 and IS1081 were used for diagnosis of TB on EPTB specimens, showed better sensitivity (90%) in EPTB than conventional technique.^[29]

Though premenstrual endometrial biopsy is the standard specimen used to diagnose genital TB, here peritoneal fluid was taken to negate the effect of blood on PCR technique. Due to financial constraints, the GeneXpert test could not be done simultaneously in both endometrial and peritoneal fluid specimen. The study though had small number of women pointed the fact that genital TB is a relevant factor leading to infertility in women. Even in women with confirmed genital TB through microscopy or laparoscopy, the peritoneal fluid was negative for *Mtb*. GeneXpert is considered indispensable tool in PTB; however, this study did not find this test useful in diagnosing genital TB in peritoneal fluid. A larger multicentric study is proposed to evaluate if GeneXpert has any role in diagnosing genital TB. Moreover, endometrial sample should also be tested concurrently to improve the results.

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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Conflicts of interest

There are no conflicts of interest.

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