BRIEF REPORT

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Molecular diagnosis of tuberculosis: a new primer design

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ABSTRACT

Background: Nucleic acid based detection of Mycobacterium tuberculosis has the potential to improve the analysis of the tuberculosis epidemiology and patient care by increasing the specificity and sensitivity of diagnosis.

Patients and methods: Fifty suspected cases were screened for M. tuberculosis by using a new primer sequence and comparing its results with the primer (IS6110) most widely used for tuberculosis diagnosis.

Results: The mean age of cases was 33.6 ± 14.3 years. Twenty one cases (42%) were positive for M. tuberculosis. Maximum positivity was found in sputum (10/19) followed by menstrual blood (4/8), blood (5/15), ascitic fluid (1/2), and pleural fluid (1/5).

Conclusion: The new designed primer was reproducible and in complete concordance with the PCR-results obtained by the standard method using IS 6110 insertion sequence in all 50 cases in which both methods were used.

Keywords: *Tuberculosis, Nucleic acid amplification, Molecular diagnosis.* (Iranian Journal of Clinical Infectious Diseases 2009;4(2):105-108).

INTRODUCTION

Tuberculosis (TB) is a clinical disease caused Mycobacterium tuberculosis and by is characterized by granuloma (1). The World Health Organization has declared tuberculosis to be global emergency causing nearly 3 million deaths yearly (2-4). India accounts for one-fifth of the global TB incident cases each year, over 19 million people in India develop TB of which around 8.7 million are infectious cases and it is estimated that annually around 325000 Indians die due to TB (5). Today several methods for the diagnosis of tuberculosis are available which include tuberculin test, radiological examination and other imaging methods and sputum smear microscopy. During the

Received: 21 April 2008 Accepted: 21 July 2008 Reprint or Correspondence: Syed Tasleem Raza. Department of Biochemistry, ERA'S Lucknow Medical College, Lucknow, India. 226003 E-mail: tasleem24@gmailcom past 10 years several molecular methods have been developed for direct detection, identification and susceptibility testing of mycobacteria. These methods can potentially reduce the diagnostic time from weeks to days. Based on this newer knowledge about the specific gene sequence, several gene probes/gene amplification systems for tuberculosis have been developed (6-10). These molecular tools and methods can be used for the confirmation of identity of isolates (11,12), direct detection of gene sequences from the clinical specimens (13) and also molecular detection of drug resistance including the use of pyrosequencing (14). The most widely used method for DNA diagnosis of tuberculosis is to detect the insertion sequences IS6110 (15).

In the present study, we have designed a new set of primers for amplifying the conserved

sequences at Rv0508 (16) region to detect M. tuberculosis and tested its utility in 50 suspected cases of tuberculosis.

PATIENTS and METHODS

Fifty specimens from suspected cases were collected from in-patient door (IPD) and outpatient door (OPD) from TB, Chest Medicine and Gynaecology departments of ERA'S Lucknow Medical College and Hospital Lucknow (North India) with prior informed consent.

Mycobacterium bacterial DNA was extracted both manually (17) and by means of a kit (Bangalore, Genei, India).

PCR amplification: PCR was performed using new primer in a MJ Mini thermal cycler (Bio Rad, UK). The final volume of 20µl containing genomic DNA 10p moles of forward and reverse primers in reaction mixture at a concentration of 1X 10mM of each dNTP's and 1 unit of Taq polymerase. The tubes were kept at 95°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and final extension at 72°C for 10 minutes. Forwards and reverse primers were as follow:

F:GAACTGGGCTTCGACATGAT (20bp) R:ATCAGGTGGGCTACCAAATG (20bp)

These primers were designed for specific amplification of conserved sequences at Rv0508 region of the M. tuberculosis. In all 50 cases, PCR amplification and primer design were achieved to check for consistency of results with the existing primer IS 6110.

Five μ l PCR products of samples was loaded along with molecular weight marker on 2% agarose gel stained with ethidium bromide and viewed with gel documentation system. The sizing of amplified 192 bp fragments was achieved using Biovis Gel ID Software (Expert Vision, Mumbai, India). The presence of 192 bp was considered as positive for M. tuberculosis. Primer design methods: Our oligonucleotide primer for Rv0508 region of the M. tuberculosis was designed by using primer analysis software (Just Bio Com) available online at: http://www.justbio.com). The Tm of the primers was calculated in the program Oligo calculator based on % GC content.

RESULTS

Fifty suspected cases were screened for the presence of M. tuberculosis. The mean (\pm SD) age of cases was 33.6 \pm 14.3 years. Specimen included blood (15 cases), menstrual blood (8), sputum (19), pleural fluid (5), cerebral spinal fluid (1) and ascitic fluid (2). Twenty one cases (42%) were positive for M. tuberculosis, among which maximum positivity was observed in sputum (10/19), followed by menstrual blood (4/8), blood (5/15), ascitic fluid (1/2), and pleural fluid (1/5).

DISCUSSION

The conventional methods for diagnosing tuberculosis (sputum microscopy, chest radiology, histopathology, and tuberculin test) have some limitations including low specificity and sensitivity as well as time-consuming. Certainly, rapidity and simplicity of amplification techniques can greatly assist TB diagnosis and treatment.

Various gene amplification techniques have demonstrated their utility in the diagnosis of pulmonary and extrapulmonary forms of tuberculosis (6-10,18-21).

The rapid detection of M. tuberculosis by PCR or other gene amplification techniques may be the standard laboratory method for diagnosing tuberculosis and offer new opportunities for laboratory testing to improve the diagnosis and management of illness in patients with tuberculosis.

The newly designed primer sequence gave reproducible results with minimal dimer formation

and less non-specific bands and may be used in low infrastructure laboratories for the nucleic acid based detection of M. tuberculosis.

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