

Diagnosis of Mycobacterium Tuberculosis Using Fluid Microscopy & PCR in Broncho-Alveolar-Fluid (BAL)

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Abstract

Objective: To measure the diagnostic yield of BAL using PCR in suspected Smear negative patient for tuberculosis.

Method: BAL specimen of 100 patient with suspected Smear negative who have 3 respective negative sputum Smear. Fiber optic bronchoscopy was done.

Result: Out of 100%, 82% patient BAL was positive for PCR, sensitivity of test=97.6% & specificity =62.5 & 24% of BAL was positive for AFB smear, sensitivity=80% & specificity=42% and 18% patient was negative for AFB smear of which. 2 were diagnosed for Lung cancer, 8 = Pneumonia, 6 Bronchitis, 2 Bronchetasis. **Conclusion:** PCR can be used as a supporting in diagnostic test providing rapid and accurate result and bronchoscopy must be considered for evaluating Tuberculosis suspected in patient from whom sputum dose not reveal Tuberculosis Bacilli so BAL fluid AFB Smear and PCR helpful in early diagnosis and treatment for tuberculosis.

Keywords: BAL: Broncho Alveolar lavage, FOB: Fiber optic bronchoscopy, PCR: Polymerase chain reaction, AFB: Acid Fast Bacilli, MTB: Mycobacterium Tuberculosis, MDR: Multi Drug Resistance

I. Introduction

Tuberculosis is considered as most serious public health problem & 1/3 of world population is infected with Tuberculosis. (1) India contributes highest number of new cases, 20% of global burden of which 1-2% occur in person with HIV (2). Early diagnosis of disease and prompt initiation of treatment are important in improving patient outcome in prevention of M.D.R.TB and in interrupting further transmission of tuberculosis bacilli (3). About 20-40% of Tuberculosis patient are smear positive. While rest of patient had either smear negative sputum scare disease (4). So Bronchoscopy with BAL is preferred in suspected case of Tuberculosis (5). Bronchoscopy with BAL is routinely performed in suspected Tuberculosis. BAL AFB stain 41% and culture is considered as the Gold standard (with 86% sensitivity) but they are expensive and take 6-8 week for diagnosis. PCR helps in detecting the MTB and is currently the most sensitive and rapid diagnostic lab method. (7). BAL PCR can rapidly detect the MTB and help in the diagnosis and management of Tuberculosis. In these studies a specific DNA sequence (IS6110) of MTB is frequently used (8). The specific (IS 6110) field is one most frequently detected common DNA fragment of MTB complex. Therefore we have searched for Fragmentation of IS6110 of Tuberculosis Complex with PCR technique (9).

II. Material and Method

Patient population: This study was conducted from 2013 – 2015 on a total of 100 patients seen in medicine and chest opd in KBN Hospital. permission was taken from hospital ethical committee.

Inclusion Criteria

- Age > 18 years
- Cough > 2 weeks
- Patient with negative sputum smear examination
- Detail evaluation to identify Etiology using HIV/CXR, sputum Examination

Exclusion criteria

- Prior H/o Tuberculosis within 2 year.
- Patient receiving treatment for recent Tuberculosis
- Smear positive case.
- Disseminated or extra Pulmonary Tuberculosis.
- HIV positive / Immuno compromised patients.

Brochoscopy

Brochoscopy was performed by trans nasal route and 100 ml of BAL was obtained in two aliquot by instillation of sterile N/S (Normal Saline. ⁽¹⁰⁾, BAL was sent for AFB stain and for PCR. ⁽¹¹⁾

Molecular technique was used for PCR and it was done in K.B.N hospital Gulbarga. We used PCR to amplify the field os IS6110 with the hybridisation technique. PCR study was done in three steps:

1 - DNA isolation

- NaOH as well as material within the Eppendorf tubes at the rate of %2 was mixed in mixer adding N-Acetyl-L-Cystein.
- After 15 minutes, 80 mcl 2 M (pH 7.4) TRIS add per 2% NaOH 100 mcl and mixed.
- The mixture was centrifuged at 12000 G for 15 minutes.
- The top part of it was thrown away and the sediment at the bottom was added 200 mcl distilled apyrogenic water and stirred.
- 200 mcl chloroform was added and stirred.
- Kept in the sterilizator at 80°C for 15 minutes.
- This material was centrifuged at 12000 G for 1.5 minute and upper layer was saved for PCR.

2-Reproduction of DNA

Taq DNA polymerase, dATP, dTTP, dGTP, dCTP, reaction buffer, the primers that codify the field of IS6110 of *M. tuberculosis* complex DNA and water were loaded in a tube and 5ml of phase which was saved in DNA isolation was added to this. The material prepared for PCR was put into the thermocycler (Minicycler MJ. Research Inc. USA) programed for 40 cycles. DNA was reproduced through the steps of denaturation in thermocycler, connection and longevity.

3-To show the reproduction

Sterilizer was set to 68 °C, mixture of prehybridization (N-loril-sarkosil, 10% sodium dedoksil sulphate, blocking reagent, 20 X SSC, distilled water) and probe (marked as digoxigein) was taken from deep-freezer.

- Membrane was prepared, loaded (2ml) and kept, under UV for 3 minutes.
- Prehybridization mixture was kept at .68 °C for 15 minutes and quickly frozen in deep-freezer.
- Probe was kept at 37 °C for 2.5 hours.
- Buffer II (5 ml blocking reagent, 45 ml buffer 1) was taken out of the freezer and let to melt. Sterilizator was set to 68°C.
- At the room temperature it was shaken twice for twenty minutes in the solution of 2 X SSC (sodium chloride + sodium citrate) + 0.1 % SDS.
- It was kept at room temperature in the solution of 0.2 x SSC + 0.1 SDS twice for 30 minutes.
- Buffer' 1 (maleic acid, sodium chloride, distilled water, sodium hydroxide, pH 7.4) was shaken at the room temperature for a minute.
- Buffer II was also shaken at the room temperature for 30 minutes.
- Buffer II (15 ml) + Dapcon (3 ml) was also shaken at the room heat for 30 minutes.
- Buffer I was also shaken at the room temperature twice for 15 minutes.
- Buffer III (1M pH 9.5 tries, sodium chloride, 1M magnesium chloride and distilled water) was also shaken at the room temperature for 2 minutes.
- We waited until seeing the color changed dark in the mixture of buffer 111(10ml) + NBT (45mcl) + X-phosphate (35 mcl) at 37 °C.
- Buffer I was also shaken for 5 minutes.
- The buffer (2M (pH 7.4) TRIS, 0.5 MpH 8.0 EDTA, distilled water) was also kept in the refrigerator.

The results were analysed comparing the positive and the negative controls on the membrane. BAL culture was not done and post bronchoscopy sputum was not done due to unavailability of sputum.

Statistic

Data was analysed and the following values were obtained

Bal Pcr

Sensitivity=97.6%

Specificity=62.5%

Positive predictive value=89.1%

Negative predictive value=75%

Bal Afb

Specificity=80%
 Specificity=42%
 Positive predictive value=37.5%
 Negative predictive value=33.3%

III. Results

AFB Smear	PCR	No of PT
-	+	58%
+	+	24%
-	-	18%

Total -----100%

Diagnosis of 18% without Tuberculosis.

Lung cancer	2	-ve	-ve
Pneumonia	8	-ve	-ve
Bronchitis	6	-ve	-ve
Bronchetasis	2	-ve	-ve

Total -----18%

Out of 100pt 82% BAL was positive for PCR & 24% of BAL was positive for AFB smear and 18% was negative for AFB smear/PCR.

Diagnosis of 18% with out Tuberculosis, 2 were diagnosed as lung cancer/ 8 were pneumonia/ 6. Bronchitis & 2 Bronchetasis.

IV. Discussion

As the number of Tuberculosis patient are increasing day by day in India thus the need for new rapid technique play a major role in rapid diagnosis & disease management⁽¹²⁾.

We have shown that Tuberculosis PCR with BAL can identify smear negative Tuberculosis patient. Clinician would otherwise fail to diagnose. Microscope examination of sputum smear for AFB by Robert Koch more than a century after continue to be the most important diagnostic test for TB (13) Even though MTB culture is gold standard but time consuming and expensive so new diagnostic test like PCR aids in diagnostic of smear-ve TB.

AFB smear sensitivity has been reported to vary from 20%-80%, in our study sensitivity is 80%. Liam ck et al got the AFB smear 80% 61. Panda et al reported sensitivity of 35%.⁽¹⁵⁾

In this study PCR sensitivity was 97.6% compared with another study BAL PCR was 88%⁽¹⁴⁾, in another study MTB PCR BAL had sensitivity of 78%.⁽¹⁶⁾

The TB PCR assay was found to be more sensitive than smear detection in BAL specimen of patient with sputum negative TB.

There were several limitations of present study, first patient of this study were highly biased, second the number of patient in this study is small, so generalizability is greatly limited.

V. Conclusion

Fibro optic bronchoscopy with BAL is useful tool for isolation of M.T.B with pulmonary TB for patient highly susceptible of PTB and BAL may increase the diagnostic yield.

References

- [1]. World Health organization Global tuberculosis control. Surveillance, planning, financing in WHO report 2005 Geneva 2005; 1 – 258.
- [2]. WHO (2009) global tuberculosis control: a short update to the 2009 report: accessed 07 may 2010.
- [3]. Garg SK, Tiwari RP, Tiwari D. Etal Diagnosis of tuberculosis, available technologies, limitation and possibilities J.Clin Lab Anal 2003;17:155-63.
- [4]. Desta K, Asrat D, Lemma E, Gebeyenu M, Feleke B, Prevalance of smear negative pulmonary tuberculosis among patients visiting St. Peter's Tuberculosis specialized Hospital Ethiopmed 2009 47:17-24.
- [5]. Kheawon N, Chuang – Ngam S, Mitsougneun S, Peam – AM.J Vislswadi J role of routine bronchial washing culture for TUBERCULOSIS in Maharat Nakhon Ratchasima Hospital, Thailand. J.med Assoc Thailand 2013;96:558-63.
- [6]. Saglam L, Akgun M, Aktas E usefulness of induced sputum & Fiber optic Bronchoscopy specimen in diagnosis of pulmonary tuberculosis J Int Med Res 2005;33:260-5.
- [7]. Del. Potillo P, Murillo LA Patarroyo ME (1991) Amplification of a species specific DNA fragment of M. Tuberculosis and its possible use in diagnosis and its possible use in diagnosis – J clin Microbiol 29:2163-2168.
- [8]. TOPCUWA, Soyletir.G Doginaoy M, Kocabas A: The injection disease, Adana – Turkey 1996: PP:418-423.
- [9]. Godfery FP: Moleccular diagnosis of tuberculosis thorax 50:709-711, (1995).
- [10]. De Gracia J, Curull V, Vidal R, Riba A, Orriois R, Martin N, Morell F Diagnostic value of Brochoalveolar lavage in suspected pulmonary tuberculosis Chest 1988;93:329-32.

- [11]. Desjardin LE, Chen Y, Perkins MD, et al Comparison of the ABI 7700 system and competitive PCR for qualification of IS6110 DNA in sputum during treatment of tuberculosis. *J.clin Microbiol* 1998;36:1964-8.
- [12]. Istans S.K Nair, M N I shiri, K K & Sarin, N.P. *Indian pediatri* 2000;37:375-382.
- [13]. American the social and center for disease control, diagnostic standard and classification of tb. *am rev respir dis.*1990=142-725
- [14]. charuena tanks, dejsomritrutat w and chaiprasser a, diagnostic role of fiber optic bronchoscopy in suspected smear negative pulmonary tuberculosis. *Respir med* 1995;89:62
- [15]. liam ck, chen yc, yap sf, srinivas p, poi pj detection of mycobacterium tuberculosis in bronchoalveolar lavage from patient with sputum smear negative ptb using pcr assay. *respirology* 1998;3:125
- [16]. value of smear&pcr in Bronchoalveolar lavage fluid in culture positive PTB *Eur respir j* 2005;26:767-772