SHORT ARTICLE

Diagnostic Value of Polymerase Chain Reaction in Female Tuberculosis Leading to Infertility and Conception Rate After ATT

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Abstract

To evaluate diagnostic value of PCR (Polymerase chain reaction) in female genital tuberculosis and to study conception rate after ATT (Anti tubercular treatment) in positive cases. Sixty infertile patients were investigated for 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.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 positive by PCR. Six PCR positive cases conceived within two years after taking ATT for 9 months. Four patients conceived spontaneously and two patients with intrauterine insemination. PCR offered increased sensitivity in determining tuberculosis etiology in female infertility and significant number of patients conceived after regular ATT.

Key Words

Genital Tuberculosis, Infertility, Polymerase Chain Reaction, Conception

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 counties partly as a result of its association with HIV virus infection and emergence of multidrug resistance. It is estimated that 5-13 percent of females presenting in infertility clinics in Indian have genital TB and majority are in 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 patients lack symptoms (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).

The abdominal and vaginal examinations 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 to 3 weeks and also rate of contamination is lower as it is a closed system. BACTEC has a sensitivity of 80-90 percent versus LJ (Lowenstein-Jensen) medium, which has

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sensitivity of 30-40 percent (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 the 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 the 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 polymerase chain reaction (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 1 ½-2 years. It has 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).

Material and Method

Sixty patients, aged 15-40 years presenting to the infertility clinic, Dayanand Medical College, Ludhiana were investigated for presence of mycobacterium tuberculosis, suspected of having genital TB. A protocol for fertility work up included complete history, examination, semen analysis, ultrasonography, endometrial biopsy and hysteroscopy with laparoscopy.

Endometrial biopsy was done on first day of menstruation within 6 hrs. Sample was taken from the endometrium especially from both cornual ends and sent for PCR amplification. Hysteroscopy and diagnostic laparoscopy was performed in all sixty patients to look for status of fallopian tubes, presence of any granulations/ caseation on tubes and uterus or presence of adhesions.

After confirmation of diagnosis of genital TB, ATT was started. Treatment is given in two phases. During the initial phase (2months), drugs used are isoniazid, rifampicin, pyrazinamide and ethambutol with vitaminB6. During continuation phase, treatment is continued for a period of further 7 months with isoniazid and rifampicin. Dosages of all the drugs are given in *table-1*.

Patients were followed up for 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 in year 2001-2004, which were followed up for 2 years and in group II, 29 patients were analyzed in 2005-2006, which we are still on follow up. *Table 2* shows clinical details of patients. Out of 31 patients in first group, 15 cases demonstrated MTB DNA and out of 29 patients in second group, 13 cases were positive by PCR amplification. *Table 3* shows comparison of various diagnostic tests.

All the PCR positive cases in 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 patients with IUI). We are still following 13 cases out of 29 and there was no conception till now.

Table 1: Dosage for Treatment of Genital TB

Drugs	Dose
Isoniazid	600 mg
Rifampicin	450 mg [a]
Pyrazinamide	1500 mg
Ethambutol	1200 mg
Vitamin B_6	10 mg[b]

[a] patients weight ³ 60kg given an extra 150mg dose rifampicin.[b] vitamin B_6 was given till pyrazinamide given. (TID)

Table 2: Clinical Details of Patients

	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
35 – 40	0	0
Infertility		
- Primary	22	16
-Secondary	9	13
Menstrual irregularity		
- Amenorrhea	1	0
- Menorrhagia	1	0
- Oligomenorrhoea	5	3
- Dysmenorrhoea	4	2
Pain abdomen	7	3
Abdominal mass	0	0
General malaise	5	4

Table 3: Comparison of Various Dignostic Tests

	Postive Test		
Test	**Group-I**	**Group-II**	**Total**
	2001-2004	**2005-2006**	
Hysterscopy	1	3	4/60(6.67%)
Laproscopy	11	8	19/60(31.67%)
PCR	15	13	28/60(46.67%)

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 damage, beyond recovery. If patients are adequately treated before their tubes are irreversibly damaged, the chance of successful pregnancy of reasonably good with a 20% pregnancy rate reported in one study (6).

In our study, PCR detected 46.67% (28/60) of the suspected cases. Hysteroscopy showed 6.67% (4/60) positive cases and Laparoscopy showed 31.67% (19/60) positive cases. Our result depicted that PCR is best method of diagnosing genital TB.

Out of 31 patients, 6 cases conceived within 2 years with conception rate of 19.35 percent. Regular and complete treatment increases the conception rate without the need of surgical treatment. It is possible only when disease is detected earlier and treatment is started immediately.

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.

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