Comparative Study of Polymerase Chain Reaction of Endometrial Aspirate/ Biopsy and Menstrual Blood in Suspected Cases of Genital Tuberculosis in Females

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Abstract: Female genital tuberculosis is an important cause of infertility in developing countries like India. Due to paucibacillary nature of genital tuberculosis, the routine diagnostic modalities do not remain conclusive; need much sensitive procedure like PCR for diagnosis. Therefore, we have planned to investigate the role of PCR in various genital specimens like endometrial aspirate (EA), endometrial biopsy (EB), and menstrual blood (MB). Total 200 patients with complaint of infertility and menstrual irregularities were enrolled. All the specimens were collected as per standard protocol and processed accordingly. Among 200 patients we were able to get all type of genital specimens (EA, EB, MB) in only 138 patients. Overall PCR positivity was 83 out of 138 patients (60%). On comparison among specimens, only MB was positive in 40/138 (29%), only EA was positive in 21/138 (15%), only EB was positive in nil patient. While both MB and EA was positive in 10/138 (7%), both EA and EB was positive in 9/138 (6.5%). All the three specimens were positive in only 3/138 (2%) cases. In the present study, PCR for tuberculosis showed a very high sensitivity to detect genital tuberculosis. Tissues from MB yielded maximum positive cases by PCR. The overall positivity of EA by PCR was nearly equivalent to MB PCR; however the positivity of EB by PCR was very less. The difference was statistically significant. Moreover, on combining the samples (EA+MB or EA+EB), the sensitivity of PCR has increased. Our study showed very low positivity by AFB (8%) and liquid culture (7%). Moreover many authors have concluded that a non invasive sample (MB) of first 12 hour may be good alternative to invasive EA or EB samples for diagnosis of genital tuberculosis by PCR.

Keywords: Genital tuberculosis, pcr, menstrual blood, endometrial aspirate, endometrial biopsy

I. Introduction

World health organization has estimated that nearly one-third of the world population is infected with Mycobacterium tuberculosis (MTB), out of them about 10% are known to progress to clinical disease [1]. Depending upon the localization of the MTB in an organ, a wide spectrum of tubercular disease is encountered in clinical practice, out of which female genital tuberculosis (GTB) is an important manifestation [2]. It is one of the commonest causes of infertility in developing countries [3]. Every patient consulting for infertility in developing countries should therefore be investigated for female genital tract tuberculosis (FGT). It is also a cause of sequelae including chronic pelvic pain, ectopic pregnancy and pelvic adhesions. Patient may present with infertility, menstrual irregularities like oligomenorrhoea or post menopausal bleeding. Genital tuberculosis represents 15–20% of extra pulmonary tuberculosis and remains to be a major public health problem in developing countries [4, 5]. Worldwide 10-85% females presenting with infertility have genital tuberculosis [6]. The fallopian tubes followed by endometrium are primarily involved in genital tuberculosis. [7].

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. Currently, the diagnosis of genital TB relies on clinical features, imaging findings and conventional microbiological tools like microscopy and culture. Microscopy is quite insensitive especially because of the pauci-bacillary nature of genital TB. Although culture is a gold standard, it has also a slow turnaround time of 6-8 weeks and sensitivity is also less. Thus, there is a need for rapid, sensitive and specific tests for the diagnosis of genital TB. Nucleic acid based tests have proven beneficial in the diagnosis of TB. The polymerase chain reaction is the most common nucleic acid amplification test among all. [8]. Therefore the present study is planned to investigate the role of PCR to diagnose the genital tuberculosis correctly on the basis of clinical suspicion in comparison to culture and microscopy.
Moreover previous studies have taken various samples like endometrial aspirate, endometrial biopsy and menstrual blood separately for diagnosing genital tuberculosis by PCR. In the present study we planned to analyze the efficacy of all the three types of samples (endometrial aspirate, endometrial biopsy and menstrual blood) simultaneously in every patient and to access the importance various specimens in the diagnosis of genital tuberculosis.

1) Material and methods

The study was conducted in department of microbiology in association with department of Obstetrics & gynecology, SN Medical college, Agra and department of microbiology, NIMS, Jaipur. Total 200 infertile women were enrolled in the study. Ethical committee approval was taken from institutional ethical committee and informed consent was taken from each and every patient. Women who presented with tubal factor infertility proved either by hysterosalpingogram (HSG) and /or laparoscopy, presence of adnexal mass diagnosed by ultrasound, cases presenting with recurrent pelvic inflammatory disease refractory to conventional therapy and those presenting with unexplained infertility were included in the study. Those cases in which infertility was due to abnormalities of ovulation, male factors, endocrine problems, sexual disorders, endometriosis and peritoneal adhesions due to previous abdominal surgery were excluded. After a detailed history and thorough clinical examination, all patients were subjected to investigations such as haemoglobin, total count (TC), differential count (DC), ESR, tuberculin test, chest X-ray, HIV I and II and abdomen and pelvic sonogram.

The material for the study was collected from pre-menstrual endometrium. Two types of material was tried to collect from each patient. Endometrial biopsy was collected as tissue sample taken from the cornal area of uterine endometrium. Endometrial aspiration was taken as extraction of tissue from uterine lining by suction following instillation of saline in the endometrial cavity as per standard protocol following universal precaution. For menstrual blood, patient was given a universal sterile container and instructed to collect the menstrual blood of initial 12 hours.

All the samples were transported to department of microbiology, SN Medical College, Agra for further processing. Endometrial aspirate, endometrial biopsy and tissues from menstrual blood were processed as according to Bhanothu V et al [6]. The sediment was decontaminated as per standard protocol by Shrinivas et al [9]. The concentrated sediment was divided in to two portions: one for ZN staining and culture and second for DNA extraction.

Culture is done in Mycobacterium growth inhibitor tube liquid culture medium and growth index is noted by micro MGIT machine as per standard protocol and identified further as per standard protocol. DNA extraction was done as per standard protocol. Polymerase chain reaction was done as per protocol followed by Thangappah RBP et al [10]. DNAs from the samples were amplified using the following primers IS6110a (5’ – CCT GCG AGC GTA GGC GTC GG – 3’) and IS6110b (5’ – CTC GTC CAG CGC CGC TTC GG – 3’). The IS6110 primers amplify a fragment with a length of 123bp. The cycling parameter used was initial denaturation at 95°C for 5 min, followed by denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 30 sec with 25 cycles and a final extension at 72°C for 5 min.

2) Results

In our study, 200 patients with complaints of infertility or menstrual irregularities with high suspicion of genital tuberculosis were included. The patients were aged between 25 to 36 years. Patients having past history of tuberculosis were excluded from the study. We took endometrial aspirate, endometrial biopsy and menstrual blood of first 12 hours from most of the patients. The duration of infertility was longer than 3 years in most cases. In 138 patients we were able to take out all the three samples. Therefore we considered only those patients in our study. We found only 11/138 (8%) positive AFB cases in our study. Liquid culture by the MGIT method showed growth in only 10/138 (7%) specimens. All were Mycobacterium tuberculosis. Two ZN smear positive specimen did not give positive culture results and one smear negative case was culture positive. Overall PCR positivity was 83 out of 138 patients (60%). We evaluated all the three samples for PCR in every patient. Out of these specimens, only menstrual blood was positive in 40/138 (29%), only endometrial aspirate was positive in 21/138 (15%), both menstrual blood and endometrial aspirate was positive in 10/138 (7%) both endometrial aspirate and endometrial biopsy was positive in 9/138 (6.5%) cases. All three samples were positive only in 3/138(2.1%) cases. We observed mauntox positive in 101 cases and mauntox negative in 37 cases.

Anti-tubercular therapy was initiated for all PCR positive women, however we did not incorporated follow up of these patient in our present study.

3) Discussion

Infertility is a health problem with very definite physiological, psychological and social implications; many times it is the complication of genital tuberculosis. Although the reported incidence of genital tuberculosis
varies worldwide; high incidence has been reported from many studies in India. Moreover, a large proportion of cases go unreported due to lack of sensitive and specific investigation [11].

Female genital tuberculosis usually affects women between the ages of 20 and 40 years and is secondary to pulmonary tuberculosis or other site [12]. In the present study, the patients were aged between 21 and 36 years. Oligomenorrhea was the common menstrual abnormality. This was in agreement with Thangappah RBP et al findings [10]. Most of the patients presented with infertility as the only complaint during recruitment in our study. In the present study tuberculosis test was positive in 101 cases however only 56% (56/101) have shown positive for PCR. Out of 37 tuberculin test negative cases 16 (44%) comes out to be positive. We therefore do not recommend tuberculin test as diagnostic test for genital TB.

In the present study, PCR showed the highest sensitivity to detect genital tuberculosis, when compared with the other methods. With the use of PCR test, we were able to detect MTB within 24 hrs compared with average 24 days required for detection by conventional method [13]. We took endometrial aspirate, endometrial biopsy tissue and menstrual blood and compared the PCR findings. Tissues from menstrual blood yield maximum positive PCR. Out of total 83 positive PCR, menstrual blood tissue yielded positive PCR in 53(64%) cases, tissue from endometrial aspirate yielded positive PCR in 43(52%)cases and endometrial biopsy tissue yielded positive PCR in only12(14.5%). The possible reason for high yield in menstrual blood in the present study that we took first 12 hours menstrual blood in which the whole endometrium is sloughed out, therefore we got good endometrial tissue in menstrual blood. The possible reason for very low yield in endometrial biopsy may be due to that we have failed to take the tissue from the right place means diseased endometrium.

The difference in the yield of MTB between menstrual blood/ endometrial aspirate and endometrial biopsy was statistically significant. Our study also showed that yield of MTB in endometrial aspirate was less than menstrual blood; however it was not statistically significant. Moreover, menstrual blood is non-invasive specimen. Therefore the present study greatly emphasized the importance of non-invasive sample first 12 hours menstrual blood for the diagnosis of MTB. However, one study from AIIMS New Delhi by Bhanu NV et al showed PCR positivity 53.3% in the endometrial biopsy and 47.6% in endometrial aspirate (14). In the present study, the sensitivity of PCR has increased to 60%, when two samples EA&MB were taken together. This is in concordance with Bhanu NV et al [14] in which the sensitivity of PCR became reasonably good when both the EA and EB sample was taken. Jindal et al had concluded very precisely in his study that detection of TB DNA by PCR in endometrium may even detect sub clinical disease and that sub clinical disease leads to infertility, which can be reversed by the institution of appropriate ATT before any damage to pelvic organs. Therefore endometrial TB PCR in infertile women could be reliably employed to diagnose endometrial TB and therefore more invasive procedure like Laparoscopy could be avoided [15]. Kulshrestha et al had also concluded that a good percentage of women with genital tuberculosis and infertility conceived after treatment with ATT solely on the basis of the PCR results [16].

Many people demonstrated the contamination of PCR leading to false positive results [17]. However, if the results of PCR are interpreted in the presence of pre test probability for TB, the chances of false positive will be less. In the present study we recruited the patients according to predefined inclusion and exclusion criteria. In our study only 8 percent sample revealed AFB on smear examination. This is in correlation with previous study by Thangappah et al 2011 who founded only 8.3 percent AFB positivity in female genital tuberculosis [10]. In another study, Namavar et al reported 12.9 percent AFB positivity in tissue biopsies [18]. We know the importance of culture in the diagnosis of MTB and consider it as gold standard. However we were able to grow MTB in only 7 percent samples in liquid culture. Similar detection rates have been reported earlier [10, 15, 19]. Bhanu NV et al concluded the possible reasons for low positivity of liquid culture in endometrial tissues could be due to paucibacillary nature and a substantial number of TB cases of the genital tract are bacteriologically mute.

In conclusion, the present study demonstrates the importance of PCR in the diagnosis of genital tuberculosis. Moreover, menstrual blood being a non-invasive specimen alone can be a specimen of choice for the diagnosis of genital tuberculosis by the TB DNA PCR.

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References


DOI: 10.9790/0853-15111015154 www.iosrjournals.org 53 | Page


DOI: 10.9790/0853-1511015154 www.iosrjournals.org 54 | Page