ORIGINAL ARTICLE

Endometrial Tuberculosis Pick By PCR; in Female Infertility

Anju Dogra, Indu Kaul, Swaran Kanta

Abstract

To evaluate the usefulness of PCR in diagnosing genital tuberculosis in female infertility and its sensitivity, specificity, positive predictive value, negative predictive value and its edge over other diagnostic modalities like histopathological examination and culture. This study included 100 infertile women who met the inclusion and exclusion criteria. After a detailed history, clinical examination and investigations, endometrial curettings were taken in premenstrual phase and sent for culture, histopathological examination and PCR. After evaluation the results were compared individually with each other or in combination to see the positive pick up percentage and their sensitivity and specificity. We found 4% cases positive for tuberculosis on culture for Mycobacterium tuberculosis, 7% of cases positive histopathologicaly and 15% cases on PCR evaluation. The sensitivity for diagnosing this disease was found highest in PCR (100%) followed by histopathological examination (75%). Culture had the least sensitivity of 42.85%. PCR represents a rapid and sensitive method for identification of Mycobacterium DNA in female genital tuberculosis. Culture and histopathology have low sensitivity compared to PCR. Therefore, when the clinical suspicion is high and culture and histopathology is negative, PCR is the method of choice for identifying the infection.

Key Word

Endometrial Tuberculosis, Infertility, PCR

Introduction

Infertility is defined as" the inability to conceive by at least one year of unprotected intercourse". It affects 10-15 percent of all couples. Infertility among couples in India is still on rise.(1)

Genital TB is an important cause of infertility. Tuberculosis is a chronic infectious disease and the morbidity associated with this condition has major health implications. The disease has a worldwide distribution, and the incidence is high in developing countries.(2) TB affects genital organ in young female, it produces devastating effect by causing irreversible damage to the fallopian tube resulting in infertility which is difficult to diagnose and cure both by medical and surgical methods.(3) The disease often remains silent or may present with non specific symptomatology. As a result, the prevalence of genital tuberculosis is largely underestimated. In developed countries, such as USA, Australia and Western European countries, the incidence of genital tuberculosis is less than 1%, but the incidence in some African countries is as high as 15-19% (4). Most common form of extra pulmonary TB is genitourinary disease, accounting for 27% (4-41%) worldwide. In India, the incidence of genital tuberculosis is nearly about 18%.(5)

From the Deptt. of Obstetrics and Gynaecology, Govt. Medical College Jammu. (J&K) -India
Correspondence to : Dr Indu Koul, Professor & Head, Department of Obstetrics and Gynaecology, Govt. Medical College Jammu. (J&K)

A positive XRAY chest for healed or active pulmonary tuberculosis, contact history, and elevated ESR and positive tuberculin test may indicate the need for further investigations.Treatment may be easier and earlier if the cause is obvious. A definite diagnosis can be made by positive mycobacterial culture and by demonstrating specific histopathological lesion in the specimen .However these methods have low detection rates and limitations as GTB is paucibacillary. Culture can detect TB if 1000 bacilli present per ml of specimen (6). Sensitivity of HPE IS 82.3 percent (7) and sensitivity of culture is 7.14 percent (8). In recent years, polymerase chain reaction [pcr] technique has evolved as a rapid technique for the diagnosis of pulmonary and extra pulmonary tuberculosis. PCR can detect TB if 10 bacilli present per ml of specimen8. Sensitivity of PCR is 96.4 percent (7). PCR is a rapid, sensitive and specific molecular biological method for detecting mycobacterial DNA in both pulmonary and extra pulmonary samples from suspected TB patients.

Any method that is used to diagnose GTB should be highly sensitive to diagnose the disease reliably in its early stages, so that treatment may improve the prospects of cure before the tubes are damaged beyond recovery. The objective of the present study was to evaluate the usefulness of PCR technique in the diagnosis of GTB in female infertility in comparison to culture and histopathological examination.

Material and Methods

This prospective study was conducted in the department of Obstetrics and Gynaecology, SMGS Hospital GMC Jammu in collaboration with INDIAN INSTITUTE OF INTEGRATIVE MEDICINE (IIIM) for one year. Patients with female infertility [primary and secondary] were recruited from both Inpatient as well as Outpatient department of Obstetrics and Gynaecology.

Exclusion Criteria -Male factor infertility: In all the patients included in this study; a detailed history was taken and thorough clinical examination was done. Investigations such as Hb, TLC, DLC, ESR, HIV1 and 2, TSH, abdominal pelvic sonogram and Semen analysis of husband were done. All the selected patients were advised to undergo DNC for taking endometrial curetting in premenstrual phase and sample divided into three parts: Two parts were collected in normal saline for PCR and CULTURE and third part was collected in formalin for HISTOPATHOLOGY and sent to different destinations: For PCR department of INDIAN INSTITUTE OF INTEGRATIVE MEDICINE (IIIM), for Histopathology Department of Pathology and culture in the Department of Microbiology.

Culture: Homogenized samples was 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.

Histopathology : For histopathological studies, a portion of endometrial tissue was fixed in 10 percent formalin and Paraffin embedded tissue sections was prepared and stained with haematoxylin-eosin and examined by a pathologist for granulomatous reactions suggestive of Mycobacterium disease.

Polymerase chain reaction technique: (1) Processing of samples-The endometrial tissue was finely chopped using a sterile scalpel and homogenized manually in TE buffer (tris-EDTA-10mm tris.cl.ph 8.0) until the solution became turbid. This was centrifuged at 11200g for 20min. The supernatant was discarded and the pellet was processed for further studies.

(2) Isolation of DNA- Pellets was 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 1 hour at 37®c .To this, 70 µl of 10 percent SDS (sodium dodecyl sulphate and 6 µl of 10 mg/ml of proteinase k was mixed and incubated for 10 min at 65®c. After incubation 100 µl of 5M NaCl was added and mixed thoroughly. The samples was further incubated with 80 µl of CTAB/NaCl (cetyl trimethyl ammonium bromide in sodium chloride) solution for 10 min at 65®c.To this prepared sample approximately equal volume(700-800 µl) of chloroform/Isoamyl alcohol was added, mixed thoroughly and centrifuged for 10 min. To the supernatant, 0.6 to the supernatant, 0.6 volume isopropanol was added

Table No.1. Outcome of Different Diagnostic Tests

Test	No. of samples	Positive (%)
Culture	100	4(4%)
HPE	100	7(7%)
PCR	100	15(15%)

Fig. 1. Outcome of different diagnostic test

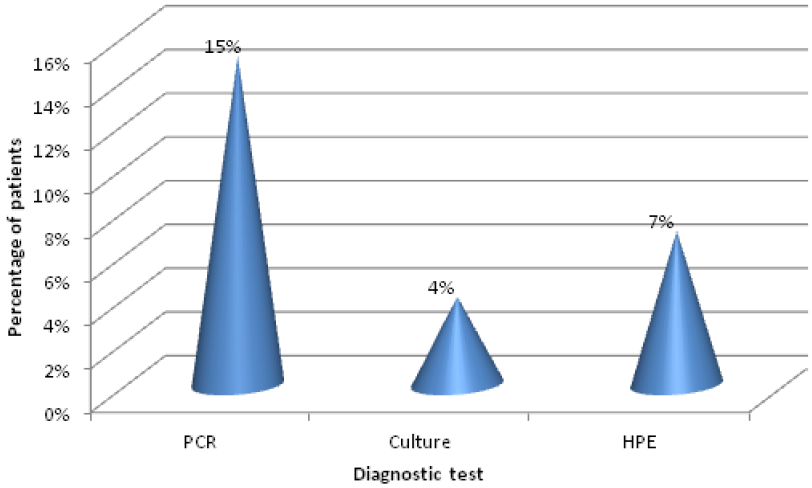
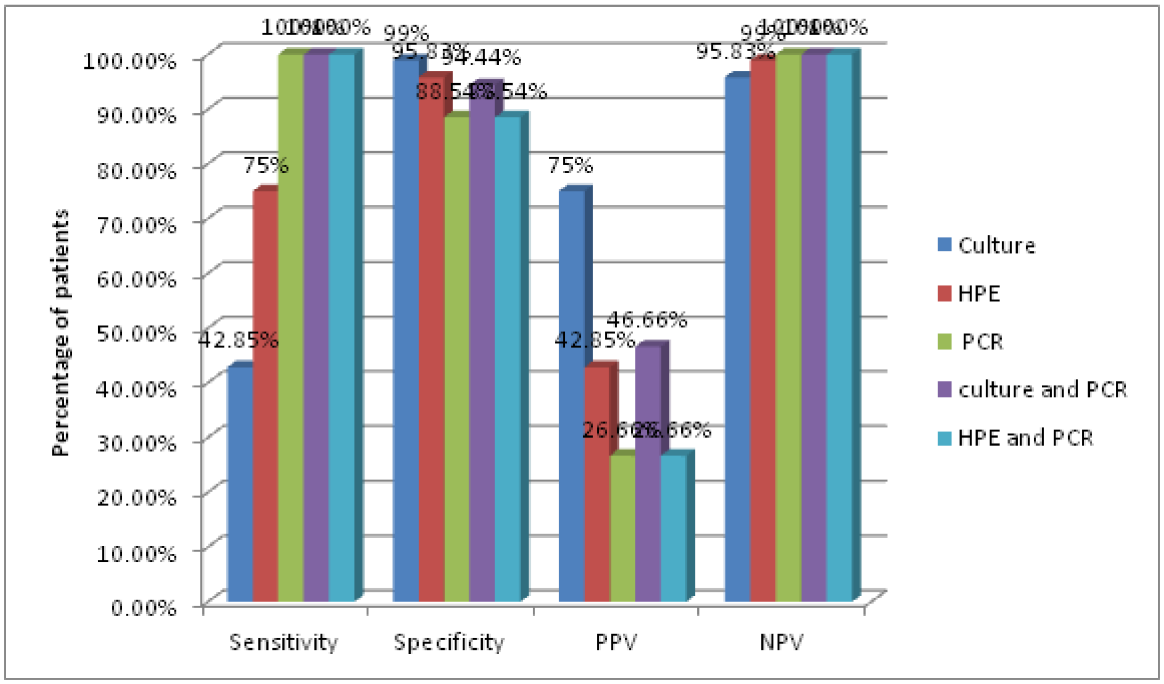


Fig.2. Bar Chart Showing Evaluation of Results of Different Diagnostic Tests



stored at 40c for future use.

(3) Amplification of mycobacterial DNA - PCR was performed using gene amplification 9700 thermal cycler with standard 25 working volume. Precautions was taken to avoid false positivity of PCR reagents, addition of template DNA and analysis of amplified products was done in three different rooms to avoid carryover contamination. Reagents were aliquoted and each aliquot to precipitate the nucleic acids and placed at -200c for 60 min. The resultant sample was spun at 16128 g for 20 min at 60c. The resulting DNA pellet was washed with 70 per cent ethanol and was carefully removed and the pellet was dried. The prepared pellet was re-dissolved in 25 ?l of TE buffer(910mm TRIS and 1mm EDTA) and

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')

and TRC4 primer 1 (5' - GAC AAC GAC GTG CGC CTA CT - 3')

TRC4 primer 2 (5' - GAC CGA ATT AGC GTA GCT CC - 3')

The IS6110 primers amplify a fragment with a length of 123bp, while the 18-mer TRC4 primers amplify a fragment with a length of 173bp.

(4) Cycling parameters - The reaction was performed on ice to minimize non-specificity. The cycling parameter used was initial denaturation at 950c for 5 min, followed by denaturation at 940c for 30 sec, annealing at 580c for 30 sec, extension at 720c for30 sec with 25 cycles and a final extension at 720c for 5 min. Detection of amplified products was done byagarose gel electrophoresis (2%) at 80 volts for 45 min. Gel was stained with ethidium bromide and viewed under UV transilluminator.

Statistical Methods

The analysis was done with help of computer software Epi - info version 6.0. The results were compared in terms of sensitivity, specificity and positive predictive value and negative predictive value of PCR vis- a- vis histopathology and culture. The positive and negative likelihood ratio along with 95% confidence interval was also reported.

Result

Among the 100 infertile patients taken in the study, 4 patients (4%) were positive on culture for Mycobacterium tuberculosis, 7 patients (7%) were positive on histopathological examination and 15 patients (15%) were positive on PCR (*Table1*).

On evaluation of different diagnostic tests, PCR was found to be highly sensitive, its sensitivity was 100% and specificity was 88.54%. Sensitivity of culture was 42.85% and specificity was 98.92%. Sensitivity of histopathology was 75% and specificity was 95.83% .

Discussion

The genitourinary tract is the second most common site for tuberculous infection , first being the lungs and it is mostly secondary to pulmonary tuberculosis. In communities where tuberculosis is still a major health problem, it is important to anticipate the possibility of genital tuberculosis in all patients presenting with infertility.

Histological diagnosis is a confirmatory test out of the various investigations available for genital tuberculosis.Histological diagnosis can be made by the presence of classical picture of granuloma, central caseation, Langhhans, giant cells and lymphocytes (9).Bacterial culture although has a specificity of 100% but a major problem observed is that weeks and months are recquired for precise identification of the species. In recent years, PCR has evolved as a rapid technique for the diagnosis of pulmonary and extrapulmonary tuberculosis.

Microbiological culture of the endometrium in Lowenstein Jenson medium in our study was found positive in only 4 samples, making it to 4% (*Table 1*) and the minimum time to see colonies in our patients was 4 weeks. Our study also shows similiar result of the culture studied by RBP Thangappah *et al* (8) (2011). In their study, they found that 4 out of 72 patients (5.6%) were culture positive. While as Roya Rozati *et al* (7) 2006 showed 12 out of 65 patients (7.8%) as culture positive. The possible reason for the low incidence of culture positivity in endometrial tissue could be due to paucibacillary nature and a substantial number of TB lesion of the genital tract are bacteriologically mute.

The low rate of positivity in culture may also be due to the presence of a bacteriostatic substance which inhibts the growth of the bacilli. In present study, sensitivity of culture found to be 42.85% and specificity was 98.92%. While as study done by Roya Rozati *et al* (7) shows sensitivity as 91.6% and specificity about 88.88% and RBP Thangappah *et al* (8) study shows

sensitivity 7.14% and specificity of 100%.

Histopathological examination is easy , cheap and not as consuming time wise as culture and provide characteristic feature of Mycobacertium tuberculosis. But due to secondary nature of the genital tuberculosis, the infecting organisms are sparse in number, the sampled site may not represent the infected area and the infected site can be easily missed. In as many as 50 percent of cases infection may be limited to fallopian tube. Moreever, due to the cyclical shedding of the endometrium, granulomas do not have enough time to form, so the endometrium may not show evidence of tuberculosis in all the cycles. The specimen obtained may be small and the blood flow may be scanty. In our study, 7(7%) endometrial samples were positive by histology (*Table 1*) similiar to that reported by R.B.P Thangappah et al (8) (2011). In our study, sensitivity of Histopathology was 75% and specificity was 95.83% (*Table 2*). Study conducted by Roya Rozati *et al* (7) shows sensitivity of histopathology about 82.3% and specificity about 84.6% while as study done by RBP Thangappah et al6 revealed sensitivity about 10.7% and specificity about 100%.

In our study, endometrial samples of all 100 patients were sent for PCR using IS6110 primer, the most widely used primer, we we found 15% patients as PCR positive. Out of these 15% PCR positive patients, 7% were histopathologicaly positive and 4% were positive on culture for Mycobacterium tuberculosis. The sensitivity of PCR found in our study was 100% and specificity was 89.47%. Study on PCR was done by different authors which reveal different results. Study by Roya Rozati (7) shows sensitivity about 96.45 and specificity about 100% while RBP Thangappah *et al* (8) revealed sensitivity about 57.14% and specificity as 90.5%.

Positivity to PCR with negative clinical findings could be due to the 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.(10) False positivity could occur by way of contamination, dead bacilli or previous infection or asymptomatic tuberculosis at another site. During this study stringent precautions were taken to avoid the problem of false positivity.

Conclusion

In countries where tuberculosis is endemic, asymptomatic patients present a big challenge for diagnosis, PCR represent a rapid and sensitive method for the detection of Mycobacterium DNA in female genital tuberculosis. Culture and histopathology has low sensitivity compared to PCR. Therefore, when the clinical suspicion is high and culture and histopathology is negative, PCR is the method of choice for identifying the infection.

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