Diagnostic Value of PCR in Genitourinary Tuberculosis

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Abstract Genitourinary tuberculosis is a disease of the genitourinary system which includes the entire urinary tract and reproductive system. Genital tuberculosis is an important cause of female infertility, especially in developing nations like India. In the present study, a total of 257 clinical specimens comprising of endometrial biopsy (109), endometrial curetting (42), menstrual blood (8), semen (17), placenta (11) and urine (70) were collected from patients and subjected for PCR, Culture and AFB detection. The endometrial biopsy, endometrial curetting, menstrual blood, semen, placenta, urine showed 30.2, 45.2, 12.5, 5.8, 27.2, 31.4 %, positivity rate for tuberculosis by PCR, 7.3, 9.5, 25.0, 0, 9, 8.5 % by culture and 1.8, 2.3, 0, 0, 0, 2.8 % respectively by AFB smear. Being a novel, rapid technique, PCR is the method of choice for rapid diagnosis and management of genitourinary tuberculosis shared with the other concerned tests. This study reveals that genital tuberculosis can occur in any age group, however, the majority of

patients were from reproductive age (nearly 75 % of them were from 20–45 years of age) group.

Keywords Polymerase chain reaction · MPB64 · Bactec 460 · Genitourinary tuberculosis

Introduction

Tuberculosis is the major public health problem across the globe [1]. More than 90 % of patients are infected in the developing nations like India (1.6–2.4 million), followed by China (1.0–1.6 million) and South Africa (0.380–57 million). 75 % of the infected individuals are in the age group of 20–45 years and economically productive, 1.4 million (15 %) of whom are HIV-positive [2]. In Indian subcontinent, genital tuberculosis is the cause of tubal damage in nearly 40 % of women experiencing tubal infertility [3]. One of eight women having pulmonary tuberculosis may also have genital tuberculosis according to studies from India and Turkey [4]. Genitourinary tuberculosis (GUTB) is caused by *Mycobacterium tuberculosis* and is almost always the result of a haematogenous spread from pulmonary to abdominal infection. There are basically three principal routes used by bacilli to reach the genital tract, the haematogenous route, descending direct spread or by lymphatic spread [4, 5]. Male genital tuberculosis, however, has not received much attention in infertility and diagnostic clinics. Infection may occur from direct sexual intercourse with infected individuals with genitourinary tuberculosis. It is very important to control this infection, since high number of patients are responsible for the spread of this disease. The most important part of the treatment is the proper and the rapid detection of the disease. Although the AFB microscopy and the conventional culture methods like

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Lowenstein Jensen (L-J) culture are available, they take 2–8 weeks for the confirmation of the result. This increases the chance of the development of the multiple drug resistance, and/or extensively drug resistance tuberculosis [6]. The present study was planned to diagnose GUTB through PCR technology and an attempt was made to compare the culture and AFB microscopy with PCR for better diagnostic efficacy and management in local population.

Materials and Methods

Two hundred and fifty-seven specimens were collected from Auroprobe Laboratories and suspected patients attending OPD and IPD of SMI Hospital Dehradun, out of which 25 were males and 215 were females. The age of these participants ranged from 20 to 45 years. The specimens included endometrial biopsy (109), endometrial curetting (42), menstrual blood (08), semen (17), placenta (11), and urine (70) respectively. This study was approved by Institutional Ethical Committee and written consent was obtained from each patient for the present study.

Sample Processing (Decontamination and Concentration of Specimens)

Decontamination of the clinical specimens were carried out to remove other bacterial flora, fungal and related contaminations which were likely to be present in all the specimens using *N*-Acetyl-L-Cysteine (NALC)/NaOH method (modified petroffe's method) [7]. Decontamination solution was prepared by adding 0.5 % NALC (w/v; Sigma) to a 1:1 mixture of 4 % NaOH and 2.9 % sodium citrate. Tissue specimens were grinded in a screw cap tube by adding silica beads in a tissue grinder. The specimens were centrifuged in a 15 or 50 ml sterile centrifuge tubes at 5,000 rpm for 20 min. Supernatant was discarded gently and the sediment was taken for PCR as well as for AFB smear preparation. 200 µl of the sediment was taken for PCR. In the remaining sediment equal volume of decontamination solution was added and was incubated at room temperature for 20 min for TB culture [8]. The samples were neutralized with sterile phosphate buffer (0.067 M, pH 6.8) and then centrifuged at 5,000 rpm for 20 min. Supernatant was discarded to get the sediment up to 2 ml and mixed with 500 µl of sterile phosphate buffer. Decontaminated specimen was used for culture (Bactec 460 TB System) and AFB smears preparation [8]. DNA was extracted with AuPrep DNA extraction kit by silica column based method [9]. PCR was performed for the amplification of MPB64 gene in *Mycobacterium tuberculosis* complex using primers (Sigma Aldrich) (forward primer 5'-TCCGCTGCCAGTCGTCTTCC and reverse

primer 5'-GTCCTCGCGAGTCTAGGCCA) [10]. A reaction mixture of 50 µl containing 10× PCR buffer (250 mM Tris HCl, 500 mM KCl), 0.2 mM dNTPS, 25 µM primers, Taq DNA polymerase (3 units/microlitre) and Mg²⁺ ions (25 mM) and milliQ was used. DNA template was added in the master mix and amplification was done on thermal cycler with initial denaturation at 95 °C for 6 min, denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and primer extension at 72 °C for 30 s with 35 rounds of repetitive cycles for denaturation, annealing, extension with final extension at 72 °C for 7 min. A 240 bp amplicon was observed for MPB64 gene on 2 % agarose gel [11].

Results

High percentage of positivity rate for tuberculosis by PCR for the different specimens in comparison to Bactec 460 TB culture system and AFB smear was observed in the present study. Our findings showed that PCR positivity rate was 30.7 %, followed by culture 8.0 % and AFB smear 1.9 % respectively (Table 1). An amplicon of size 240 base pair for MPB64 gene was used as a target (Fig. 1).

Discussion

Genital tuberculosis in females manifests a wide spectrum of clinical presentation and involves all age groups and socio-economic classes. However, it continues to be a diagnostic dilemma and is associated with injudicious and overuse of anti-tuberculosis treatment (ATT) leading to the development of drug resistance [12–14]. Although genital tuberculosis can occur in any age group, the majority of patients considered were in reproductive age nearly i. e., 75 % being in 20–45 years of age [15]. In the present

Table 1 Depicting specimen type and positivity rate by PCR, Bactec 460 TB culture and AFB smear

Clinical specimen types	Techniques employed		
	Positive by PCR	Positive by culture (Bactec 460 TB culture)	Positive by API smear
Endometrial biopsy (109)	33 (33.2 %)	08 (73 %)	02 (1.8 %)
Endometrial curetting (42)	19 (45.2 %)	04 (9.5 %)	01 (2.3 %)
Menstrual blood (08)	01 (12.5 %)	0 (0 %)	0 (0 %)
Semen (17)	01 (5.8 %)	0 (0 %)	0 (0 %)
Placenta (11)	03 (27.2 %)	01 (9.0 %)	0 (0 %)
Urine (70)	22 (31.4 %)	06 (8.5 %)	02 (2.8 %)
Total clinical specimen (257)	79 (30.7 %)	21 (8.1 %)	05 (1.9 %)

Fig. 1 Gel picture showing 240 bp amplified product of MPB64 gene

study endometrial biopsies and endometrial curetting showed 30.2 and 45.2 % positivity rate for tuberculosis by PCR. Primary infertility, secondary infertility, ectopic pregnancies were clinical manifestations with these patients. Even though diagnostic facilities are available, diagnosis of genital tuberculosis still remains difficult since, histology may not be conclusive in some individuals and culture needs a long time [16]. AFB staining is not sensitive enough and both culdoscopy and laparoscopy carry a significant risk of bowel injury. Chest X-ray is less effective for genital tuberculosis [17]. The tuberculin skin test is not specific and may be affected by various systemic illnesses and immune-suppressions [18]. WHO has banned serology, ELISA and currently available commercial blood tests for detecting TB because of their inaccurate findings which adversely affect patient safety, claiming that these tests are unreliable and could lead to incorrect diagnoses, putting millions of lives at risk [19]. The present observations could further confirm that PCR is the method of choice for rapid diagnosis of genitourinary tuberculosis. Confirmed diagnosis of genital tuberculosis is of great value before starting anti tuberculosis treatment (ATT). False diagnosis of genital tuberculosis may lead to failure of timely intervention, thus, further aggravating the morbidity in terms of reproductive health. Undiagnosed and untreated female genital tuberculosis may also lead to repeated IVF failures. Sometimes, on strong clinical suspicion but without confirmatory diagnosis, empirical ATT is started which puts the patients on high risk of ATT drug resistance and stigmatization. Also with the advent of Multi Drug Resistance, the duration of treatment required and response to the ATT in microbiological confirmed cases of genital tuberculosis needs to be periodically assessed with repeat tests. With PCR, the detection of TB bacilli can be done in 5–6 h as compared to culture which takes 3–8 weeks. Our observations are an addition and further confirmation of the already existing knowledge on

the use of PCR for the proper diagnosis of GUTB. This study exhibited that the majority of patients were from reproductive age group.

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Conflict of Interest None.

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