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ORIGINAL ARTICLE

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Evaluation of women with infertility and genital tuberculosis

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OBJECTIVE(S) : To study the genital tuberculosis by identifying mycobacterial DNA with polymerase chain reaction (PCR) and with different laboratory methods available for its diagnosis.

METHOD(S) : Biopsy or curettage samples from 65 women clinically suspected to have genital tuberculosis were investigated with smear microscopy, histopathology, culture, and PCR for the mycobacterium.

RESULTS : Of the 65 clinically suspected patients investigated, only eight were acid fast bacilli (AFB) smear positive, 12 were culture positive, 17 were histology positive, and 28 were positive by PCR.

CONCLUSION(S) : A combination of PCR with the other available technics is the best method of achieving sufficient sensitivity and specificity for the diagnosis of female genital tuberculosis.

Key words : infertility, acid fast bacilli, female genital tuberculosis, polymerase chain reaction

Introduction

Infertility is defined as the inability to conceive by at least one year of unprotected intercourse. Treatment may be started earlier in case of an obvious cause or advanced age of the couple. Genital tuberculosis is an important cause of subfertility, more so in endemic zones such as South India. Still, the true epidemiology of this disease remains unknown due to lack of highly sensitive and specific tests. Genital tuberculosis not only causes tubal obstruction and dysfunction but also impairs implantation due to endometrial involvement and ovulatory failure from ovarian involvement [1]. Female genital tuberculosis (FGTB) is still a major cause of infertility in South India in spite of the availability of specific therapy. The prevalence of FGTB in infertility clinics shows marked variations in different countries ranging between 15 and 25% [2]. In 80-90%of cases, FGTB affects young women between 18 and 38 years of age and is an important cause of infertility [2,3].

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It is often a secondary complication as a result of reactivation of a silent bacillemia, primarily from lungs, affecting most commonly the fallopian tubes (92-100%), ovaries (10-30%), cervix (5%), endometrium (50%) [2,4], and vagina and vulva (<1%), but in some instances also from kidney, intestines, etc [5]. However a few reports have found endometrium to be the most commonly involved site. Direct inoculation of tubercle bacilli can also take place over vulva or vagina during sexual intercourse with a partner suffering from tuberculous lesions of genitalia. Primary infection of the female genital organs is very rare [6]. Establishment of the true incidence and prevalence of FGTB is difficult because asymptomatic latent cases predominate over symptomatic ones [6,7].

Diagnosis is often limited to clinical suspicion in countries where facilities for mycobacterium culture and histopathology are not available [8]. Abdominal and vaginal examinations may be normal. A high erythrocyte sedimentation rate and a positive mantoux test are non-specific [9]. AFB staining is not sensitive enough and both culdoscopy and laparoscopy carry a significant risk of bowel injury [2]. Surgery is disadvantageous as it is highly invasive and carries a risk of activating silent infection [8]. A pelvic ultrasound and hysterosalpingography may be of some help. Only histopathological evidence in biopsy

of premenstrual endometrial tissue or demonstration of tubercle bacilli in culture of menstrual blood or endometrial curettings can provide diagnosis with certainty .

Method

Sixty-five consecutive women visiting the gynecology clinic between February 2002 and November 2004 complaining of infertility and suspected of having genital tuberculosis (TB) on clinical grounds were included in the study. Symptoms included pelvic pain and irregular menstrual bleeding, scanty menstruation and amenorrhea. A pelvic mass in variable combination aroused a suspicion.

Constitutional symptoms such as sweating and weight loss were not major complaints while local organ dysfunction manifested in amenorrhea, and bilateral tubal blockage seen on hysterosalpingographic study. The median age of the subjects was 28 (range 18-39) years. We investigated the value of different diagnostic technics for the diagnosis of FGTB. Different diagnotic technic should be combined judiciously and correlated with the clinical profile prior to instituting the antituberculosis treatment (ATT) [9]. Institutional ethical committee had approved this study.

Specimen collection

In 47 women tubal biopsy was taken at laparotomy and in 18 endometrium was obtained by curettage for smear microscopy, histopathology, culture, and PCR for mycobacterium.

Blood samples were collected in heparinised bottles. Tissue specimens taken from each subject were collected in phosphate buffer saline (PBS) to clear it of any adhering blood and debris, transfered to 10% formalin, and transported immediately to the laboratory.

Culture

Homogenized samples were cultured on Lowenstein Jensen egg medium for acid fast bacilli and incubated for 3 to 8 weeks. Ziehl-Neilsen staining was used to identify the bacilli

Histology

Paraffin embedded tissue sections were prepared and stained with hematoxylin-eosin and examined by a pathologist for granulomatous reactions suggestive of mycobacterium disease.

DNA extraction

DNA was extracted from tissue samples as described by van Soolingen using chloroform/isomylalcohol extraction method and the DNA was treated with RNAse (2mg/mL) for 1hour at 37°C and stored at –20°C.

Primers

The gene sequence that encodes for ESAT-6 protein was obtained from the Tuberculist genome database and was used to design deoxyoligonucleotide primers. The forward primer designated FtesaBhis is constructed with a Bam…. Restriction site (5 9 – CCCCGGATCCCATGACAGAGCAGCAGTGG-3 9).

The reverse primer was constructed with an *Eco*... restriction site Reset Eco (5 9- CTCGGAATTCCCCTATGCGAACATCCC-3 9). The designed primers were synthesized by Gibco BRL (Paisley, UK).

PCR amplification

Ten micromoles of each primer, 100ng DNA and distilled water were added to a Ready-To-Go- Bead (Pharmacia P –L Biochemicals Inc., Uppsala, Sweden) in a total volume of 25mL and subjected to amplification in three steps as follows – the first step of denaturing at 95°C for 2 minutes one cycle; the second step of denaturing at 95°C for 15 minutes primer annealing at 60°C for 30 minutes and primer extension at 72°C for 1 minute for 25 cycles; and the third step of prolonged primer extension at 72°C for 7 minutes for one cycle. The PCR was run using a Hybid Omini-gene thermocycler (Hybid Ltd., UK).

The PCR product was then loaded on a 2% gel stained with ethidiumbromide, later visualized under UV light, and photographed using Polaroid film cassettes. The test system is sensitive enough to detect as few as 10 bacilli (Figure 1).

Statistical analysis

Statistical analysis was carried out using the Mann-Whitney rank sum test. Fisher's exact test was used to compare proportions. Differences among groups were considered to be significant at $P<0.05$.

Results

Among the 65 patients suspected of suffering from FGTB, 32 were confirmed to have the evidence of M. tuberculosis

infection by either AFB smear microscopy (8), or positive culture (12), or histopathology (17), or PCR (28) or a combination of these. Twenty-eight patients were positive by PCR alone. The number of positive cases identified by PCRfrom the biopsy specimens was 26.17% (17/47) and that from the curettage specimens 61.17% (11/18).

Table 1. Comparison of the diagnosis yield of genital tuberculosis by different methods.

Specimen	Numbers tested	Number positive by			
		Smear microscopy	Culture	Histoloty	PCR
Curettage	18	0	0	7	11
Biopsy	47	8	12	10	17
Total	65	8	12	17	28

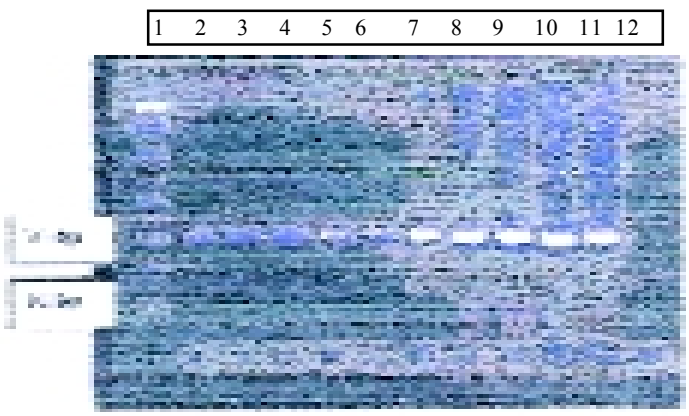

Figure 1. PCR showing amplified ESAT-6 protein encoding gene of Mycobacterium DNA from selected, strongly positive clinical samples. Lane 1,100 base pair DNA ladder, lanes 2-10 from clinical samples (DNA extracted from tissue); lane 11, positive control (known M. tuberculosis DNA); and lane 12, negative control (no template added).

Table Evaluation of diagnostic procedures.

Procedure	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Smear microscopy	87.5%	86.36%	70%	95%
Histopathology	82.3%	84.6%	87.5%	78.5%
Culture of mycobacterium	91.6%	88.88%	84.6%	94.1%
PCR	96.4%	100%	100%	66.66%
Combination of culture and PCR	100%	100%	100%	100%

Discussion

The genitourinary tract is the second most common site for tuberculous infection after the lungs. Genital tuberculosis is usually secondary to renal tuberculous infection. Effective chemotherapy had reduced all forms of TB including FGTB [7] until the HIV epidemic reversed the trend over the last decade. The proportion of extra-pulmonary TB is increasing in South India and currently stands slightly higher than smear-positive pulmonary TB [10]. Of the four laboratory diagnostic technics used, AFB staining gave the lowest detection rate (8/65; 5.2%). Culture also gave a low detection rate (12/65; 7.8%) and required a long time to obtain an answer. In our study the minimum time to see colonies was 4 weeks.

Histopathology is often not satisfactory. In our study we found only 11.05% (17/65) samples to be positive by histology. Interestingly, four of the seven histology positives were not supported by PCR, usually considered as a more sensitive method. Moreover, one patient was negative by histology at the first curettage and was positive at the second sampling. The second sampling was done because of inadequate specimen obtained at the first attempt. This indicates that inadequate specimens contribute to false negative results. Other reasons for false negative results include nonrepresentative tissue samples, technical failure in processing biopsy, period of specimen collection in relation to disease stage and effect of HIV coinfection [11,12]. These reasons may explain why classical granuloma formation was found in only one patient out of the seven histology positives.

PCR detected 43.1% (28/65) of the suspected cases. Using PCR as a additional tool a total of 22 positives are obtained consistent with the clinical diagnosis of FGTB. Of these, 12 were positive by PCR alone and the other 10 were positive by at least one of the other technics. This shows that without PCR, 50% of the clinically suspected cases would have been missed (Table 1). The sequence of the ESAT-6 gene is selected as the target of Mycobacterial DNA for amplification in our PCR system because this gene is presenting the Mycobacterium tuberculosis complex and is absent in BCG and thus is suitable for distinguishing between infection with M. tuberculosis and BCG vaccination [13]. Each PCR run is controlled by adding negative controls (without templates) to monitor false positive results. We encountered no positive bands in the controls in any of the PCRs.

36.17% (17/47) of the biopsy specimens taken from the fallopian tube gave positive findings on PCR while 61.17% (11/18) of the endometrial curettages did so. Histology of the endometrium provides lesser diagnostic information

viz., 21.3% (10/47) because the endometrium is sloughed monthly and the time for granuloma formation at this site is often inadequate [14] or the endometrium is severely damaged resulting in amenorrhea [15]. Therefore, diagnosis based on histology from endometrial curettage alone and a report based on a single sample could result in false negativity. FGTB occurs in relatively young females in the reproductive age group [2]. This is also the case in our study. Fifty-two out of our 65 patients were in the age group of 21-39 years. This could be because, after puberty the blood supply to the pelvic organs is increased and as a result, more bacilli could reach this site and infect the reproductive organs [3].

Our results as depicted in Table 1 demonstrate that PCR is the best method of diagnosing genital tuberculosis in women and a combination of PCR and culture for mycobacterium is totally dependable for the diagnosis.

In clinically suspected symptomatic subjects, PCR could be the technic of choice for its higher sensitivity and specificity. However PCR requires biopsy material obtained after invasive procedures. Therefore, a noninvasive way of getting samples for use in PCR is beneficial. One such material, when available, could be menstrual blood.

Conclusion

PCR represents a rapid and sensitive method for the detection of mycobacterium DNA in FGTB women. AFB screening has low sensitivity compared to culture and histology. Therefore, when the clinical suspicion is high and smear result is negative, and yet the signs and symptoms of M. tuberculosis are apparent, PCR is the method of choice for identifying the infection.

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