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ORIGINAL ARTICLE

Comparison of Polymerase Chain Reaction (PCR), Microbiological and Histopathological Observations in the Diagnosis of Endometrial Tuberculosis

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Abstract

Background Female genital tuberculosis often faces diagnostic challenges due to the asymptomatic nature of the disease. Our study aims at comparing the microbiological and histopathological results with PCR in diagnosing genital tuberculosis in endometrial curettage specimens.

Methods Around 139 patients with diverse gynaecological complaints were recruited for the study, and endometrial curettage specimens were collected. The specimens were subjected to microbiological culture and staining, histopathological examination and PCR to look for the presence of *M. tuberculosis.* Statistical analyses of the PCR results include calculating sensitivity, specificity, positive and negative prediction values and positive and negative likelihood ratios.

Results PCR yielded a detection rate of 41.7% (58/139) when compared to the microbiology (2.15%) and histopathology results (1.43%). PCR with *hsp65* and *cfp10*, in combination, detected 20% of the cases. Statistical analyses were suggestive that PCR with *hsp65* showed a higher sensitivity and specificity of 50% and 92.59% respectively.

Conclusion The results obtained in this study suggest that for a definitive diagnosis, combinations of the results from various diagnostics techniques can only be considered.

Keywords PCR · Female genital tuberculosis · Culture · Histopathology

Introduction

Female genital tuberculosis continues to be an important cause for infertility, especially in developing countries like India accounting for about 7–15% [1]. This is a form of secondary tuberculosis after a primary encounter of tuberculosis, either pulmonary or extrapulmonary [2]. *Mycobacterium tuberculosis* remains abeyant for a longer duration, reactivates and causes harmful effects on female genital tract which develops as female genital tuberculosis. Among the various sites, fallopian tubes and endometrium are affected the most, followed by ovary, cervix and vagina [3]. Tubal obstruction, impairment of implantation and ovulatory

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failure occur as a result of genital tuberculosis [4]. Therefore, infertility, chronic abdominal pain and menstrual dysfunctions are often found associated with this disease. On the other hand, 11% of women affected are asymptomatic [5] and so remain underdiagnosed.

Various diagnostic procedures like microbiological culture, smear microscopy, histopathology, hysterosalpingography (HSG) and ultrasonography are in use, though each of which had proven unhelpful in diagnosing certain cases. PCR is emerging as an important tool in view of its rapidness, sensitivity and specificity reported by many researchers [6, 7]. Since endometrial tuberculosis leaves only traces of bacilli, molecular techniques are potentially considered important for mycobacterial demonstration.

In our study, we have made an attempt to compare the results between PCR, microbiology and histopathology as well as correlate its results with the phenotype displayed by the patients. This investigation would help us in understanding the epidemiology of this disease, commonly appeared clinical evidences and superiority of techniques that can be employed in its diagnosis.

Materials and Methods

Patient Enrollment

Around 139 women belonging to the reproductive age group who attended the Obstetrics and Gynecology clinic in PSG Hospitals featuring symptoms suggestive of genital TB like chronic pelvic inflammatory disease, infertility, tubo-ovarian mass, secondary amenorrhoea and history of pulmonary tuberculosis were recruited for this study. Ethical committee approval was obtained for this study from Institutional Human Ethics Committee, PSG Institute of Medical Sciences & Research, Coimbatore (IHEC No: 10/214). Pregnant women and patients in whom dilatation and curettage (D&C) were contraindicated were excluded in this study. Informed consent was obtained from all individual participants included in the study and detailed history, and gynaecological examination were done on the patients. Endometrial curettings collected from the patients were subjected to microbiological culture in Lowenstein–Jensen's (LJ) medium, acid fast staining, histopathological examination and PCR for detecting *M. tuberculosis.*

Microbiological Examinations

The endometrial curettings suspended in saline were vortexed in the presence of glass beads to obtain an even suspension, and the samples were subjected to Ziehl Neelson (acid fast) staining technique for smear positivity. The samples were inoculated onto Lowenstein–Jenson's medium and incubated for 4 weeks at 37 °C. To confirm the absence of growth, incubation was extended to 2 weeks more.

Histopathological Observations

The endometrial curettings were suspended in 10% formalin and processed. The samples were stained with haematoxylin and eosin to look for the presence of caseating granulomas with epitheloid cells, lymphocytes, plasma cells and giant cells which is suggestive of tuberculosis.

Polymerase Chain Reaction from Endometrial Curettings

The endometrial curettings were suspended in lysis buffer (50 mM KCl, 10 mM TrisHCl pH 8.3, 1.5 mM $MgCl_2$, 0.1% Nonidet P-40, 0.5% Tween-20) and homogenized. DNA was extracted using the standardized protocol [8]. PCR for amplifying three mycobacterial gene segments was carried out. The primer sequences and the amplicon length are mentioned in Table 1. 20 µl of PCR mix consisted of 50 ng of DNA mixed with 0.2 mM of each of the four dNTPs (dATP, dCTP, dGTP, dTTP), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5 mM MgCl2, 0.05 µM of each forward and reverse primers, 1 U/µl *Taq* polymerase and nuclease free water. The reaction was supplemented with positive and negative controls. The PCR parameters followed were 35 cycles of denaturation (94 °C for 5 min), primer annealing (60 °C for 50 s) and extension (72 °C C for 1 min). The final extension was carried out at 72 °C C for 5 min, and the samples were put on hold at 4 °C C. The PCR products were run on 1.5% agarose gel, and the bands were visualized under UV light using a gel documentation system.

Table 1 Primer details of three mycobacterial gene segments

S. no	Gene segment	Primer sequences (FP – Forward primer, RP – Reverse primer)	Size of PCR product (bp)
1	*Hsp65*	FP-5′ ACCAACGATGGTGTGTCCAT 3′ RP-5′ CTTGTCGAACCGCATACCCT 3′	441
2	*Cfp10*	FP-5′ GGCAGAGATGAAGACCGATG 3′ RP-5′ GCTTATTGGCTGCTTCTTGG 3′	191
3	*Esat6*	FP-5′ CATGACAGAGCAGCAGTGG 3′ RP-5′ CCCTATGCGAACATCCC 3′	291

Statistical Parameters

Statistical parameters like sensitivity, specificity, positive and negative prediction values and positive and negative likelihood ratios were calculated.

Results

Clinical Presentations of the Patients

The common presenting symptoms found in the study population were primary infertility (60%) and irregular cycles (47.5%). Secondary infertility and abdominal pain account for about 16.5% and 10%, respectively. White discharges (7.2%), secondary amenorrhoea (6.5%), dysmenorrhoea (5.7%) and fibroid uterus (4.3%) were manifested by the patients too. Hypomenorrhoea (1.4%), oligomenorrhoea (3.6%), menorrhagia (2.9%), ectopic pregnancy (1.4%) and history of tuberculosis (1.4%) constituted to a meagre amount (Fig. 1).

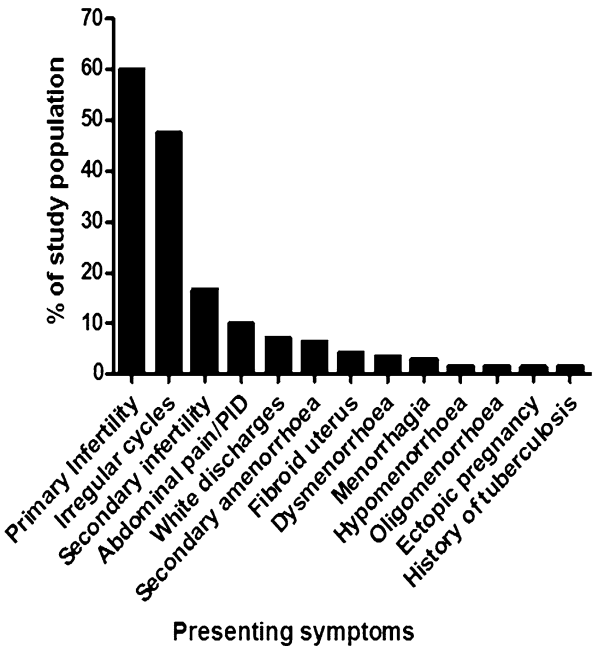

Fig. 1 Frequency of presenting symptoms in the study population. The percentage of different clinical presentations observed in the patients recruited for this study was depicted. Primary infertility and irregular cycles were presented more frequently, while ectopic pregnancy and oligomenorrhoea were presented the least

Correlation of Microbiological and Histopathological Results with PCR

For PCR detection, primers specific for three mycobacterial gene segments, namely *hsp65, cfp10* and *esat6*, were used. *Hsp65* is the mycobacterium genus-specific gene segment was employed for the diagnosis of genital TB [9]. *cfp10* and *esat6* were the gene segments present in *M. tuberculosis*, *M. africanum* and virulent *M. bovis,* thus comprising the *M. tuberculosis* complex [10, 11].

Overall, 58/139 samples were positive at least for one primer through PCR with a detection rate of 41.7%. On the contrary, only 2/139 samples were positive for histopathology with a detection rate of 1.43%. Microbiological examinations detected two cases of *M. tuberculosis* and a case of non-tuberculous mycobacteria with a positivity rate of 2.15%. Based on the PCR results, positive samples were grouped into four categories depending on the number of gene segments amplified for each sample (Table 2).

Out of 139 samples, four samples were positive for all the three gene segments. Both histopathological findings and microscopic techniques (acid fast staining & culture) yielded the negative results for these samples. *Hsp65* and *cfp10* gene segments were identified in 28/139 samples. Among 28 samples, only one sample detected tubercle bacilli through culture on LJ medium. Histopathological examinations yielded the negative results. In 12/139 samples, *hsp65* alone was detected. In 1/12 sample, mycobacterial culture and histopathology results were positive for *M. tuberculosis*. Non-tuberculous mycobacterium was detected in one sample. *cfp10* was detected in 14/139 samples which showed the negative results for microbiology and histopathology. There were 81/139 samples which gave the negative results for PCR. Out of this, one sample was positive for histopathology.

Overall, it is clear from the data that *hsp65* and *cfp10* either alone or in combination have been successful in detecting 58/139 samples. In combination, *hsp65* and *cfp10* have detected up to 20% of cases which microbiological and histopathological assays were unable to detect (Table 2).

Table 2 Analysis of PCR-positive samples

Gene segments amplified	No of samples positive	Percentage
hsp65, cfp10 and *esat6*	4/139	2.9
hsp65 and *cfp10*	28/139	20
hsp65	12/139	8.6
cfp10	14/139	10

Correlation of Clinical Symptoms with PCR Results

The varied clinical clues exhibited by the patients and their respective PCR results were compared (Table 3). The predominant clinical symptoms associated with the PCR-positive samples were irregular menstrual cycles and primary infertility accounting for 50–75% of the cases. Secondary infertility, secondary amenorrhoea, hypomenorrhoea, dysmenorrhoea, oligomenorrhoea, chronic abdominal pain and white discharges were found in 10–25% of the cases. History of tuberculosis and fibroid uterus constitute a very meagre percentage of 3.5–7% (Table 3).

The patients in whom all the three gene segments of mycobacteria were amplified belonged to the reproductive age group 27–31, marked with irregular menstrual cycles, primary infertility, hypomenorrhoea, dysmenorrhoea, oligomenorrhoea and white discharges.

The 28 patients in whom *hsp65* and *cfp10* gene segments were amplified belonged to the age group of 19–47. They indicated gynaecological irregularities like primary infertility (75%), secondary infertility (21.4%), secondary amenorrhoea (7.1%), white discharges (10.7%), fibroid uterus (7.1%) and ectopic pregnancy (7.1%). 39% of these 28 patients had irregular menstrual cycles. A previous history of abdominal tuberculosis was found in a patient. 3.5% of these patients had chronic pelvic inflammatory disease (PID). Other menstrual problems like menorrhagia, hypomenorrhoea and dysmenorrhoea were also associated in these patients.

Hsp65 alone was amplified in 12 samples. Primary infertility (66%), irregular cycles (25%), 8.3% each of secondary infertility, secondary amenorrhoea, abdominal pain, fibroid uterus and oligomenorrhoea were the clinical symptoms associated in these patients.

In 14 samples, only *cfp10* was amplified. A history of pelvic tuberculosis was also associated with a case. 50% of these patients had irregular cycles and primary infertility. White discharges, chronic abdominal pain and PID were observed in 21.4% of patients. 20% of patients had secondary infertility and oligomenorrhoea. 7.1% of the patients had complaints like secondary amenorrhoea, fibroid uterus and dysmenorrhoea.

Table 3 Correlation of clinical symptoms with PCR results

Clinical features	PCR amplicons found			
	hsp65, cfp10 and *esat6*	*hsp65* and *cfp10*	*hsp65*	*cfp10*
Primary infertility (%)	50	75	66	50
Secondary infertility (%)	–	21.4	8.3	20
Secondary amenorrhoea (%)	25	7.1	8.3	7.1
Hypomenorrhoea (%)	25	3.5	–	–
Oligomenorrhoea (%)	25	–	8.3	20
Dysmenorrhoea (%)	25	7.1	–	7.1
Menorrhagia (%)	–	3.5	–	–
White discharges (%)	25	10.7	–	21.4
Irregular cycles (%)	75	39	25	50
Abdominal pain/PID (%)	–	3.5	8.3	21.4
Ectopic pregnancy (%)	–	7.1	–	–
History of tuberculosis (%)	–	3.5	–	3.5
Fibroid uterus	–	7.1	8.3	7.1

Sensitivity and Specificity of TB PCR

Microbiological culture was considered as the gold standard technique to diagnose endometrial TB. All culture-positive cases in our study were considered as positive cases. Based on this, the sensitivity, specificity and positive and negative predictive values for the TB PCR were calculated (Table 4). Using any one of the primers, the sensitivity and specificity of PCR were found to be 75% and 59.2%, respectively. The

Table 4 Sensitivity, specificity and predictive values for the TB PCR

PCR primers	Statistical parameters					
	Sensitivity (%)	Specificity (%)	Positive predictive values (%)	Negative predictive values (%)	Positive likelihood ratio	Negative likelihood ratio
PCR Positive (for any one of the primer)	75	59.26	5.17	98.77	1.84	0.42
PCR positive for all the three primer sets (*hsp65*+ *esat6*+ *cfp10*+)	0.00	97.04	0.00	97.04	0.00	1.03
PCR positive for two primer sets (*hsp65* and *cfp10*)	20	82.91	3.57	97.04	1.17	0.96
PCR positive for one primer set (*hsp65* alone)	50	92.59	16.67	98.43	6.75	0.54
PCR positive for one primer set (*cfp10* alone)	0.00	89.63	0.00	96.80	0.00	1.12

sensitivity and specificity were high for primer set *hsp65* when compared with other combinations of primers.

Discussion

Our results indicated lower detection rates for culture (2.15%) and histopathological analyses (1.43%) and higher identification rates for PCR. The similar results were obtained for histopathology (1.87%) and microbiology (2.05%) findings recorded earlier [12]. Other studies reported a higher detection rate of 65% for PCR with endometrial biopsies [13].

PCR-positive four samples (for all three gene segments) showing the negative results for microbiology and histopathology may be due to the lower sensitivity of culture and histopathological analyses. The possible reason for culture insensitivity may be poor recovery of bacilli as the sparse number of bacilli is a characteristic feature of genital TB [14]. The false-negative histopathology results may be due to the shedding of endometrium leaving insufficient time for granuloma formation or damaged endometrium [2, 15].

A PCR and culture negative sample displaying positive result may be due to the co-presence of other pathogens which might have precipitated the lesions. This also confirms the fact that the PCR results correlate with that of culture and have shown reliability.

Review of available data on microbiological diagnosis of genital TB indicates that BACTEC 460 TB culture had a sensitivity of 7.14–40% and a specificity of 90–100% [16–18] which improved upon usage of PCR as an additional tool in diagnosing genital TB. The histopathological method showed a sensitivity of 10.7% and specificity of 100% [16]. These figures reveal that there was a need for development of a better sensitive method for genital TB with subtle presentations and lower bacterial load. In our setting, around 20% of the positive cases were spotted positive by *hsp65* and *cfp10*. Statistical parameters indicate a higher sensitivity and specificity of PCR when any one of the primers was used. Among the primer combinations, *hsp65* primers demonstrated higher probabilities of finding mycobacteria with higher specificity of 92.59% as it is a mycobacterium genus-specific gene, but it showed a lower sensitivity rate of 50% possibly because of the presence of both the endometrial DNA and the mycobacterial DNA in the sample which might give rise to more inhibitory effects on PCR. Lower sensitivity range of 55% in detecting *M. tuberculosis* through PCR was also reported earlier [19] where the usage of low-cost PCR reagents was the reason for lower sensitivity, though it was developed for applications in low income settings [20]. Similarly, lower sensitivity of PCR (57%) in diagnosing genital TB was reported in another study where combination of many sets of primers was suggested to be used to improve the sensitivity.

Conclusion

The gold standard test to diagnose endometrial tuberculosis remains the culture technique: LJ medium and MGIT liquid culture. In the current scenario, all the clinical decisions are best made depending on the culture results although collaborative tests may be supportive. Therefore, PCR can only be used as an additional method along with histopathology, microbiology and imaging tools.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Institutional Human Ethics Committee, PSG Institute of Medical Sciences and Research, Coimbatore (IHEC No: 10/214).

Informed Consent Informed consent was obtained from all patients for being included in the study.

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