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Evaluation of Xpert MTB/ RIF Assay for the Detection of Female Genital Tuberculosis in a Tertiary Care Center- A Descriptive Cross-sectional Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author AF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DZ and MM managed the analyses of the study. Author FK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Female genital tuberculosis (FGTB) is a silent disease and patients may present with non-specific symptoms irrespective of disease severity and duration. It is a major cause of severe tubal disease leading to infertility. A high degree of suspicion is required for its diagnosis. Conventional diagnostic tests have low sensitivity due to the paucibacillary nature of FGTB. Polymerase chain reaction (PCR) based methods like Xpert MTB/RIF assay hold potential for the early diagnosis of FGTB. The objective of this study was to evaluate the utility of Xpert MTB/RIF assay for the diagnosis of FGTB.
Methodology: Laparoscopy directed endometrial biopsies of 87 samples of females suspected of genital tuberculosis were included in the study. The specimen were subjected to Ziehl Neelson staining, culture on Lowenstein-Jensen media and nucleic acid amplification by Xpert MTB/RIF assay.
Results: Infertility was the most common presenting complaint in 59.77% of cases. None of the

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samples included in the study was positive by Ziehl – Neelson staining. Culture was positive in 4.6% of the cases. Xpert MTB/RIF assay was positive in 8.05% of cases. Compared with microscopy and culture, Xpert MTB/ RIF assay demonstrated improvement rate in detection of FGTB by 55.55%.

Conclusion: Xpert MTB/RIF assay is a useful tool for the early detection of female genital TB with the advantage of rapid results, minimal hand on time and detection of drug resistance.

Keywords: Female genital tuberculosis; Xpert MTB/ RIF assay; culture.

1. INTRODUCTION

Tuberculosis is an important cause of morbidity and mortality around the world despite the availability of effective anti-tubercular drugs [1]. Female genital tuberculosis is one form of extrapulmonary TB [2]. It is by no means uncommon, particularly in communities where pulmonary as well as extrapulmonary tuberculosis are prevalent. The global prevalence of genital tuberculosis is estimated to be 9% of the total extrapulmonary tuberculosis with a rising incidence, particularly as a result of HIV pandemic and the emergence of drug-resistant strains [3].

Genital tuberculosis in females affects fallopian tubes, uterine endometrium, ovaries, cervix, uterine myometrium and vagina/vulva. This disease not only causes tubal obstruction and dysfunction but also impairs implantation and ovulatory failure due to endometrial and ovarian involvement. It is well recognized as an important etiological cause for infertility in areas with high prevalence of tuberculosis. A study on FGTB among patients with infertility from India has shown an incidence of 3- 16%. Besides infertility, genital TB causes menstrual irregularity and pregnancy loss in women [4-6].

Genital TB may be asymptomatic and therefore the burden of tuberculosis is largely underestimated. Most of the patients are usually diagnosed during an evaluation for infertility. Despite the availability of various techniques, the diagnostic dilemma for genital TB still exists. A high index of suspicion and elaborate history and clinical examination are essential for diagnosis [7]. Any diagnostic method used to detect genital TB should be sensitive enough to diagnose the disease in early stages, so that appropriate treatment can be initiated. Although tuberculosis culture and histopathology are gold standard tests for the diagnosis of FGTB; the utility of these tests is compromised by their low sensitivity owing to the paucibacillary nature of genital tuberculosis. Over the years, PCR has been evaluated for the rapid and reliable diagnosis of tuberculosis [8]. The world health organization has recommended the use of a novel, automated, cartridge-based nucleic acid amplification technique, the Xpert MTB/RIF assay (Cepheid, USA), for the diagnosis of pulmonary TB, pediatric TB and extrapulmonary TB [9]. However data involving the use of Xpert MTB/ RIF assay for the diagnosis of genital tuberculosis is limited. The objective of this study was to evaluate the performance of Xpert MTB/RIF assay in the diagnosis of female genital tuberculosis in comparison to conventional methods i.e. microscopy and culture.

2. MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Government Medical College Srinagar. After taking informed consent, samples of women attending associated gynaecology clinics and suspected of having genital tuberculosis were included in the study. Ethical clearance was obtained from the institutes' ethical clearance committee. These included women in the reproductive age group who presented with clinical symptoms such as infertility, chronic pelvic pain, and menstrual irregularities such as oligomenorrhoea, amenorrhoea, menorrhagia, dysmenorrhoea and abnormal vaginal discharge. The study also included women presenting with postmenopausal bleeding and persistent leucorrhoea.

After a detailed history and examination, laparoscopy directed endometrial biopsies were obtained in all women. Whenever possible, fluid from the pouch of Douglas was also aspirated at the time of laparoscopy. Also during laparoscopic procedures, features suggestive of tuberculosis such as frank tubercles, caseation granulomas and beaded tubes were noted. All samples were sent to the department of microbiology for microscopy, culture and Xpert MTB/ RIF assay testing.

2.1 Sample Processing

Samples received in the microbiology laboratory were grounded well in 5 ml of distilled water. The specimen was centrifuged, the supernatant

discarded and the sediment was decontaminated by 5% H_2SO_4 method. The decontaminated specimen was used for preparing smear and AFB (Acid Fast Bacilli) staining was done by Ziehl Neelson method. Culture on Lowenstein-Jensen medium was done using a standard protocol. All samples were examined within 48 hours to detect gross contamination. Thereafter samples were examined weekly for the detection of growth. Growth on Lowenstein-Jensen media was identified as *Mycobacterium tuberculosis* by biochemical tests. Cultures were incubated for 8 weeks before being declared as negative. Cultures with gross contamination (those where the surface has been completely contaminated or where the medium has been liquefied or discolored) were discarded. However, drug susceptibility testing was not done in cases where *Mycobacterium tuberculosis* was isolated from culture.

2.2 Analysis by Xpert MTB/RIF Assay

The assay was performed using version 4 cartridges according to the manufacturers' recommendations. Briefly, the sample-reagent (containing NaOH and isopropyl alcohol) was added to decontaminate clinical specimen in the ratio of 2:1. The sample-SR (Sample Reagent) mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another 10 minutes. Two ml of the digested material was transferred to the cartridge. The cartridge was subsequently loaded in the GeneXpert instrument where all subsequent steps occurred automatically. In case the results were reported as invalid, error or no result, the sample was reprocessed and rerun, if sufficient material was available.

2.3 Statistical Analysis

Data was entered into a Microsoft excel sheet. Categorical variables were summarized as frequency and percentage. Continuous variables were summarized as a mean and standard deviation. Data analysis was done using EpiInfo 7.0.

3. RESULTS

A total of 87 patients were included in the study. Mean age of the patients was 33.11 years (range 21-50 years). All patients included in the study were HIV negative. None of the patients included in the study had symptoms of active pulmonary tuberculosis or any other comorbid illness. Most common presenting symptoms were infertility (59.77%) followed by menstrual abnormalities (24.14%). Primary infertility was seen in 33 (37.93%) patients while secondary infertility was seen in 19 (21.84%) women. Menorrhagia was the commonest reported menstrual abnormality followed by oligomenorrhea. Other abnormalities included ectopic pregnancy (8.05%), increase in endometrial thickness (4.12%) on Ultrasonography and abortions (3.45%) (Table 1).

Table 1. Clinical findings of women suspected of female genital tuberculosis

Clinical findings	No. (%)
Infertility	52 (59.77)
Primary infertility	33 (37.93)
Secondary infertility	19 (21.84)
Menstrual abnormalities	19 (21.84)
Menorrhagia	11 (12.64)
Oligomenorrhoea/ amenorrhoea	6 (6.9)
Abnormal vaginal discharge	2 (2.3)
Postmenopausal bleeding	2 (2.3)
Ectopic pregnancy	7 (8.05)
Abortions	3 (3.45)
Increase in endometrial thickness	4 (4.6)
Total	**87**

Of the 87 patients recruited, none of their samples was positive on smear microscopy for acid-fast bacilli (AFB). Culture on Lowenstein-Jensen medium revealed 4 (4.6%) samples to be positive for *Mycobacterium tuberculosis*, while 4 (4.6%) samples were contaminated. Non-tubercular mycobacteria were isolated from one sample. Rest of the samples were reported as negative after 8 weeks of incubation.

Seven (8.05%) samples were positive by Xpert MTB/RIF assay. Two of the 4 culture-positive samples were also Xpert MTB/RIF assay positive while other 2 samples were Xpert MTB/RIF assay negative. 2 samples that came positive on Xpert MTB/RIF assay had contaminated cultures. However, the other 3 samples positive by Xpert MTB/RIF assay were culture negative. The correlation of Xpert MTB/RIF assay with other diagnostic techniques is given in Table 2.

4. DISCUSSION

Female genital tuberculosis is a major health problem which usually goes unnoticed due to its varied clinical presentations. In most cases, the disease is asymptomatic and can present with

Table 2. Correlation of Xpert MTB/ RIF assay with other diagnostic techniques

	No. of samples	Xpert MTB/RIF assay	
		Positive (n=7) (%)	Negative (n=80) (%)
ZN staining			
Positive	0	0	0
Negative	87	7(8.05)	80 (91.95)
Culture			
Positive	4	2 (2.3)	2 (2.3)
Negative/ contaminated	83	5(5.75)	78(89.66)

few non-specific symptoms among which, infertility is the most common. Conventional methods like Ziehl-Neelsen staining and culture on Lowenstein-Jensen media are less sensitive for detection due to the paucibacillary nature of the gential TB. Besides, culture may take upto 8 weeks for growth of mycobacteria. The use of polymerase chain reaction (PCR) holds potential for detection of female genital TB due to high detection rate and rapid results [10]. Several studies have assessed the utility of Xpert MTB/RIF assay for the diagnosis of extra pulmonary tuberculosis including body fluids, tissues and biopsy specimen [11]. However very few studies evaluating the utility of Xpert MTB/RIF assay for the diagnosis of genital tuberculosis has been found in literature. This study was performed to evaluate Xpert MTB/RIF assay for the diagnosis of female genital TB and to compare the technique with conventional methods.

Most of the women included in the study were in the reproductive age group. 73.56% of the women were in the age group of 25-35 years. The presenting complaints were infertility (59.77%) followed by menstrual abnormalities (24.14%). These findings are consistent with other studies [12,13]. However, in most of the studies, oligomenorrhea is the commonest menstrual abnormality while as in our study, menorrhagia was the most common presenting complaint.

As there is no gold standard for the diagnosis of FGTB, a battery of bacteriological and molecular tests are required for the definitive diagnosis, and in many cases, it may still remain elusive [14]. In our study, a composite reference standard including clinical suspicion, laparoscopic findings and conventional methods were taken.

Out of 87 patients, female genital tuberculosis was diagnosed in 9 (10.34%) of the patients by any of the three diagnostic methods i.e. microscopy, culture and Gene Xpert. However, none of the specimen included in the study gave a positive result on acid-fast bacilli (AFB) microscopy. Few studies have reported the sensitivity of acid-fast bacilli (AFB) smear in the range of 0.1-8%. Microscopic examination of the endometrium requires the presence of 10^4 organisms per ml in the sample. The paucibacillary nature of the endometrial samples accounts for the negative results on acid-fast bacilli (AFB) smear in our study [15].

4.6% of the specimens were positive for culture. Low positivity of culture in endometrial specimen can be attributed to following factors: (i) paucibacillary nature of extrapulmonary samples; (ii) uneven distribution of tubercle bacilli in the specimen; (iii) substantial number of the bacilli in the specimen being bacteriologically dormant and (iv) the presence of antitubercular substance which inhibit mycobacteria to grow in the medium [16]. Additionally, culture takes 6-8 weeks for positive results during which the bacteria can permanently damage the genital tract. Another problem is the risk of contamination on culture media. In our study, 4.6% of the isolates gave contaminated cultures. A high rate of contamination could be explained by the use of a milder decontaminant (H_2SO_4) in this study. Also, one isolate yielded a non-tubercular mycobacteria in the study. The significance of isolation of non-tuberculous mycobacteria (NTM) in endometrial specimen needs to be ascertained.

Polymerase chain reaction (PCR) has been found as a reasonably good alternative for the diagnosis of extrapulmonary TB. Xpert MTB/RIF assay unlike other nucleic acid amplification tests (NAAT) has the advantage of detecting tuberculosis and rifampicin resistance within two hours with minimal hand on technical time**.** Polymerase chain reaction (PCR) based tests have also been associated with false positivity [17]. In the present study, Xpert MTB/ RIF assay detected an additional 55.55% of the patients with female genital TB. Two samples that were

culture positive were negative on Xpert MTB/ RIF assay. It could be possible due to the blood contamination of endometrial biopsy samples which could be inhibitory for Gene Xpert. Studies found in literature assessing the efficacy of Xpert MTB/RIF assay for detection of female genital TB are limited. A study by Sharma et al. found the assay to be highly specific although with a low sensitivity [18]. The authors recommended the use of assay as a useful adjunct in the diagnosis of female genital TB. All samples found positive by Gene Xpert were found to be rifampicin sensitive excluding the possibility of MDR- TB. However, in our study, the sensitivity of the assay was higher when compared to the conventional methods.

5. CONCLUSION

Female genital TB is a silent disease wherein patients present with non-specific symptoms irrespective of the severity and duration of the disease. Currently, there are no standard guidelines for the diagnosis of genital tuberculosis. None of the available techniques can pick up all cases of genital TB. The Xpert MTB/RIF assay has a higher detection rate compared to conventional methods. We recommend the use of Xpert MTB/ RIF assay for the early detection of female genital TB with an added advantage of early results, minimal technical expertise and detection of drug-resistant TB.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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