Genital tuberculosis: Comparative study of the diagnostic modalities

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ABSTRACT

BACKGROUND: Genital tuberculosis (GTB) is one of the major causes for severe tubal disease leading to infertility. Unlike pulmonary tuberculosis (TB), the clinical diagnosis of GTB is difficult because in the majority of cases the disease is either asymptomatic or has varied clinical presentation. Routine laboratory tests are of little value in the diagnosis. The objective of this study was to compare the modalities of polymerase chain reaction (PCR) technique, acid fast bacilli (AFB) culture and AFB staining. **MATERIALS AND METHODS:** The women visiting *in vitro* fertility center during December 2012 and May 2013 were included in this study. A total of 227 aseptically collected endometrial tissue samples were processed. AFB staining, AFB culture and PCR were carried out using standard procedures. **RESULT:** Out of 227 patients suspected of GTB, 133 were found to be positive either by AFB smear microscopy, culture or PCR. Out of 133 samples, two samples (1.5%) were found to be positive by all three methods, i.e. microscopy, culture and PCR, 11 (4.8%) were found to be positive by both PCR and culture, whereas 126 (86%) samples were found to be positive only by PCR. The PCR has failed to detect seven cases that were positive by conventional culture method. **CONCLUSION:** Our study showed that the conventional methods of diagnosis like microscopy and culture are less sensitive when compared with PCR. PCR also helped in early diagnosis of infection. However simultaneously, false negative results were an important limitation of this method. PCR negative samples were found to be positive by culture methods. Deoxyribose nucleic acid PCR is not reliable for TB due to false positive or negative result. Thus, we suggest both culture and PCR as important diagnostic methods for detection of GTB.

KEY WORDS: Acid fast bacilli culture, acid fast staining, genital tuberculosis, polymerase chain reaction

INTRODUCTION

Tuberculosis (TB), once thought to be a disease of poor countries and nearly completely eradicated in the western world, has resurged world-wide and has become a global issue.[1] It is a leading cause of death and infects more than a third of the world's population,[2] the etiological agent being *Mycobacterium tuberculosis* (MTB). 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%).[3,4] It is almost always secondary to a tubercular lesion elsewhere in the body. The exact incidence of the disease remains unknown, as the majority of the cases remain undiagnosed due to asymptomatic presentation of genital TB and paucity of investigations.

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.[5,6] Although culture for *Mycobacterium* is more sensitive, it still needs 10-100 bacilli/ml of sample for the diagnostic yield and requires 2-4 weeks for the growth of *Mycobacterium.* A diagnostic method that is less time-consuming and at the same time has high sensitivity and specificity is therefore desirable.

Nucleic acid amplification (NAA) tests represent a major advance in the diagnosis of TB.[7] With the use of amplification systems,

nucleic acid sequences unique to MTB can be detected directly in clinical specimens, offering better accuracy than microscopy and greater speed than culture. Advanced molecular methods such as polymerase chain reaction (PCR), a type of NAA system, have shown very promising results for early and rapid diagnosis of the disease due to its detection limit of one to ten bacilli in various clinical samples.[8]

MATERIALS AND METHODS

Study design and settings

The present prospective study was conducted between December 2012 and May 2013 in the Department of Microbiology, of a teaching tertiary care hospital.

Sample size

A total of 227 endometrial tissue samples were collected from every women who presented with infertility was tested for genital tuberculosis (GTB).

Collection of samples

Tubal biopsy was taken at laprotomy and Endometrium tissue was obtain by curettage and collected in normal saline. All the samples were kept at 4°C before processing. Tissue were 3-4 mm long delicate. All samples were processed in the microbiology department.

Inclusion criteria

Endometrial samples of every woman who visited IVF center with complain of Infertility were included in this study.

Exclusion criteria

Clinical and Histopathological finding were excluded.

Methods

All the electron microscopy (EM) tissue samples were microscopically examined after performing the ZN staining of direct and concentrated smears (N-acetyl-L-cystine-NaoH concentration method) (LYFECTOL Tulip Diagnostics). The concentrated samples were simultaneously inoculated on Lowenstein-Jensen medium (Hi Media, India) and incubated at 37°C temperature in biochemical oxygen demand incubator under aerobic conditions.[9] The inoculated media were examined after 24 h, 48 h and then weekly for 8 weeks. Species identification of the positive culture samples were done by MPT64Ag card test (SD Bioline Standard Diagnostics Inc.).

The deoxyribose nucleic acid (DNA) extraction was carried out using Qiagen. QUamp DNA mini kit. All the EM tissue were homogenized in a pestle and mortar followed by centrifugation at 6,000 rpm for 10 min. The supernatant was discarded and 3 ml of tris-buffer was added to the pellet obtained. All steps of PCR were performed in separate rooms to minimize the chance of contamination.

The homogenized tissues were centrifuged again at 6,000 rpm for 10 min and to the resultant pellet, 250 µl of lysis buffer I and 20 µl of proteinase K was added. Then after mixing by vortexing, all the samples were kept in dry bath at 90°C for 20-25 min and then centrifuged at 10,000 rpm for 10 min. Into 200 µl of supernatant, 200 µl of lysis buffer II (containing internal control at the concentration of 10 µl/ml) was added in a 1.5 ml eppendorf tube and incubated at 70°C for 10 min. Next, 200 µl of 96-100% ethanol was added and mixed by vortexing. This mixture was added to a spin column placed in a 2-ml collection tube and centrifuged at 6000 rpm for 3 min. The spin column was kept in a new 2-ml collection tube and washed twice with wash buffer (provided in the kit) and final centrifugation was performed at 14,000 rpm for 2 min to ensure complete removal of the wash buffer. Then the spin columns were kept in a 1.5-ml tube and 100 µl of pre-warmed (50°C) elution buffer (provided in the kit) was added. After incubating at room temperature for 5 min, it was centrifuged at 10,000 rpm for 2 min to elute the DNA. The DNA samples were kept at −20°C until further analysis.[10]

Two-step nested PCR was performed by commercial kit method from applied Biosystem Bangalore (India) for IS6110 of MTB in PTC-Applied Biosystem 2720, Thermocycler Inc., USA.

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.[10] Each PCR run is controlled by adding negative (without templats) and positive controls (with templats) to maintain false positive and negative results.

RESULTS

Out of 227 patients suspected of suffering from GTB, 133 were found to be positive by either of the three diagnostic methods i.e., AFB smear microscopy, culture and PCR. Out of 133 positive samples, 2 (1.5%) samples were found to be positive by all the three methods, i.e. microscopy, culture and PCR, 11 (4.8%) were found to be positive by both PCR and culture. A total of 126 samples were found to be positive only by PCR, whereas seven samples were found to be positive only by culture method. None of the samples were found to be positive by microscopy alone. Two samples that were positive by microscopy were also positive by PCR. Out of 11 culture positive samples, nine were MTB and 2 samples were positive for *Mycobacterium*

Table 1: Percentage positivity of the various EM samples detected by various diagnostic methods

Total samples	Total positive samples	Microscopy+ Culture− PCR−	Microscopy− Culture+ PCR−	Microscopy− Culture− PCR+	Microscopy+ Culture+ PCR−	Microscopy+ Culture− PCR−	Microscopy+ Culture− PCR+	Micro scopy+ Culture+ PCR+
227	133 (58.5%)	0 (0%)	07 (5.2%)	115 (86.4%)	0 (0%)	0 (0%)	11 (8.2%)	2 (1.5%)

EM=Electron microscopy, PCR=Polymerase chain reaction

other than tuberculosis (MOTT). PCR positive samples were all detected as MTB. Overall positive predictive value of PCR was 126 (86%) and culture was 18 (12%) [Table 1].

DISCUSSION

The genitourinary tract is the second most common site for tuberculous infection after the lungs. GTB is usually secondary to renal tuberculous infection.[11]

In communities where TB is still a major health problem, it is important to anticipate the possibility of GTB in patients presenting with infertility.[12] Most of time it is undiagnosed due to lack of awareness and lack of diagnostic modalities which are prone to false positive as well as false negative results.

Histopathological diagnosis of TB is not specific for TB as it can be present in the variety of other conditions such as sarcoidosis, syphilis, leprosy, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus and pneumoconiosis.[13] Therefore, to confirm the diagnosis of TB, either acid-fast staining or culture of tissues *Mycobacterium* must be performed. Both of these tests have poor sensitivity because of paucibacillary tissue samples. Furthermore culture is a very laborious and time consuming procedure. Recent molecular techniques such as PCR have high sensitivity for diagnosis of TB.[14,15]

In this prospective study, we have compared the performance of various diagnostic methods for GTB. PCR showed the highest sensitivity when compared with other methods. With the use of PCR test, we were able to detect MTB in 115 cases which were negative by culture method. PCR test detected MTB with in 24 h, compared with average 24 days required for detection by conventional method, as supported by earlier studies.[16]

In our study, PCR has shown 82% sensitivity with the sequence (IS6110) ranging from endometrium tissue which co-relate well with the study done by Cheng *et al*. and Chakravorty *et al*.[5,17]

The present study has shown good results of PCR. We noticed that PCR produced true positive results when performed in careful, clean and uncontaminated condition. On the other hand if PCR shows negative results when TB is suspected, PCR results should be co-related with culture reports. In our study, PCR failed to detect MTB in seven samples, which were found positive in culture. Similar observations were made by Rozati *et al*.[18] It may be possible that portion of specimen processed by PCR lacked AFB. The possible explanation for these false negative results of PCR could be the presence of PCR inhibitors or blood[18] in the specimen or it may be possible that the mycobacterial DNA amplification may be compromised if human bacterial genome ratio is at least 190:1 (Restrepo *et al*.)[19] PCR result may be negative as it was not able to detect the *Mycobacterium* species whereas we differentiated between MTB and MOTT by culture method. Out of 11 culture positive samples nine were MTB and two were MOTT. The two MOTT culture positive samples were found to be negative by PCR as our PCR test detected only MTB. PCR also has some limitations as it fail to distinguish between live and dead bacilli. This sophisticated technique is also limited by the need for a suitable infrastructure and high cost of the test. To conclude, molecular diagnosis of TB by PCR has a great potential to improve the ability of diagnosis of GTB. PCR is a rapid, sensitive and specific test that can be used for early diagnosis of GTB. Though culture is a time consuming method, early PCR can enable the consultant to diagnose GTB and start treatment. But in this study PCR missed seven cases of GTB. Hence when the clinical suspicious is high and smear result is negative and get the sign and symptoms of Mycobacterium are apparent, PCR is use for identify the infection because other methods are time consuming. However as there is no gold standard methods in diagnosis of GTB and to compare PCR.

However, we propose both culture and PCR for diagnosis of GTB to avoid any kind of false negative result occurred during different steps of diagnosis.

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