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Original Research Article

Evaluation of endometrial polymerase chain reaction in diagnosis of female genital tuberculosis

Manvi Dua[1]*, Kiran Pandey[1], Sangeeta Arya[1], Anil Verma[2]

[1]Department of Obstetrics and Gynecology, GSVM medical College, Kanpur, Uttar Pradesh, India
[2]Department of Anesthesiology, GSVM Medical College, Kanpur, Uttar Pradesh, India

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***Correspondence:**
Dr. Manvi Dua,
E-mail: drmanvi@rediffmail.com

ABSTRACT

Background: Genital tuberculosis also known as tuberculous pelvic inflammatory disease can affect any age group, most common being reproductive women of 20-40 years. Clinical diagnosis of genital tuberculosis is a big challenge as the disease is either asymptomatic or has varied presentations. Conventional methods for diagnosis including AFB smear, endometrial histopathology and culture have limitations of low detection rate because of paucibacillary nature of disease. Laparoscopy generally detects macroscopic changes such as peritubal adhesions, tubercles and tubo-ovarian mass but it fails to diagnose disease at early stage. The objective of this study was to evaluate efficacy of TB DNA PCR in diagnosis of genital tuberculosis.

Methods: A total of 127 patients (between 2013-2016) who presented in gynecologic OPD with symptoms suggestive of tuberculosis were included in the study. All patients were subjected to endometrial histopathology and TB DNA PCR of endometrial tissue and peritoneal fluid. Since there is no gold standard test available for diagnosis of genital tuberculosis, a diagnostic criteria was adopted in the study based on laparoscopic findings, clinical history and other investigations. Patients were divided in two groups. Group A included patients positive of tuberculosis based on diagnostic criteria. Group B included patients negative for tuberculosis based on diagnostic criteria.

Results: In our study sensitivity of endometrial PCR, peritoneal PCR and endometrial histopathology were 73.8%,17.8% and 10.7% respectively. Endometrial histopathology and peritoneal fluid PCR was found to be highly specific (100%) while endometrial PCR was found to be 93% specific. Endometrial PCR although has highest sensitivity and specificity amongst the groups evaluated but high false negative rate was its major limitation.

Conclusions: No single test fulfills all criteria to emerge as sole diagnostic test, hence a high degree of suspicion with a detailed history and investigating with a variety of tests is all that is required to diagnose geniatal tuberculosis.

Keywords: Chronic pelvic pain, Genital TB, Infertility, Laparoscopy, Polymerase chain reaction, Sensitivity, Specificity

INTRODUCTION

Tuberculosis is a chronic infectious disease and morbidity associated with this condition has major health implications. Incidence of genital TB has been estimated to range from 1 to 26.7% in various studies from India.[1,2] Incidence of genital TB varies greatly depending upon the geographical location ranging from 10.3% in India to being less than 1% in USA.[3] It is endemic in India with an incidence of 58%.[4] Genital tuberculosis can affect any age group, most common being reproductive women of 20-40 years. Infertility, menstrual irregularities and chronic pelvic pain are the most common manifestations of female genital tuberculosis.[5] Others included fever,

ascites, irregular vaginal bleeding, chest pain and pain in the flanks.[6] Genital tuberculosis appears to be an important and common cause of Asherman's syndrome in India, causing oligomenorrhoea or amenorrhoea with infertility.[7] Fallopian tubes are the first and the most commonly affected genital organ followed by endometrium, ovary and cervix. It causes irreversible damage to the fallopian tubes leading to infertility which is difficult to treat both by medical and surgical methods.[8] Endometrial involvement in genital TB is secondary to tubal involvement. It occurs in 50-80% of genital TB. Since, there is no way to take the fallopian tubes out, sampling from the ovaries and endometrium was suggested for the detection of female genital tuberculosis.[9] Use of menstrual blood for bacteriologic or molecular diagnosis has been recommended but was reported to show low sensitivity.[10,11] Granulomas in endometrial tissue biopsy are better seen in premenstrual phase or within 12 hours after onset of menstruation. Focal collection of chronic inflammatory cells or presence of proliferative endometrium in the premenstrual week in a patient with past history of TB in other parts of the body or a family history of TB would favour a diagnosis of female genital TB.[12] Ovaries are involved in 15-25% of cases. Presence of tubo-ovarian mass, tubo-ovarian cyst with adhesions surrounding them is found in such cases. Cervical tuberculosis occurs in 5-15% of cases while TB of vagina and vulva occurs in 1% of cases. Genital TB was an important aetiological cause in patients with unexplained infertility with repeated IVF failure.[4]

Laparoscopy usually detects macroscopic changes such as peritubal adhesions, tubercles on the tubes and small tubo-ovarian masses that are commonly seen in chronic cases. Female genital tuberculosis also presents distinctive diagnostic challenges including subtle clinical manifestations that were over-looked in laparoscopy during early stages of infection. The presence of periovarian adhesions, cornual block, tubal beading, tubercles, intrauterine adhesions, and ostial fibrosis had very strong association with positive TB PCR. Total predictive value of endoscopic evaluation in diagnosis of genital TB was 42.52%.[13]

METHODS

Inclusion criteria

- The All patients who presented with infertility, menstrual disturbances, chronic pelvic pain, recurrent pelvic inflammatory disease refractory to conventional therapy were included in the study.

Exclusion criteria

- Antenatal patients, unmarried female and those with any other medical disorder were excluded from the study.

This is a prospective study conducted between 2013 to 2016.

The study was conducted on 127 female patients attending gynecologic OPD of UISEMH, Department of Obstetrics and Gynecology, GSVM Medical College, Kanpur. Detailed history and examination was done. All patients underwent investigations such as CBC, ESR, tuberculin test, chest X-ray and pelvic sonogram. Endometrial biopsy within 12 hours of menstruation and diagnostic laparoscopy on day 9 of menstrual cycle was done in all patients. Semen analysis and HSG was done only in patients presenting with infertility. Sample was taken from the endometrium especially from both cornual ends and sent for PCR amplification and histopathology. Diagnostic laparoscopy was performed to look for beaded tubes, tubal block, hydosalpinx, presence of any tubercles on the tubes and other pelvic viscera, pelvic adhesions and tubo-ovarian mass. During laparoscopy peritoneal fluid from pouch of douglas else peritoneal washings was collected and sent for DNA PCR for detection of mycobacterium tuberculosis.

A diagnostic criteria adopted in the study is as follows

Findings suggestive of tuberculosis on laparoscopy along with any one of the following findings: Past history of TB, Raised ESR, Positive mantaux. Based on diagnostic criteria patients were divided into two groups. Group A included patients positive for tuberculosis based on diagnostic criteria. Group B included patients negative for tuberculosis based on diagnostic criteria. Endometrial tissue was collected in two vials -one in 10% formalin and other in normal saline. Formalin fixed tissue was sent to pathology where routine processing was done and tissue was stained with hematoxylin and eosin and observed under microscope. Presence of caseating granulomas surrounded by lymphoid cells, plasma cells, lymphocytes and giant cells were diagnostic of tuberculosis. Tissue from normal saline vial was sent for DNA PCR.

Statistical analysis

Sensitivity, specificity, positive predictive value negative predictive value of different tests were calculated using standard formula.

Polymerase chain reaction technique

All the available molecular tests are based on the principle of polymerase chain reaction (PCR). PCR based diagnosis of TB has been evaluated to be useful and important in the detection of pulmonary as well as extra-pulmonary TB. PCR assays targeting various gene segments such as 64kDa protein encoding gene4, the IS6110 element 5 and mpt646 have considerably reduced the delay in laboratory diagnosis for definitive mycobacterial detection.

Processing of samples

The endometrial tissue was finely chopped using a sterile scalpel and homogenized manually in TE buffer (Tris-EDTA -10 mM Tris.1 mM EDTA pH 8.0) until the solution became turbid. This was centrifuged at 11200 g for 20 min. The supernatant was discarded and the pellet was processed for further studies. POD aspirate and urine samples were centrifuged at 700 g for 15 min. Supernatant was discarded and the pellet was used to extract DNA.

Isolation of DNA

Pellets were re-suspended in 500 µl of TE buffer by repeated pipetting. Then 50 µl of 10 mg/ml of lysozyme was added, mixed well and incubated for one hour at 37 0C. To this, 70 µl of 10 percent SDS (sodium dodecyl sulphate) and 6µl of 10 mg/ml of proteinase K were mixed and incubated for 10 min at 65^0C.After incubation 100 µl of 5 M NaCl was added and mixed thoroughly. The samples were further incubated with 80 µl of CTAB/NaCl (Cetyl trimethyl ammonium bromide in sodium chloride) solution for 10 min at 65^0C. To this prepared sample approximately equal volume (700 - 800 µl) of chloroform/isoamyl alcohol were added, mixed thoroughly and centrifuged for 10 min. To the supernatant, 0.6 volume isopropanol was added to precipitate the nucleic acids and placed at -200^0C for 60 min. The resultant sample was spun at 16128 g for 20 min at 60^0C. The resulting DNA pellet was washed with 70 per cent ethanol to remove residual CTAB. The supernatant was carefully removed and the pellet was dried. The prepared pellet was re-dissolved in 25 µl of TE buffer (910mM TRIS and 1mM EDTA) and stored at 40^0C for future use.

Amplification of mycobacterial DNA

PCR was performed using gene amplification 9700 Thermal cycler with standard 25 µl working volume. Precautions were taken to avoid false positivity. Preparation of PCR reagents, addition of template DNA and analysis of amplified products were done in three different rooms to avoid carry over contamination. Reagents were aliquoted and each aliquot was used only once. Wax beads were added to minimize nonspecific amplification. DNAs from the samples were amplified using the following primers.

- IS6110a (5’ - CCT GCG AGC GTA GGC GTC GG - 3’)
- IS6110b (5’ - CTC GTC CAG CGC CGC TTC GG - 3’)
- TRC4 primer 1 (5’ - GAC AAC GAC GTG CGCCTA CT - 3’)
- TRC4 primer 2 (5’ - GAC CGA ATT AGC GTA GCTCC - 3’)
- The IS6110 primers amplify a fragment with a length of 123bp, while the TRC4 primers amplify fragment with a length of 173bp

Cycling parameters

The reaction was performed on ice to minimize non-specificity. The cycling parameter used was initial denaturation at 95^0C for 5 min, followed by denaturation at 94^0C for 30 sec, annealing at 58^0C for 30 sec, extension at 72^0C for 30 sec with 25 cycles and a final extension at 72^0C for 5 min. Detection of amplified products was done by agarose gel electrophoresis (2%) at 80 volts for 45 min. Gel was stained with ethidium bromide and viewed under UV transilluminator.

RESULTS

The present study assesses the role of PCR in the diagnosis of genital TB using endometrial and peritoneal samples. In our study 26-30 years was the most common age of presentation which correlates well with the results of other available studies. Infertility (n=82, 64.5%) was found to be the most common symptom followed by menstrual disorders (n=71, 56%), chronic pelvic pain (n=52, 41%) and refractory PID (n=18, 14.1%). Abdominal pain and altered bowel habits were the least common presenting symptoms. Constitutional symptoms (fever, anorexia, weight loss) was present only in 16.5% (n=21) patients (Table 1).

Table 1: Distribution of patients according to symptomatology.

Complaints	No of patients (n=127)	%
Infertility	82	64.5%
0-3 years duration	15 (n=82)	18.2%
4-6 years duration	39 (n=82)	47.5%
7-9 years duration	28 (n=82)	34.3%
Menstrual disorders	71	56%
Hypomenorrhea	35 (n=71)	49.2%
Oligomenorrhea	23 (n=71)	32.3%
Secondary amenorrhea	10 (n=71)	14%
Menorrhagia	3 (n=71)	4.2%
Chronic pelvic pain	52	41%
Refractory PID	18	14.1%
Altered bowel habits	12	9.4%
Constitutional symptoms (fever, anorexia, night sweats, weight loss)	21	16.5%
Total	127	100%

It was found that 62 (73.8%) out of 84 patients (Group A) were found to be positive (true positive) on Endometrial Tissue PCR. Peritoneal fluid PCR and endometrial histopathology was found to be positive in 17.8% and 10.7% patients respectively. Thus, sensitivity of

endometrial PCR was found to be higher than the other two. We concluded that 40 (93.13%) patients were found to be negative when tested by endometrial tissue PCR (true negative) while peritoneal fluid PCR and histopathology could detect all the true negatives. Thus, endometrial histopathology and peritoneal fluid PCR were found to be 100% specific while endometrial tissue PCR specificity was moderately high (93.02%) (95% CI 80.9%-98.5%). Positive predictive value of histopathology, peritoneal fluid PCR and endometrial PCR was found to be 100%,100% and 95.3% respectively but their negative predictive value were 36.4%, 38.3% and 64.5% respectively. False negative rate of 26.1% (22 patients in Group A) of endometrial PCR was found to be significantly high which was found to be major limitation of our study. Because of this high false negative rate it cannot be relied upon as sole diagnostic test. Endometrial histopathology and peritoneal fluid PCR was found to have high false negative rate of 89.2% and 82.1% respectively (Table 2, 3) which means that majority of patients will be missed if we rely only on one of these tests for the diagnosis. False positive rate of endometrial histopathology and peritoneal fluid PCR was found to be 0% which implies that no single patient may be falsely labelled as diseased. Thus there is minimum possibility of misdiagnosing the patient as suffering from genital tuberculosis when endometrial histopathology and peritoneal fluid PCR is used as diagnostic test but false positive rate of endometrial TB PCR was 6.9% which could be due to early disease with low number of bacilli or with latent infection which are picked by PCR when women are still asymptomatic and before the structural damage to the tube has taken place.

Table 2: Comparison of endometrial tissue PCR, peritoneal fluid PCR and endometrial histopathology with diagnostic criteria.

Endometrial tissue PCR/diagnostic criteria	Group A diagnostic criteria positive (number)	Group A diagnostic criteria positive (percentage)	Group B diagnostic criteria negative (number)	Group B diagnostic criteria negative (percentage)
Endometrial tissue PCR positive	62 (true positive)	73.8%	3 (false positive)	6.97%
Endometrial tissue PCR negative	22 (false negative)	26.2%	40 (true negative)	93.13%
Peritoneal fluid PCR positive	15 (true positive)	17.8%	0 (false positive)	0
Peritoneal fluid PCR negative	69 (false negative)	82.1%	43 (true negative)	100%
Endometrial histopathology positive	9 (true positive)	10.7%	0 (false positive)	0%
Endometrial histopathology negative	75 (false negative)	89.2%	43 (true negative)	100%
Total	**84**	**100**	**43**	**100%**

Table 3: Comparison of endometrial PCR, peritoneal PCR and endometrial histopathology.

Diagnosis	Endometrial PCR	Peritoneal PCR	Endometrial histopathology
Sensitivity	73.8%	17.8%	10.7%
Specificity	93%	100%	100%
Positive predictive value	95.3%	100%	100%
Negative predictive value	64.5%	38.3%	36.4%
P-value	<0.0001	0.0024	0.0278

DISCUSSION

According to Sharma JB et al, most women presented with infertility (90.6% primary 72.9%; secondary 17.6%) while the rest had chronic pelvic pain (9.4%). The mean duration of infertility was 6.2 years. A total of 49 (57.6%) women had normal menses, while hypomenorrhea, oligomenorrhea, secondary amenorrhea and menorrhagia were seen in 25 (30.1%), 3 (3.5%), 5 (5.9%), and 2 (2.4%) women respectively.[7] Sahu C et al, concluded that polymerase chain reaction (PCR) showed higher sensitivity and specificity than conventional techniques.[14] Kumari G et al analysed that there isn't any clear cut diagnostic value of the methods other than biopsy.[15] Recently, the PCR method is known to have an important diagnostic value. Conclusively the data obtained from this PCR based investigation reflected that conventional methods of diagnosis namely, HPE, AFB smear and

culture have low sensitivity.[14] Endo TB-PCR had high specificity to diagnose GTB, as did laparoscopy. Laparoscopy may therefore be avoided in TB-PCR-positive patients for diagnosis but may still be required to rule out GTB in PCR-negative cases.[16] PCR was found to be useful in diagnosing early disease as well as confirming diagnosis in clinically suspected cases.[17] One should be cautious about false positivity by way of contamination, dead bacilli, previous infection or asymptomatic TB at other site. Thus, 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.[18] PCR showed good correlation with laparoscopic findings in diagnosis of female genital tuberculosis.[14]

CONCLUSION

Genital TB has varied presentations, difficult to diagnose on the basis of clinical history and examination. Conventional methods of diagnosis have several limitations. Newer molecular methods including endometrial polymerase chain reaction (PCR) showed higher sensitivity and specificity than conventional techniques. But it has high false negative rate of 26% which prevents it from emerging as sole diagnostic test. Peritoneal fluid PCR was found to be highly specific but it has very low sensitivity and it is invasive, hence endometrial PCR is found to be better route of investigation. Since no single test fulfills all criteria to emerge as sole diagnostic test, hence a high degree of suspicion with a detailed history and investigating with a variety of tests is all that is required to diagnose geniatal tuberculosis.

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