

CASE REPORT

Utility of PCR in the Diagnosis of Female Genital Tuberculosis

Gill Manmeet Kaur, Aggarwal Aruna

Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab

ABSTRACT

Genital tuberculosis (GTB) is one of the major causes of infertility among females. Since the infection is asymptomatic in most of the cases, it makes the clinical diagnosis quite difficult. We report a case of a 24 years old female with infertility that was discovered to have endometrial tuberculosis, detected by PCR using IS6110. The culture report was negative and histopathological examination indecisive. She was given anti-TB therapy and subsequently had a pregnancy with a live birth. Although conventional methods like culture on Lowenstein-Jensen medium and histopathology are important in the diagnosis of such cases but the molecular methods like PCR can be a highly useful aid in the establishment of a rapid and early diagnosis of genital and other forms of extra-pulmonary tuberculosis. *J Microbiol Infect Dis 2014;4(2): 72-74*

Key words: Genital tuberculosis, Mycobacterium tuberculosis, nested polymerase chain reaction, IS6110 sequence.

Kadın genital tüberkülozunun tanısında PCR kullanımı

ÖZET

Genital tüberküloz (GTB) kadınlarda kısırlığın en önemli nedenlerinden biridir. GTB'nin klinik tanısı, olguların çoğunun asemptomatik veya çok farklı klinik görünümünde olması nedeniyle, zordur. Biz 24 yaşında, PCR'da IS6110 probu kullanılarak endometrial tüberküloz tanısı konulan, infertil bir kadın hastayı sunduk. Hastanın kültür sonuçları negatifti ve histopatolojik incelemesi tanı koyduruyordu. Hastaya antitüberküloz tedavi verildi ve ardından hamile kalarak canlı bir bebek doğurdu. Lowenstein-Jensen besiyerinde kültür ve histopatoloji gibi geleneksel yöntemler bu gibi olguların tanısında oldukça önemlidir; ancak PCR gibi moleküler metotlar, genital tüberküloz ve diğer akciğer dışı tüberküloz olgularında hızlı ve erken tanı koymada çok faydalı olabilmektedirler.

Anahtar kelimeler: Genital tüberküloz, Mycobacterium tuberculosis, nested polimeraz zincir reaksiyonu, IS6110 sekansı

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease with a worldwide distribution and the incidence is high in developing countries. Genital tuberculosis (endometrial and salpingoophoritic) is an important cause of infertility in women.¹ Genital tuberculosis is mostly asymptomatic but can sometimes present as infertility. Various Indian studies have shown that tuberculous endometritis and salpingitis account for 4%-9% of all infertility cases.^{2,3}

Since the clinical diagnosis of genital tuberculosis is difficult due to the asymptomatic nature/ varied clinical presentation; so for definite diagnosis a positive culture or tissue biopsy revealing specific histopathological lesion in the specimen is required. However, these methods have their limita-

tions as GTB is paucibacillary in nature. In recent years, polymerase chain reaction (PCR) technique has evolved as a useful tool for rapid and accurate diagnosis of pulmonary and extra-pulmonary tuberculosis.⁴ We report a case of a 24-year old female with infertility that was discovered to have genital tuberculosis by PCR using IS6110, with culture report negative and histopathological examination indecisive.

CASE

A 24 years old female presented at the Gynecology Clinic of our hospital with the complaints of inability to conceive even after 5 years of her marriage despite adequate unprotected sexual intercourse. She had no history of chronic cough or fever suggestive

Correspondence: Manmeet Kaur Gill, 61, Gill Niwas, Guru Ram Das Avenue, Ajnala road, Amritsar, Punjab
Email: minna_mahl@yahoo.com

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of tuberculosis. Her erythrocyte sedimentation rate (ESR) was 25 mm for first hour, Mantoux test was negative and skiagram chest did not show any pleural or parenchymal abnormality. However, Enzyme Linked Immunosorbent Assay (ELISA) was reactive for TB IgM. Ultrasonography report of uterus showed highly echogenic endometrial cavity.

Dilation & curettage (D&C) was done and the endometrial curetting were received in our microbiology lab in normal saline. A portion of the endometrial tissue, fixed in 10 percent formalin was also sent for histopathological studies. However histopathology report was indecisive and was advised to repeat the biopsy sample.

The tissue was apportioned and was processed for Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB), culture on Lowenstein-Jensen (LJ) medium and single tube nested PCR using primers targeting IS6110 gene sequence of Mycobacterium tuberculosis which amplify a fragment with a length of 123bp. Nested primers were obtained from Bangalore Genei, Bangalore, India.



Lane 1. DNA molecular weight marker (500-100bp above downwards)

Lane 2. Negative control with no band

Lane 3. Sample 1 (endometrial biopsy) showing band at 340bp and 123, result is positive

Lane 4. Sample 2 (endometrial biopsy) showing band at 340bp only, result is negative

Lane 5. Sample 3 (pleural fluid) showing band at 340bp only, result is negative

Smear was negative for acid fast bacilli, culture on LJ medium showed no growth even after six weeks of incubation. PCR assay was positive.

Throughout the PCR processing, recommended precautions were taken to avoid contamination also appropriate positive and negative controls were included in the test to avoid false positive results.

The patient was referred to the RNTCP center in the institute where she was treated for TB using the Directly Observed Treatment Short Course (DOTS) strategy. Eleven months after initial visit and commencement of anti-TB therapy she conceived.

DISCUSSION

Genital tract TB accounts for about 9 percent of all extra-pulmonary tuberculosis cases. It is a chronic disease that often presents with low grade symptomatology and very few specific complaints. Presenting symptoms are generally varied; infertility being the most frequent clinical presentation (43-74%).⁵ The diagnostic tests like imaging, laparoscopy, histopathology, bacteriological and serological tests, also have their limitations regarding sensitivity and specificity.

Extra-pulmonary lesions are paucibacillary in nature therefore conventional techniques like microscopy for AFB and culture suffer from low sensitivity. In this patient also both were negative. Histopathological examination is easy, quick and cheap and provides characteristic features of MTB but due to the 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. This could have been the reason for indecisive histopathology in this case also.

However molecular diagnostic methods like PCR hold the key to better and efficient diagnosis of genital and other forms of extra-pulmonary TB. Several authors have explored the application of PCR based diagnosis of female genital tract TB infections to aid in rapid and improved diagnosis. Bhanu et al processed endometrial biopsy specimen using mpt64 based PCR, Kumar et al used nested PCR for hupB DNA target, Thangappah et al used two sets of primers IS6110 and TRC4.⁶⁻⁹ A recent study by Jindal et al also reported favorable infertility treatment outcome following anti-tubercular treatment, prescribed on basis of positive PCR.¹⁰

We used the most widely used primers i.e. IS6110 to detect Mycobacterium tuberculosis by single tube nested PCR and it was positive in this case. However, it has already been reported that some strains of M. tuberculosis isolated from pa-

tients may have only few copies of IS6110 or may not carry even a single copy.¹¹ But to overcome this problem multiplex PCR with combination of suitable primers targeting repetitive sequences and species specific gene sequences can be used.

To conclude, this case highlights the need to consider the possibility of genital tuberculosis in the etiology of infertility even in apparently low risk patients. A good history taking, correct sampling and use of multiplex PCR can help in the diagnosis of the disease in its early stage, so that anti-tubercular treatment improves the prospects of cure and permanent damaged can be avoided.

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