Iranian Journal of Clinical Infectious Diseases
2009;4(2):105-108

BRIEF REPORT

Molecular diagnosis of tuberculosis: a new primer design

Syed Tasleem Raza*, Gulraiz Ali, Sikandar Hasan, Farzana Mahdi
Department of Biochemistry, ERA'S Lucknow Medical College, Lucknow, India

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 by Mycobacterium tuberculosis and 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 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

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

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 out-patient 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 µl 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 (±SD) age of cases was 33.6±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.

ACKNOWLEDGEMENT

The study was achieved by the financial support of Intramural Grant from ERA'S Lucknow Medical College, Lucknow, India.

REFERENCES

1. Centers for Disease Control and Prevention: Case definitions for infectious conditions under public health surveillance. MMWR Morb Mortal Wkly Rep 1997;46:1-55.

2. Centers for Disease Control and Prevention: Trends in tuberculosis in United States, 2004. MMWR Morb Mortal Wkly Rep 2005;54:245-9.

3. Centers for Disease Control and Prevention: Epidemiologic notes and reports expanded tuberculosis surveillance and tuberculosis morbidity, United States, 1993. MMWR Morb Mortal Wkly Rep 1994;43:361-6.

4. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 1999;282(7):677-86.

5. Communicable Diseases and Disease Surveillance tuberculosis, 2008. Available via DIALOG. http://wwwwhoindiaorg/en/Section3/Section123htm Accessed March 27, 2008.

6. Suffys PN, Silva Rocha AD, Oliviera MD. Rapid identification of Mycobacteria to the species level using INNO-LIPA mycobacteria a reserve hybridization assay J Clin Microbiol 2001;39:4472-82.

7. Traore H, van Deun A, Shamputa IC, Rigouts L, Portaels F. Direct detection of Mycobacterium tuberculosis complex DNA and rifampin resistance in clinical specimens from tuberculosis patients by line probe assay. J Clin Microbiol 2006;44(12):4384-8.

8. Ritis K, Tzoanopoulos D, Speletas M, Papadopoulos E, Arvanitidis K, Kartali S, et al. Amplification of IS6110 sequence for detection of Mycobacterium tuberculosis complex in HIV-negative patients with fever of unknown origin (FUO) and evidence of extrapulmonary disease. J Intern Med 2000;248(5):415-24.

9. Katoch VM. Newer diagnostic techniques for tuberculosis. Indian J Med Res 2004;120:418-28.

10. Rogerio CN, Sibelle B, Odir AD. Molecular inversion probes for sensitive detection of Mycobacterium tuberculosis. Journal of Microbiological Methods 2008;72:60-6.

11. Gascoyne-Binzi DM, Barlow RE, Frothingham R, Robinson G, Collyns TA, Gelletlie R, et al. Rapid identification of laboratory contamination with Mycobacterium tuberculosis using variable number tandem repeat analysis. J Clin Microbiol 2001;39(1):69-74.

12. Huard RC, Lazzarini LC, Butler WR, van Soolingen D, Ho JL. PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. J Clin Microbiol. 2003;41(4):1637-50.

13. Yam WC, Cheng VC, Hui WT, Wang LN, Seto WH, Yuen KY. Direct detection of Mycobacterium tuberculosis in clinical specimens using single-tube biotinylated nested polymerase chain reaction-enzyme linked immunoassay (PCR-ELISA). Diagn Microbiol Infect Dis 2004;48(4):271-5.

14. Zhao JR, Bai YJ, Wang Y, Zhang QH, Luo M, Yan XJ. Development of a pyrosequencing approach for rapid screening of rifampin, isoniazid and ethambutol-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2005;9(3):328-32.

15. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993;31(2):406-9.

16. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998;393(6685):537-44.

17. Amita J, Tiwari V, Guleria RS. Qualitative evaluation of mycobacterial DNA extraction protocols for polymerase chain reaction. Molecular Biology Today 2002;3:43-49.

18. Abe C, Hirano K, Wada M, Kazumi Y, Takahashi M, Fukasawa Y, et al. Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J Clin Microbiol 1993;31(12):3270-4.

19. Clarridge JE 3rd, Shawar RM, Shinnick TM, Plikaytis BB. Large-scale use of polymerase chain reaction for detection of Mycobacterium tuberculosis in

a routine mycobacteriology laboratory. J Clin Microbiol 1993;31(8):2049-56.

20. Ichiyama S, Iinuma Y, Tawada Y, Yamori S, Hasegawa Y, Shimokata K, et al. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and Roche PCR-microwell plate hybridization method (AMPLICOR MYCOBACTERIUM) for direct detection of mycobacteria. J Clin Microbiol 1996;34(1):130-3.

21. Gamboa F, Manterola JM, Viñado B, Matas L, Giménez M, Lonca J, et al. Direct detection of Mycobacterium tuberculosis complex in nonrespiratory specimens by Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J Clin Microbiol 1997;35(1):307-10.