

PCR in tuberculosis: An extremely useful tool?

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Abstract

Introduction: Tuberculosis is a global air borne infectious disease with significant increase in incidence and prevalence. Conventional methods take longer time for diagnosis and drug susceptibility with less sensitivity and specificity. So rapid diagnosis and detection of multi, extensive and total drug resistant bacilli are of up most importance for therapeutic purpose.

Materials and Methods: It was a retrospective study in tertiary care. All clinical samples suspected for tuberculosis received by laboratory were included in the study. The samples were processed for microscopy by ZN stain, culture by LJ media and RT-PCR.

Results: Total 566 sample were tested out of 177 (31%) were positive for tuberculosis. 62% samples were PCR positive, 51% were culture positive and 49% were smear positive. The most common sample received with maximum positivity rate was pleural fluid (34%). Male predominance was seen with young adults as the predominant group affected.

Conclusion: The present study shows good specificity of PCR with sensitivity of 50%. It detects 50% times more cases in relation to conventional results where they fail to produce correct results. More such comparative studies are needed to establish importance of PCR in early diagnosis and treatment.

Keywords: Mycobacteria, RT-PCR, Diagnosis, Detection, Infection.

Introduction

Tuberculosis is a great global epidemic and communicable infectious disease.¹ Incidence and prevalence of the disease has been increasing significantly. Its rapid diagnosis and detection of multi, extensive and total drug resistant disease is still today a challenging task. If diagnosis is delayed, these resistant bacilli can spread person to person rapidly without fail. This will ultimately give rise to an era where majority of the patients will have total drug resistant tubercular bacilli and clinicians left with choice of supportive treatment only. There are few conventional methods of diagnosis like microscopy with ZN staining, fluorescent staining, culture on solid or liquid media, CBNAAT etc. Staining method is relatively fast, inexpensive and specific for tuberculosis in high incidence area with a varying sensitivity from 20% to 80 % depending upon quality of the specimen and training of the laboratory personnel.²⁻⁴ Culture is the gold standard method for diagnosis of tuberculosis but it takes too long time for isolation of bacteria, with LJ solid media 8 weeks and liquid media 14-21 days.^{5,6} Molecular method like CBNAAT is a semi quantitative nested real time PCR which is recommended under RNTCP. It detects 131 CFU/ml with turnaround time of 2-3 hours.^{7,8} Our laboratory does not have CBNAAT instead we have real time PCR instrument ABI 7700. So we have used RT-PCR as a molecular test for diagnosis of tuberculosis and compared it with conventional methods.

Materials and Methods

It was a retrospective analytical study in a tertiary care for 18 months of period. All consecutive clinical samples (like pleural fluid, ascitic fluid, cerebrospinal fluid, pericardial fluid, any other body or cavity fluids, sputum, biopsy material- endometrial, lymph node biopsy, bladder wall biopsy, pus from any abscess, bronchoalveolar lavage, D & C material, urine, ICD drain, drain from any other site, scrapping material etc.) of suspected tuberculosis patients were included in the study. The samples were decontaminated by 4% NaOH and 2.9 % Sodium citrate method. The process was done in the bio safety cabinet using necessary personal protective equipment's. 4 % NaOH and 2.9% Sodium citrate was taken into similar amount with final concentration of NaOH being 2%. Collected samples were transferred to Falcon tubes and equal amount of above mixture was added to it. The tubes were put at room temperature for 20 minutes and phosphate buffer solution was added to neutralize the pH. After that tubes were centrifuged and supernatant was discarded. From the sediment culture on solid media- Lowenstein Jensen media, smear for ZN microscopy and RT-PCR were done. Grading for ZN microscopy was given according to RNTCP guidelines. Inoculated LJ media was put into the incubator at 37°C temperature. Reading was taken every weekly and growth observed. If growth seen, it was confirmed by microscopy and biochemical reactions. Contamination was ruled out. The follow up of culture was taken up to 8 weeks to give result as negative. RT-PCR was performed according to user's

manual and results were interpreted as per the kit literature. 85B mRNA was detected with TaqMan detector probe with the sequence 5'-(5-carboxyfluorescein [FAM])-TCGAGTGACCCGGCATGGGAGCGT-(N,N,N',N'-tetramethyl-6-carboxyrhodamine [TAMRA]). All data of the patients were decoded and analysis of the results was done using Microsoft Excel Sheet.

Results

177 (31 %) out of 566 samples were positive for tuberculosis. The most common sample received were

body fluids with maximum positivity rate in pleural fluid (34 %). Fig. 1 shows distribution of received samples. Table 1 shows positivity rate of different methods. Table 2 shows sample wise positivity rate of different methods. Male predominance was seen with 111 (63 %) positive samples. The male to female ratio was 2:1. The most common age group of isolation of tuberculosis was 20-29 years followed by 30-39 years and 40-49 years with isolation rate of 30 %, 21 % and 17 % respectively.

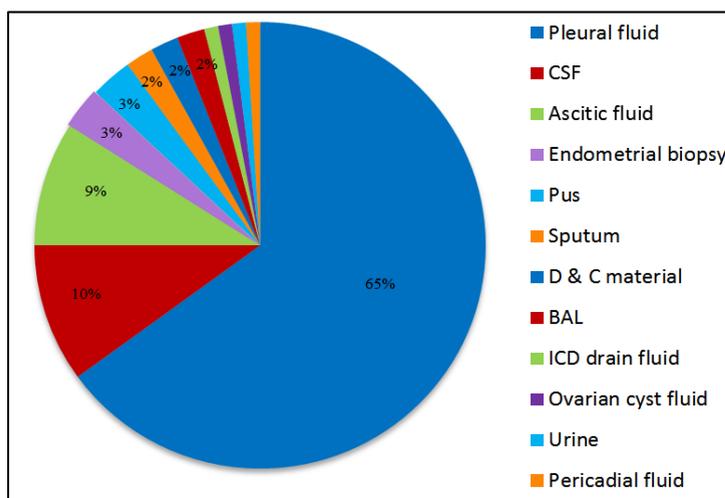


Fig. 1: Distribution of received samples

Table 1: Comparison of different methods

| Type of method | ZN microscopy | LJ solid culture | RT-PCR |
|----------------------|---------------|------------------|--------|
| Positivity rate in % | 49 % | 51 % | 62 % |

Table 2: Sample wise positivity rate of different methods

| Type of sample | % PCR positivity | % ZN microscopy positivity | % LJ solid culture positivity |
|--------------------|------------------|----------------------------|-------------------------------|
| Pleural fluid | 35 | 20 | 20 |
| Ascitic fluid | 15 | 5 | 6 |
| CSF | 10 | 2 | 2 |
| Endometrial biopsy | 25 | 5 | 6 |
| Pus | 30 | 10 | 12 |
| Sputum | 20 | 15 | 15 |
| ICD drain fluid | 50 | 20 | 25 |

Discussion

Tuberculosis is a worldwide public health issue as in this advanced era of technologies, a perfect rapid and completely reliable method for diagnosis is still a great challenge. In the present study, results of PCR showed 62% sensitivity and 100% specificity which show similar results with Gholoobi et al,⁹ Prakash et al,¹⁰ Tiwari et al.¹¹ Although gold standard method, sensitivity of culture was 51% only, which is similar to Tiwari et al.¹¹ The sensitivity and specificity of

microscopy by ZN staining were 49% and 100 % respectively. This correlates with S.-H. Park et al,¹² Jobayer et al,¹³ Kavita modi-Parekh et al.¹⁴

PCR showed 75 % positivity in culture and microscopy positive samples while other studies showed 95-100 % positivity rate Abe et al,¹⁵ Jonas et al,¹⁶ Miler et al,¹⁷ Gupta et al.¹⁸ It shows 30% positivity in smear positive cases. 41% showed positive results in culture negative, smear positive samples which is similar to Zakhm et al,¹⁹ Gupta et al.¹⁸ This may be

due to non viable mycobacteria or very low bacillary load which may have lost during decontamination. Around 50 % showed positive results where both the conventional methods- culture and microscopy- were negative. It suggests that only dependence on conventional methods, 50% of the times false negative results can be conveyed to the patients and physicians. This is too risky as actual positive patients are labeled negative and can spread infection to others until they have appropriate treatment. PCR showed 13% positivity with smear negative, culture positive samples which correlates with Jobayer et al,¹³ Bodmer et al,²⁰ Gupta et al.¹⁸ Smear negativity may be because of high bacillary load needed for microscopy (10^3 - 10^4) as compared to culture (10-100) whereas PCR needs only 1-10 bacilli.²¹

The study showed male predominance which matches with. Male: Female ratio was 2:1 while reproductive age group was the high risk group for the disease with predominance in 20-29, 30-39 and 40-49 years in descending order which is similar to Silva, R.M. et al,²² Kiran Chawla et al,²³ Zakham et al.¹⁹ This may be due to their exposure to environment more frequently as they are the earning members of the family.

Conclusion and Limitations

The present study shows good specificity of PCR with sensitivity of 50%. It detects 50% times more cases in relation to conventional results where they fail to produce correct results. Another advantage is quicker results. Because of its low sensitivity in the present study, it can be used as supplement test rather than the primary test. Besides this, high cost is the main disadvantage in developing countries. More such comparative studies are needed to establish importance of PCR in early diagnosis and treatment.

As it was a retrospective study, clinical correlation of the patients could not be done. The authors could not done comparison of PCR with fluorescent microscopy as it was not done routinely. Drug susceptibility test was also not done. Further we could not analyze cost effectiveness of different methods.

References

- Munshi SK, Rahman F, Mostofa Kamal SM, Noor R. Comparisons among the diagnostic methods used for the detection of extra-pulmonary tuberculosis in Bangladesh. *Int J Mycobacteriology [Internet]*. 2012;1(4):190–5.
- Ryu YJ. Diagnosis of pulmonary tuberculosis: Recent advances and diagnostic algorithms. *Tuberc Respir Dis (Seoul)*. 2015;78(2):64–71.
- Kivihya-Ndugga L, Cleeff M Van, Juma E, Kimwoni J, Githui W, Oskam L, et al. Comparison of PCR with the Routine Procedure for Diagnosis of Tuberculosis in a Population with High Prevalences of Tuberculosis and Human Immunodeficiency Virus Comparison of PCR with the Routine Procedure for Diagnosis of Tuberculosis in a Population wi. *J Clin Microbiol*. 2004;42(3):3–7.
- Kivihya-Ndugga L, Cleeff M Van, Juma E, Kimwoni J, Githui W, Oskam L, et al. Comparison of PCR with the Routine Procedure for Diagnosis of Tuberculosis in a Population with High Prevalences of Tuberculosis and Human Immunodeficiency Virus Comparison of PCR with the Routine Procedure for Diagnosis of Tuberculosis in a Population wi. *J Clin Microbiol*. 2004;42(3):3–7.
- Abdul M, Siddiqui M, Anuradha PR, Nagamani K, Vishnu PH. Original article Comparison of conventional diagnostic modalities, BACTEC culture with polymerase chain reaction for diagnosis of extra-pulmonary tuberculosis. 2013;3(2):53–8.
- Albuquerque YMM de, Lima ALM de A, Lins AK, Magalhães M, Magalhães V. Quantitative Real-Time Pcr (Q-Pcr) for Sputum Smear Diagnosis of Pulmonary Tuberculosis Among People With Hiv/Aids. *Rev Inst Med Trop Sao Paulo*. 2014;56(2):139–42.
- Kim SW, Kim SI, Lee SJ, Lee JH, Ryu YJ, Shim SS, et al. The effectiveness of real-time PCR assay, compared with microbiologic results for the diagnosis of pulmonary tuberculosis. *Tuberc Respir Dis (Seoul)*. 2015;78(1):1–7.
- Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. *J Clin Diagnostic Res*. 2016;10(5):DC09-DC12.
- Gholoobi A, Masoudi-Kazemabad A, Meshkat M, Meshkat Z. Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens. *Jundishapur J Microbiol*. 2014;7(2):1–6.
- Prakash RC, Fathima F, Venkatesh VN, Rao A. Evaluation of PCR for early diagnosis of Tuberculosis. Editor-in-Chief Message. 2012;1(2).
- Tiwari S, Nataraj G, Kanade S, Mehta P. Diagnosis of pediatric pulmonary tuberculosis with special reference to polymerase chain reaction based nucleic acid amplification test. *Int J Mycobacteriology*. 2015;4(1):48–53.
- Park SH, Kim CK, Jeong HR, Son H, Kim SH, Park MS. Evaluation and Comparison of Molecular and Conventional Diagnostic Tests for Detecting Tuberculosis in Korea, 2013. *Osong Public Heal Res Perspect*. 2014;5(S):S3–7.
- Mtb By Gold Probe 2012 J Med Micro Bangladesh.
- Modi-Parekh K, Inamdar V, Jog A, Kar A. a Comparative Study of the Diagnosis of Pulmonary Tuberculosis Using Conventional Tools and Polymerase Chain Reaction *. *Indian J Tuberc*. 2006;53:69–76.
- Abc C, Hirano K, Wada M, Kazumi Y, Takahashi M, Fukasawa Y, Yoshimura T, Miyagi C, Goto S. Detection of Mycobacterium tuberculosis in clinical specimens by polymerase chain reaction and Gene Probe amplified Mycobacterium tuberculosis direct test. *J. Clin. Microbiol* 1993;31:3270.
- Jonas V, Alden MJ, Curry JJ, Kaamisango K, Knott CA, Lankford R, Wolfe JM, Moore DF. Detection and identification of Mycobacterium tuberculosis directly from sputum sediments by amplification of RNA. *J. Clin. Microbiol*. 1993;31:2410.
- Miller N, Hernandez SG, Cleary TJ. Evaluation of Gene-Probe amplified Mycobacterium tuberculosis direct test and PCR for direct detection of Mycobacterium tuberculosis in clinical specimens. *J. Clin. Microbiol*. 1994;32:393.
- Gupta P, Singh KP, Tripathi AK, Jain A. Role of Polymerase Chain Reaction As a Diagnostic Tool in Pulmonary Tuberculosis. 2013;(January 2012):19–24.
- Zakham F, Lahlou O, Akrim M, Bouklata N, Jaouhari S, Sadki K, et al. Comparison of a DNA based PCR

- approach with conventional methods for the detection of Mycobacterium tuberculosis in Morocco. *Mediterr J Hematol Infect Dis.* 2012;4(1).
20. Bodmer T, Gurtner A, Schopfer K, Matter L. Screening of respiratory tract specimens for the presence of Mycobacterium tuberculosis by using the Gen-Probe amplified Mycobacterium tuberculosis direct test. *J. Clin. Microbiol.* 1994;32:1483.
 21. Querol, J.M.; Farga, M.A.; Granda, D.; Garcia de Lomas, C.J. (1995). The utility of Polymerase Chain Reaction (PCR) in the diagnosis of pulmonary tuberculosis. *Chest.* 107 (6),1631-1635.
 22. Journal B. Submitted: December 12, 2010; Approved: January 16, 2012. 2012;261–5.
 23. Article O, Chawla K, Johar R, Vishwanath S, Mukhopadhyay C. Role of PCR in the Diagnosis of Pulmonary and Extra-Pulmonary Tuberculosis. 2015;(August 2011):2013–6.