The Journal of
OBSTETRICS AND GYNECOLOGY of India

J Obstet Gynecol India Vol. 59, No. 1 : January/February 2009 pg 67-70

Original Article

Diagnostic value of PCR in female genital TB and its therapeutic implications

Puri Suman[1], Bansal Bhavana[2]

[1] Asstt. Professor, [2] Senior Resident,

Department of Obstetrics & Gynaecology, Dayanand Medical College and Hospital, Ludhiana

Abstract

Objectives: To evaluate the diagnostic value of polymerase chain reaction (PCR) in female genital tuberculosis and to study conception rate after antituberculosis chemotherapy (ATT) in positive cases. *Method:* Sixty infertile patients were investigated for the presence of mycobacterium tuberculosis (MTB) by PCR of endometrial curettings. PCR demonstrated MTB DNA in 28 patients. ATT was started in diagnosed cases which were followed up for two years for conception. *Results:* All patients with laparoscopy suggestive of tuberculosis, more than 50% of those with a probable diagnosis and significant number of those with incidental findings were found positive for TB by PCR. Six PCR positive cases conceived within two years after taking ATT for 9 moths. Four patients conceived spontaneously and two patients with intrauterine insemination. *Conclusion:* PCR offered increased sensitivity in determining tuberculosis in female infertility and significant number of patients conceived after regular ATT.

Key words: polymerase chain reaction, genital tuberculosis, infertility, anti-tubercular treatment.

Introduction

Tuberculosis (TB) is an increasing public health concern worldwide. Genital TB is one form of extrapulmonary TB and is not uncommon. The global prevalence of genital TB is estimated to be 8-10 millions cases, with a rising incidence in the industrialized and developing countries partly as a result of its association with HIV virus infection and emergence of multidrug resistance. It is estimated that 5-13 percent of the females presenting in infertility clinics in India have genital TB and majority are in the age group of 20-40 years [1]. The actual incidence may be under reported due to asymptomatic presentation of genital TB and paucity of investigations. Genital TB frequently presents without symptoms and diagnosis requires a high index of suspicion [2]. It is estimated that at least 11% of the patients lack symptoms and genital TB is often detected in diagnostic workup of women attending infertility clinics [3]. The typical presentation of genital TB includes pelvic pain, menstrual irregularity, general malaise and infertility. Diagnosis of early TB is very difficult. Early diagnosis may be associated with a more favorable result before extensive genital damage occurs [4].

Paper received on 07/04/2007 ; accepted on 19/09/2008

Correspondence :
Dr. Puri Suman
66-C, Udham Singh Nagar
Ludhiana - 141 001
Tel. 0161-2300041, 098157-45300
Email : drsandeeppuri@yahoo.com

The abdominal and vaginal examination may be normal. A high erythrocyte sedimentation rate and a positive Montoux test are non-specific. The chest X-ray is normal in most cases. A pelvic ultrasound and hysterosalpingography may be of some help. Microscopic examination of acid-fast bacilli (AFB) requires presence of at least 10,000 organisms/ml in the sample. Mycobacterial culture is more sensitive compared to AFB microscopy, requiring as little as 10-100 organisms/ml. BACTEC radiometric culture had decreased the time required for bacteriologic confirmation to 2-3 weeks and also the rate of contamination is lower as it is a closed system. BACTEC has a sensitivity of 80-90% versus LJ (Lowenstein-Jensen) medium, which has a sensitivity of 30-40% [5]. Whether cultured by LJ medium or BACTEC, the detection of a positive culture depends on various factors: 1) Number of organisms in the specimen – heavy smear positive specimens may turn positive as early as 48 hours, but if bacterial load is low, it takes longer to grow the bacilli 2) Treatment status of the patient – if the patient is already on treatment, the bacilli are debilitated and may require a longer time to grow. All BACTEC cultures are maintained for 6 weeks and LJ culture for 8 weeks before being reported as negative. Besides technical drawbacks in demonstrating MTB in laboratory, a substantial number of TB lesions of genital tract are bacteriologically mute [3]. Culture is gold standard for diagnosis of genital TB. Rapid culture techniques like BACTEC can be used to save time in diagnosis. With new diagnostic molecular tests like PCR it is now possible to pick up latent endometrial TB [5]. Rapid nucleic acid amplification techniques such as PCR allow direct identification of M. tuberculosis on clinical specimens. It can detect less than 10 bacilli per ml of the specimen and the results are available within 1-2 days [5]. False positive cases reported in TB PCR are basically because of contamination from air inside the laboratory. Techniques like Real time PCR have markedly decreased the incidence of false positive cases because amplification and detection takes place in the same reaction tube. This is known as Mycoreal PCR and this method has been adopted by many laboratories since 1½-2 years. It has a sensitivity of 90-94% and specificity of 70-78%. Therefore it can be applied to specimens, where culture is difficult due to bacterial load [5].

Medical treatment is the main mode of therapy and availability of effective drugs has significantly decreased the requirement of surgical treatment in genital TB [4].

Method

Sixty patients, aged 15-40 years suspected of having genital TB and presenting to the infertility clinic of Dayanand Medical College, Ludhiana were investigated for the presence of mycobacterium tuberculosis. A protocol for fertility work up included complete history, examination, semen analysis, ultrasonography, endometrial biopsy and hysteroscopy with laparoscopy. Endometrial biopsy was done on the first day of menstruation within 6 hours. Sample was taken from the endometrium especially from both cornual ends and sent for PCR amplification. Hysteroscopy and diagnostic laparoscopy was performed in all the sixty patients to look for the status of fallopian tubes, presence of any granulations/caseation on the tubes and uterus or presence of adhesions.

After confirmation of the diagnosis of genital TB, ATT was started. Treatment was given in two phases. During the initial phase (2 months), the drugs used were isoniazid, rifampicin, pyrazinamide and ethambutol with vitamin B_6. During the continuation phase, treatment was continued for a period of further 7 months with isoniazid and rifampicin. Dosages of all the drugs are given in Table 1.

The patients were followed up for a period of two years for conception. In order to improve pregnancy success rates, in some patients, ART like intrauterine insemination (IUI) was done.

Results

Sixty patients were analyzed to rule out genital TB in unexplained infertility. In group I, 31 patients were analyzed during the years 2001-2004, which were followed up for 2 years and in group II, 29 patients were analyzed during the years 2005-2006, which we are still following up. Table 2 shows clinical details of the patients. Out of the 31 patients in the first group, 15 cases demonstrated MTB DNA and out of 29 patients in the second group, 13 cases were positive by PCR amplification. False positive rate was 7.14% and false negative rate was 3.84%. Table 3 shows the comparison of various diagnostic tests.

All the PCR positive cases in the first group (15) were followed up for 2 years for conception after giving ATT for 9 months. Six patients conceived within two years (4 spontaneously and 2 with IUI). We are still following 13 cases out of the 29 and there was no conception till

Table 1. Dosage for treatment of genital TB.

Drugs	Three times a weeks
• Isoniazid	600mg
• Rifampicin	450 mg [a]
• Pyrazinamide	1500 mg
• Ethambutol	1200 mg
• Vitamin B6	10 mg [b]

[a] patients with weight =60 kg were given an extra 150mg dose of rifampicin
[b] vitamin B_6 was given till pyrazinamide was given.

Table 2. Clinical details of patients.

Parameter	Number	
	Group I	Group II
Age (years)	2001-2004	2005-2006
15-20	1	0
21–25	16	8
26-30	12	18
31-35	2	3
36-40	0	0
Symptoms		
Infertility		
- Primary	22	16
- Secondary	9	13
Menstrual irregularity		
- Amenorrhea	1	0
- Menorrhagia	1	0
- Oligomenorrhea	5	3
- Dysmenorrhea	4	2
Pain abdomen	7	3
Abdominal mass	0	0
General malaise	5	4

Table 3. Comparison of various diagnostic tests.

Test	Positive cases	
	Group I	Group II
	2001–2004	2005-2006
Hysteroscopy	1	3
Laparoscopy	11	8
PCR	15	13

now. Tubal patency was present in 55/60 (91.67%) and was absent in 5/60 (8.33%) of the cases.

Discussion

Female genital tuberculosis is an important cause of infertility. Early diagnosis and treatment in young patients with genital TB may improve the prospects of care, before the tubes are damaged beyond recovery. Culture of Mycobacterium TB remains the gold standard of diagnosis of genital TB, but early TB being a paucibacillary disease, can be missed in culture which can only be detected in PCR. PCR can detect less than 10 bacilli per ml of specimen. If the patients are adequately treated before their tubes are irreversibly damaged, the chance of successful pregnancy is reasonably good with a 20% pregnancy rate reported in one study[6].

In our study, out of total of the 60 patients investigated for unexplained infertility, 46.67% i.e. 28/60 cases came out to be positive for MTB by PCR. Hysteroscopy suggested evidence of TB in 6.67% i.e 4/60 cases and laparoscopy suggested evidence in 19 out of 60 positive cases i.e. 31.67%. While laparoscopy generally detects macroscopic changes such as peritubal adhesions, tubercles on the tubes and small tubo-ovarian masses that commonly are seen in chronic cases, GTB presents unique diagnostic challenges including subtle clinical manifestations that may be overlooked on laparoscopy during early stages of infection[3].

In group I, out of the 31 patients on follow-up, 6 cases conceived within 2 years with conception rate of 19.35%. Regular and complete treatment increases the conception rate without the need for surgical treatment. It is possible only when the disease is detected earlier and treatment is started immediately.

Gene amplification techniques are highly sensitive and under optimum conditions may detect 1-10 organisms. If systems are adequately standardized, evaluated and precautions for avoiding the contamination are taken, these assays can play a very useful role in early confirmation of diagnosis in paucibacillary extra-pulmonary forms of tuberculosis. A variety of PCR methods have been developed for detection of specific sequences of M. tuberculosis and other mycobacteria. These PCR assays may target either DNA or rRNA/ and these could be based on conventional DNA based PCR, nested PCR and RT-PCR. Targets include insertion and repetitive elements, various protein encoding genes, ribosomal rRNA etc. Indian laboratories have

been active in the development of PCR methods for detection of M. tuberculosis. Different Indian investigators have used separate gene targets like MPB 64, repetitive sequences, GC repeats, devR, 38kD, TRC 4, IS 1081 and a system patented by Central Drug Research Institute (CDRI), Lucknow. Some of these assays (CDRI) have been repeatedly found to be reproducible, highly sensitive and specific in double blind evaluations. IS-1081 based system has been further modified and a new nested PCR target of this gene has been developed at the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad. These assays have been reported to be quite promising in confirming the diagnosis of different forms of tuberculosis. A PCR assay system for tuberculosis which is commercially available has been found and reported to be reproducible, sensitive as well as specific. These methods can also be adapted for in situ application for confirmation of histological diagnosis. Real time PCR has been investigated for rapid and specific detection of M. tuberculosis in the clinical specimens. This strategy can be used for confirming the diagnosis and also monitoring the progress.

There has been a genuine concern of false positivity due to contamination occurring in clinics and laboratories. The problems of false positivity can be substantially reduced by proper laboratory design, strict discipline about collection and processing of the specimens, handling of reagents and use of certain blocking reagents. Further, the application of in situ PCR approach removes the doubts about contamination and will be very useful to the pathologists for arriving at a confirmed diagnosis. In case of false negative results several strategies can be used to improve the sensitivity. While there are individual problems of appropriate sample collection, extraction and assay, very small number of organisms and inhibitors in paucibacillary specimens are specially important. It has been observed that by using immunomagnetic beads and capture resins, the sensitivity of PCR assays can be significantly improved.

Conclusion

As we know that female genital TB is a paucibacillary disease and if detected in the early state and treated, can improve conception rate significantly, PCR represents rapid and sensitive method for detection of mycobacterium DNA in early female genital TB and may be a useful adjunct to diagnostic modalities in genital TB.

References

1. Agarwal J, Gupta JK. Female genital tuberculosis – a retrospective clinicopathologic study of 501 cases. Indian J Pathol Microbiol 1993;36:389-97.
2. Arora VK, Gupta R, Arora R. Female genital tuberculosis – need for more research. Indian J Tuberc 2003;50:9-11.
3. Bhanu NV, Singh UB, Chakraborty M et al. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. J Med Microbiol 2005;54:927-31.
4. Gatongi DK, Gitah G, Kay V et al. Female genital tuberculosis. Obstet Gynaecol 2005;7:75-9.
5. Shetty J, Kumar P, Ramkumar V et al. Management of female genital tuberculosis: re-appraised. Obstet Gynaec Today 2006;11:506-9.
6. Sin SY. Female genital tuberculosis: an update. Hong Kong Pract 1995;17:18-25.