RESEARCH PAPER | Medical Science | Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

A Clinico-Microbiological Study of Extrapulmonary Tuberculosis in Patients Attending a Tertiary Care Centre

KEYWORDS: Extrapulmonary Tuberculosis, Lowenstein Jensen medium, Microscopic examination, Real Time PCR

Mr. Rakesh Prasad Sah	Dr. Anahita V. Bhesania Hodiwala	Dr. Harapriya Kar
Post Graduate, Microbiology, MGM Medical College, Navi Mumbai-410209	Professor, Microbiology department MGM Medical College, Navi Mumbai-410209	Asst. Professor, Microbiology department, MGM Medical College, Navi Mumbai-410209

Dr. A.D. Urhekar	Mr. Rakesh Kumar Mukhia	B. L. Chaudhary
HOD, Professor, Microbiology department, MGM Medical College, Navi Mumbai.	Post Graduate, Microbiology, MGM Medical College, Navi Mumbai	Post Graduate, Microbiology, MGM Medical College, Navi Mumbai

ABSTRACT *Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis. Annually, about nine million people contract tuberculosis and nearly two million are killed by the disease. Extra-pulmonary tuberculosis (EPTB) increases world over with advent of HIV. The sensitivity of the conventional methods ranges from 40-80% whereas, sensitivity of PCR ranges from 90-100%. 60 clinically suspected patients of EPTB lesions were analyzed over a period of one year. Samples were subjected for ZN staining, Lowenstein-Jensen culture media and Real Time PCR respectively. Lymph nodes were the most common site of clinically suspected EPTB lesions. The sensitivity and specificity of ZN staining was 67% & 100% and RT-PCR was 100% & 96% respectively, Culture was the "Gold Standard". Real Time PCR with the two conventional diagnostic modalities of ZN staining and Culture definitely enables to diagnose a challengeable diagnosis of extra-pulmonary tuberculosis which certainly helps to initiate timely anti-tubercular treatment.*

Introduction

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*, has been recorded in history since the Greco-Roman and Egyptian civilizations, with evidence of spinal tuberculosis being recorded as long ago as 3400 BC. Ancient Indian scriptures also mention this disease, with the first known description of tuberculous spondylitis being written in Sanskrit sometime between 1500 and 700 BC [1]. However, the modern name of the disease has been attributed to Laennec in the 1800s [2].

It has been postulated that M. tuberculosis existed as an unimportant pathogen to man until the coming of the industrial revolution [3]. With resulting urbanization and propinquity of living, a new epidemic, described as 'a great white plague', evolved. Tuberculosis is the second most common cause of death from infectious disease (after HIV) [4]. Annually, about nine million people contract tuberculosis and nearly two million are killed by the disease [5]. 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 [6].

In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% (range 63–68%) of the estimated number of incident cases in 2010. India and China accounted for 40% of the world's notified cases of TB in 2010, Africa for a further 24% and the 22 high-TB burden countries (HBCs) for 82% [7]. Despite the accelerated efforts to control the disease for decades, it remains the seventh leading cause of death globally [8].

Tuberculosis typically affects the lungs but can affect other sites as well [9]. Broadly, TB may be classified as pulmonary (occurring in lungs) or Extra-pulmonary (occurring outside the lungs) [10]. EPTB is the result of dissemination of tubercle bacilli from an initial focus in the lungs soon after primary infection [11]. The dissemination is primarily by way of the lymphohematogenous route with seedling of virulent tubercle bacilli in almost all of the organs and tissues of the body [12].

HIV/AIDS AND TUBERCULOSIS

It is well established that the impairment of the immune system as a result of human immunodeficiency virus (HIV) infection predisposes to the development of tuberculosis and the disease is now regarded as a "sentinel" manifestation of the progression from HIV to AIDS [13-15].

Materials and Methods:

This comparative study was conducted over a period of one year in the department of microbiology, MGM Medical College, Navi Mumbai. 60 clinically suspected extra-pulmonary tuberculosis samples were taken & processed for ZN staining, Culture and Real Time PCR.

Specimen processing

The samples were decontaminated by NALC (N-acetyl-L-cysteine) -NaOH (sodium hydroxide) method. Briefly, the NALC-NaOH methods involved the decontamination and digestion of the clinical samples with 2 percent NaOH (final concentration) in 0.5 percent NALC and concentrated by centrifugation at 3600g for 15 min. Supernatant was discarded and to sediment, 1-2 ml of sterile phosphate buffer of *p*H 6.8 (1 to 2 ml) was added and centrifuged for 15 minutes at 3600g [16,17]. Deposit was used for smear examination by Ziehl-Neelsen method, culture on Lowenstein Jensen Medium slant and DNA extraction for Real Time PCR. The inoculated LJ slants were incubated at 37^{0}C for 6-8 weeks and examined every second day during the first week and then weekly for up to 6 weeks for presence of growth. The growth if present was confirmed by colony morphology and ZN staining.

Polymerase chain reaction

DNA extraction from specimens

DNA extraction was done by following the protocol of com-

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

mercially available QIAGEN DNA extraction kit.

Real Time PCR
Real Time PCR for detection of Mycobacterial DNA was performed according to the manufacturer's kit instruction (Professional Biotech Ltd) using Roche Light Cycler 480 (LC 480).

Preparation for the PCR amplification:
Depending upon the number of samples the following pipetting scheme was followed e.g.

MTC/MOTT MASTER MIX	1 REACTION	10 REACTIONS
MTC/MOTT SUPER MIX (R1)	12 µl	120 µl
MTC/MOTT Mg SOLUTION (R2)	2.5 µl	25 µl
IC-1 (R3) RG	0.5 µl	5 µl
TOTAL	15 µl	150 µl

NOTE :- MTC = Mycobacterium Tuberculosis Complex, MOTT = Mycobacterium Other than Tuberculosis.

15 µl of the Master Mix was pipetted into the each labelled PCR tube. Then 10 µl of the earlier extracted DNA was added to each sample tube and mixed by pipetting up and down. Correspondingly, 10 µl of the Positive Control was used as a positive control and 10 µl of water (PCR grade) as a negative control. The PCR tubes were closed and tubes were transferred into the rotor of the instrument.

Amplification Cycle: It consists of following steps:-

S. No.	Steps	Temperature	Time
1.	Holding	95^{0}C	10 min.
2.	Denaturation	95^{0}C	15 sec.
3.	Annealing	55^{0}C	20 sec.
4.	Extension	72^{0}C	15 sec.

The above steps were run for 45 cycles.

Result : A total of 60 clinically suspected patients of EPTB lesions were analyzed. The ZN smear detected AFB in 6 samples, LJ culture showed growth on 9 samples and 11 samples showed positivity by RT-PCR.

Number of Samples and Positive Cases:

Type of Sample	No. of Specimens	Smear positive	Culture positive	PCR positive
Lymph node aspirate (pus)	22	1	2	4
Pleural Fluid	10	2	4	4
CSF	8	0	0	0
Urine	8	1	1	1
Ascitic Fluid	4	0	0	0
Stool	3	1	1	1
Synovial Fluid	3	0	0	0
Peritoneal Fluid	2	1	1	1

Comparison of results of the three diagnostic tools:

ZN	CULTURE	PCR	NO. OF CASES
Positive	Positive	Positive	6
Negative	Positive	Positive	3
Negative	Negative	Positive	2
Negative	Negative	Negative	49
Total			60

Out of 60 total suspected cases, only 6 cases were positive for EPTB lesion by all three diagnostic tools. Whereas, 49 cases were confirmed negative for EPTB lesion by all three diagnostic techniques.

Comparison of sensitivities and specificities of ZN staining and PCR. Culture is the gold Standard.

METHODS	SENSITIVITY	SPECIFICITY	PPP	NPV
ZN	67%	100%	100%	94.4%
PCR	100%	96%	82%	100%

The results of the conventional methods i.e. ZN staining and PCR technique were compared with gold standard "Culture" results. Taking in consideration the above values, the most sensitive diagnostic tool is PCR with 100% sensitivity, However it can give false positive results and the most specific diagnostic method is Culture with 100% specificity which still remains the gold standard, but major drawback of conventional culture remains as it is time consuming.

Discussion
EPTB is on the increase world over. The diagnosis of EPTB is challenging for a number of reasons: the lack of adequate sample amounts, the apportioning of the sample for various diagnostic tests (microbiology and PCR), and resulting in non uniform distribution of microorganisms. The poor performance of conventional techniques (ZN staining, Culture) in extra-pulmonary tuberculous specimens has stimulated the increased use of PCR tests in the laboratory diagnosis of tuberculosis.

Comparison of sensitivity and specificity of PCR technique with other studies:

AUTHOR	SENSITIVITY	SPECIFICITY
R.C. Kesarwani *et al.*[18] (2004)	97.87%	100%
SS Negi *et al.*[19] (2005)	74.4%	97.29%
Kiran chawla *et al.*[20] (2009)	74.1%	96.1%
Sharma kusum *et al.*[21] (2012)	82.22%	100%
Present Study	100%	96%

In our study, the sensitivity of PCR method was 100% and Specificity 96%.

Conclusion
RT-PCR with the two conventional diagnostic modalities of ZN staining and Culture definitely enables to diagnose a challengeable diagnosis of extra-pulmonary tuberculosis which certainly helps to initiate timely anti-tubercular treatment and prevent progression to irreversible changes.

The application of such rapid, sensitive and quiet specific technique is certainly a boon to our country, India, which carries a heavy burden of tuberculosis in the form of Pulmonary or Extra-pulmonary Tuberculosis.

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

REFERENCE

1. Duraiswami P.K., Tuli S.M. Five thousand years of orthopedics in India. Clin Orthop, 1991;75: 269-280. | 2. Dhillon M.S., Tuli S.M. Osteoarticular tuberculosis of the foot and ankle. Foot Ankle Int, 2001; 22: 679-686. | 3. Davies P.D.: Tuberculosis and migration. The Mitchell Lecture, 1994. J R Coll Physicians Lond, 1995, 29: 113-118. | 4. Dolin, (edited by) Gerald L. Mandell, John E. Bennett, Raphael (2010). Mandell, Douglas, and Bennett's principles and practice of infectious disease (7th edition). Philadelphia, PA: Churchilll Livingstone/Elsevier. Pp. Chapter 250. | 5. World Health Organization, WHO Report, Geneva, Switzerland, 2005. | 6. Communicable Diseases and Disease Surveillance tuberculosis, 2008. Available via DIALOG. http://wwwwhoindiaorg/en/Section3/Section123htm Accessed March 27, 2008. | 7. World Health Organization, Global tuberculosis control: Executive Summary Chapter 3, WHO report 2011. ISBN 978 92 4 156438 0. | 8. World Health Organization, The global burden of disease: 2004 update. World Health Organization, Geneva, Switzerland, 2004. | 9. Introduction Chapter 1, Global tuberculosis control: WHO report 2011, ISBN 978 92 4 156438 0 | 10. Noertjojo K, Tam CM, Chan SL, Chan-Yeung MM, et al. Extra-pulmonary and pulmonary tuberculosis in Hong Kong. Int J Tuberc Lung Dis 2002; 6(10):879–86. | 11. Youmans G. Tuberculosis. Philadelphia: WB Saunders Co. 1979. | 12. Simon H, Weinstein A. Genitourinary tuberculosis - clinical features in a general hospital population. 1977; Am J Med 63:410-419. | 13. Raviglione M., Norain J.P., Kochi A.: HIV associated tuberculosis in developing countries: clinical features, diagnosis and treatment. Bull World Health Organ, 1992, 70: 515-526. | 14. Festenstein F., Grange J.M.: Tuberculosis and the acquired immune deficiency syndrome. J Appl Bacteriol, 1991, 71:, 19-30. | 15. Watson J.M., Gill O.N.: HIV infection and tuberculosis. BMJ, 1990, 300: 63-65. | 16. Washington Winn, Jr., Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger & Gail Woods, Mycobacteria, Chapter 19, Koneman's Color Atlas and Textbook of Diagnostic Microbiology, Sixth Edition: 1065-1117. | 17. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld, Mycobacteria, Chapter 45, BAILEY & SCOTT'S Diagnostic Microbiology, Twelfth Edition: 478-508. | 18. R.C. Kesarwani, Anjana Pandey, Ashutosh Mishra et al. Polymerase chain reaction (PCR): Its comparison with conventional techniques for diagnosis of extra-pulmonary tubercular diseases. Indian Journal of Surgery, Vol. 66, No. 2, Mar-Apr, 2004, pp. 84-88. | 19. SS Negi, SFB Khan, S Gupta et al. Comparison of the conventional diagnostic modalities BACTEC culture and Polymerase Chain Reaction test for diagnosis of Tuberculosis. Indian Journal of Medical Microbiology, (2005)23(1):29-33. | 20. Kiran Chawla, Soham Gupta, Chiranjay Mukhopadhya et al. PCR for M. tuberculosis in tissue samples. Journal of Infect Developing Countries 2009; 3(2)83-87. | 21. Sharma Kusum, Modi Manish, Goyal Kapil et al. Evaluation of PCR using MPB64 Primers for Rapid diagnosis of Tuberculosis Meningitis. 1:204.doi: 10.4172/scientificreports.204. Vol 1. Issue 3.2012