Original Research Article

Genital Tuberculosis: Evaluating Microscopy, Culture, Histopathology and PCR for Diagnosis “All Play Their Role”


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ABSTRACT

Genital Tuberculosis (GTB) is a major socioeconomic burden in India, afflicting 14 million people, mostly in the reproductive age group. It affects 5–16% of cases of infertility among Indian women. Diagnosis remains difficult due to asymptomatic nature or a variable clinical presentation. This study was done to compare Microscopy, Culture, Histopathology and PCR technique in the diagnosis of GTB in female infertility. A total of 225 endometrial samples were collected and 218 samples were processed for Acid Fast Bacilli (AFB) smear microscopy by Ziehl Neelsen (ZN) staining, AFB culture on Lowenstein Jensen media, Histopathology & Polymerase Chain Reaction (PCR). Out of these, AFB smear microscopy was positive in one (0.45%) sample, culture was positive in 12(5.5%) samples, histopathology was evident of tuberculosis in 3(1.37%), while PCR gave positive result in 84(38.5%) samples. Two culture positive cases revealed negative PCR results. This study revealed that although Positivity rate of Conventional method of AFB smear, culture and histopathology was very less as compared to PCR, it cannot be used as the only test for diagnosis due to its false positive & false negative results. Thus a combination of AFB smear microscopy, Culture, histopathology & PCR should

Keywords
Genital Tuberculosis; AFB; LJ Media; histopathology; PCR.

Introduction

The global prevalence of genital tuberculosis (GTB) is estimated to be 8-10 million cases annually. Reportedly about nine per cent of all extra pulmonary tuberculosis cases are genital tract TB. It is the root cause in 5–16% of cases of
infertility among Indian women. (Bose M, 2011; Malhotra et al., 2012). Genital tract TB is a chronic disease that often presents with low grade symptomatology and very few specific complaints. Presenting symptoms are generally varied; infertility being the most frequent clinical presentation (43-74%). Other clinical presentations include oligomenorrheoa (54%), amenorrhoea (14%), menorrhagia (19%), abdominal pain (42.5%), dyspareunia (5-12%) and dysmenorrheoa (12-30%). (Bose M, 2011) The tubercle bacilli reach the genital tract mainly by haematogenous spread from foci outside the genitalia. Haematogenous spread of TB bacilli to the fallopian tubes results in involvement of the sub mucosa (endosalpingitis) at the outer ends with gradual spread medially to the endometrium. Direct spread of infection to the fallopian tubes results in exopsalpingitis with tubercle on the surface. The fallopian tube is the initial site of involvement, affected in almost all cases, followed by endometrium in 50-90 per cent of cases (Thangappah et al.2011). Diagnostic dilemma arises because of the varied clinical presentation of the disease, confounded by diverse results on imaging, laparoscopy, histopathology and bacteriological tests, each of which has its limitation in diagnostic sensitivity and specificity. Any method that is used to diagnose GTB should be highly sensitive to diagnose the disease reliably in its early stage, so that treatment may improve the prospects of cure before the tubes are damaged beyond recovery ( Bhanu et al.,2005). A definite diagnosis can be made by positive mycobacterial culture which still is the gold standard test but as GTB is bacteriologically mute, newer methods like PCR aids in diagnosis. The objective of this study was to compare smear, culture, histopathology and PCR technique, in the diagnosis of GTB in female infertility.

Materials and Methods

The present prospective study was carried out in the Departments of Microbiology, Histopathology and Central Research Laboratory of a tertiary care hospital located in Central India. A total of 225 endometrial samples were collected from February 2013 to July 2013 from patients with provisional clinical diagnosis of infertility made in the InVitroFertility (IVF) centre of our institute. All samples received in the laboratory were processed for Acid Fast Bacilli (AFB) smear microscopy by Ziehl Neelsen (ZN staining), AFB culture on Lowenstein Jensen media, histopathology and Polymerase Chain Reaction (PCR).

The samples were processed for microscopy and culture for which Endometrial tissue biopsy were received in a sterile container and was crushed as finely as possible with a homogenizer for 1 min. and a direct smear was prepared from it. The grinded tissue was digested and decontaminated by Lyfectol kit (Tulip Diagnostics). Both the direct and concentrated smears were stained by ZN staining method. The concentrated samples were also inoculated on LJ media. The LJ media were incubated at 37°C under aerobic conditions and monitored for eight weeks regularly. (Bailey and Scott’s 9th ed.) The Mycobacterial species were identified in positive cultures by biochemical test and MPT64Ag card test (SD Bioline Standard Diagnostics Inc ).

Further for histopathological studies, a portion of the endometrial tissue was fixed in 10 per cent formalin; routine processing
was done and stained with haemotoxylin and eosin. Presence of caseating granulomas surrounded by epitheloid cells, lymphocytes, plasma cells and giant cells were diagnostic of tuberculosis (Thangappah et al., 2011).

The endometrial samples were also processed for PCR. Deoxyribose Nucleic Acid (DNA) extraction was done using Qiagen. QUamp DNA mini kit. Nested PCR against the most conserved region insertion sequence IS6110 of M. tuberculosis was performed by a commercial kit method from Appliae Biosystem, Bangalore (India) in PTC – Applied Biosystem 2720, Thermocycler Inc., USA. Following amplification, gel electrophoresis of the products was done using 1.5% agarose gel and the bands were observed under ultraviolet light. The PCR product giving a clear band at 123bp base pair indicated positivity of the sample. (Chawla et al., 2009)

Results and Discussion

Out of the 225 endometrium samples considered for study, 218 samples were further tested (seven samples were rejected being inappropriate or insufficient) by microscopy, culture, histopathology and PCR methods. Among these, 86 (39.45%) samples were found to be positive by at least one of the test methods and only one (0.45%) sample was found to be positive by all the four methods.

Out of 218 samples tested, only one (0.45%) sample was positive by microscopy, 12(5.5%) samples were positive by culture only, three (1.3%) samples were positive by histopathology alone and 84(38.5%) samples were positive by only PCR (Figure 1). Out of total positive 86 samples, 1.16% (1/86) were positive by smear microscopy, 13.9% (12/86) were positive by Culture, 3.48% (3/86) were positive by histopathology and, 97.6% (84/86) were positive by PCR. Among the 12 culture positive samples, only one sample was positive by microscopy, two were positive by histopathology and ten were positive by PCR also. Out of the three histopathology positive samples, only one sample was positive by microscopy, two were culture positive and all three were positive by PCR also. Out of the 84 PCR positive samples, only one sample was positive by microscopy, ten (4.5%) were culture positive while three (1.3%) were positive by histopathology method also. [Table 1]

Among culture negative 206 samples all were negative by smear microscopy, histopathology was positive in one sample and PCR yielded positive result in 74 samples. Thus among culture positive samples PCR was not able to detect Mycobacterium in 2 cases while it additionally detected positivity in 74 culture negative samples.

As culture is considered the gold standard, the comparative evaluation of the various methods with respect to culture has been done in Table 2.

Genitourinary tract is the second most common site for Mycobacterium tuberculosis infection after lungs. Such infections usually occur secondary to renal tuberculosis. (Ron et al., 1996) In communities where TB is still a major health problem, the possibilities of GTB in patients presenting with infertility cannot be ruled out( Luqman et al., 1997).

Demonstration of Mycobacteria on smear and culture, used as a gold standard, is the most specific test for diagnosis of TB. Although mycobacterial demonstration in
examination of sputum is the mainstay of the diagnosis of pulmonary TB where there is a high bacterial burden, the very low sensitivity for diagnosis of paucibacillary disease limits its practical utility in extra-pulmonary TB (Jassawalla, 2006).

Microscopy for AFB can provide a quick diagnosis of poor sensitivity while conventional bacteriology for isolation and identification of Mycobacteria has its specific advantages of being a conclusive diagnostic test. This study reveals that overall detection rate of smear microscopy was low as 0.45% which was similar to the finding of other studies i.e. 0.4%, 1.23%, 1.6% and 2.7% reported by Agrawal et al. (1993), Misra et al. (1996), Bhanu et al. (2005) and Malhotra et al. (2012) respectively.

Culture positivity was found to be 5.5% in this study, which was in agreement with 5.2% reported by Thangappah et al. (2011) 3.2% reported by Bhanu et al.(2005), 8.28% by Malhotra et al. (2012) and other studies (3.3% - 10.6%) (Bose M, 2011). This difference could be attributed to the use of liquid culture medium which increases the positivity rate. The radiometric culture BACTEC has a sensitivity of 80- 90% whereas the LJ medium has a sensitivity of only 30- 35%. (Malhotra et al., 2012) This high sensitivity is particularly useful in cases of genital TB as traditional methods show poor recovery of AFB.

Three samples (1.37%) were found to be positive by histopathology in our case, out of which one sample was found to be culture negative. Histopathological examination is easy, quick and cheap and provides characteristic features of M. tuberculosis. But due to the secondary nature of the genital tuberculosis, the infecting organisms are sparse in number, the sampled site may not represent the infected area and the infected site can be easily missed. Histology demonstrates the typical caseous granulomatous lesions with giant epitheloid cells. Such lesions are highly suggestive of TB but are not diagnostic, as these also appear in fungal infections, syphilis, leprosy, rheumatoid arthritis, systemic lupus erythematosus, pneumoconiosis and sarcoidosis (Misra et al., 1996). Chhabra et al.(1986) suggested that histology and bacteriology both are complementary and neither is completely dependable.

PCR was found to be positive in 38.5% of the samples consistent with the studies done by Malhotra et al.(2012) who reported 23.78% positivity, Thangappah et al.(2011) who reported 36.7% positivity, but still higher positivity rate of 56% was seen in studies by Bhanu et al. (2005).

PCR was found to be seven times more sensitive than culture in the present study. However Malhotra et al.(2012) reported nine times more sensitivity, and Bhanu et al. (2005) reported PCR to be 14 times more sensitive than culture. This difference could be attributed to use of real time PCR by Malhotra et al. (2012) and use of mpt64 based PCR by Bhanu et al.(2005). Use of multiplex PCR with genus specific and MTb primers, detection of mRNA instead of DNA, can further help in proper diagnosis of PCR (Chhabra et al., 1986).

However, there were two samples which were culture positive but PCR negative. Similar observation was also made by Rozati et al.(2006). This could be due to contamination of the sample with heparin which is a known PCR inhibitor, absence of even a single AFB in the sample
Table 1 Results of samples by various detection methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Total: 218 (Positive: 84 i.e 38.5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td></td>
</tr>
<tr>
<td>Culture+PCR</td>
<td>10</td>
</tr>
<tr>
<td>Culture+Histo-Pathology+PCR</td>
<td>02</td>
</tr>
<tr>
<td>Culture+PCR</td>
<td>03</td>
</tr>
<tr>
<td>Culture+Histo-Pathology+PCR</td>
<td>03</td>
</tr>
<tr>
<td>Microscopy+Culture+Histo-Pathology+PCR</td>
<td>01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample size</th>
<th>Culture positive (12)</th>
<th>Culture negative (206)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear microscopy</td>
<td>(218)</td>
<td>1 (0.45%)</td>
<td>0</td>
</tr>
<tr>
<td>Positive (01)</td>
<td></td>
<td>1 (0.45%)</td>
<td>0</td>
</tr>
<tr>
<td>Negative (217)</td>
<td></td>
<td>11 (5.04%)</td>
<td>206 (94.4%)</td>
</tr>
<tr>
<td>Histopathology</td>
<td>(218)</td>
<td>2 (0.92%)</td>
<td>1 (0.45%)</td>
</tr>
<tr>
<td>Positive (03)</td>
<td></td>
<td>2 (0.92%)</td>
<td>1 (0.45%)</td>
</tr>
<tr>
<td>Negative (215)</td>
<td></td>
<td>10 (4.5%)</td>
<td>205 (94.03%)</td>
</tr>
<tr>
<td>PCR(218)</td>
<td></td>
<td>10 (4.5%)</td>
<td>74 (33.94%)</td>
</tr>
<tr>
<td>Positive (84)</td>
<td></td>
<td>10 (4.5%)</td>
<td>74 (33.94%)</td>
</tr>
<tr>
<td>Negative (314)</td>
<td></td>
<td>2 (0.92%)</td>
<td>132 (60.55%)</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the methods used for detection of Mycobacterium

Figure 1 Pie Diagram showing percentage positivity by various methods
collected, presence of species of Mycobacterium other than tuberculosis and high salt concentration of a specimen which interferes with the PCR results. It is also shown that mycobacterial DNA amplification was compromised when the human: bacterial genome ratio was at least 190:1. As endometrial samples are always mixed with blood, this could possibly explain the false negative results in this study. A negative PCR may result in missing the diagnosis in a few cases. This sophisticated technique is also limited by the need for a suitable infrastructure and high cost of the test. Though PCR is a very rapid and sensitive test for diagnosis of GTB in clinically relevant time but the false negative result is an important limitation in this study. Also PCR cannot distinguish between live and dead bacilli and there is a small risk of false positive results. (Kaul, 2001; Soini et al., 2001; Park, 2003). To conclude, rapid, sensitive and specific molecular diagnostic modalities like PCR play an important role in diagnosis of GTB. Therefore, when GTB is suspected clinically, but the PCR results are negative, it indicates the need for further evaluation using other diagnostic tests and repeat testing to confirm/exclude diagnosis and it is at this step at which culture histopathology plays an important role.

Further there were few limitations of the present study which were use of solid culture media which has less positivity as compared to liquid media. Detailed clinical profile of the patient were not evaluated and Laproscopic correlation if done, would have helped for specific diagnosis.

Acknowledgment

Author like to thank the Management and Dean of SAIMS & PG Institute to provide an opportunity and laboratory facilities for carrying out the present study.

References


