



Original Research Article

Utility of MTP40 nested PCR in diagnosis of tubercular pleural effusion

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ARTICLE INFO

Article history:

Received 01-05-2020

Accepted 09-05-2020

Available online 31-07-2020

Keywords:

Mycobacterium tuberculosis

Tubercular pleural effusion

ABSTRACT

Extra pulmonary TB is present in 15% of all TB cases. This incidence is higher about 50% in HIV affected patients. Tuberculous pleural effusion accounts for 4% of all TB cases.¹

Though we have lot of diagnostic methods to diagnose pulmonary TB, we are lacking of a good diagnostic method to identify tubercular pleural effusion.

In this issue we have studied the utility of Nested PCR (MTP40) in diagnosis of tubercular pleural effusion. We have included 200 pleural effusion patients. The sensitivity of Nested PCR test is 19% and specificity 100%, Diagnostic Accuracy 59.5%.

This study conducted from May 2017 to June 2019. This is a cross sectional study done in a tertiary care center in Varanasi included 200 patients of pleural effusion.

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1. Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*, affecting the lungs mainly. MTB infection can be silent or it can cause a progressive disease. Burden of TB is one of the important health issue in developing countries. Two thirds of Global TB cases were in 8 countries. India accounts for 27% of Global TB cases.² Tubercular pleural effusion is a major health seeking problem in developing countries.

Tubercular pleural effusion is difficult to diagnose as being a reaction phenomenon, mycobacterium culture is usually negative. A very small microbial load in the pleural fluid disfavor the role of direct microscopic examination by executing Ziehl-Neelsen staining. Different other biochemical tests including Adenosine deaminase enzyme, fluid protein level (Light's criteria), LDH level in pleural fluid might help in the diagnosis. The definitive diagnosis is still depends on the identification of MTB or demonstration of caseous granulomas in pleural biopsy.³

PCR techniques were emerging as a useful tool in these settings.⁴ Although PCR techniques have been demonstrated adequate accuracy in pulmonary TB, their role in pleural TB is not clear.⁵

Recently different molecular targets are being used for the rapid diagnosis of tubercular pleural effusion.

2. Materials and Methods

A total of 200 cases of pleural effusion were enrolled in this cross sectional study. The diagnosis of Tuberculosis confirmed by elevated pleural fluid protein levels, lymphocytic predominance & level of adenosine deaminase enzyme, clinical diagnosis and the treatment effectiveness on anti-tubercular therapy. A 15ml pleural fluid was collected in the falcon tube and was sent for molecular workup and routine microscopy & ADA.

2.1. DNA extraction and PCR setup

Pleural fluid was taken in a conical falcon tube and centrifuged at 10000rp for 15 minutes. A 0.5ml of sediment was suspended in 400 µl of TE buffer and boiled at 100°C

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for 40 minutes. Subsequently, After boiling the suspension was vortexed for 10 minutes. Then 30 μ l of SDS and 2 μ l of proteinase-K were added and mixed by gentle inversion and the sample suspensions were incubated at 37°C for 2 hours. After that, 100 μ l of 5M NaCl was added and vortexed for 15 seconds. Then 80 μ l of 10% CTAB was added in the suspension and all the components were mixed well. Subsequently the samples were incubated at 60°C in hot water bath to deactivate the proteinase K and CTAB activity. After incubation, equal volume of Phenol: chloroform: isoamyl alcohol (P:C:I; 25:24:1) mixture was added and vortexed briefly for 15 seconds. Next the samples were centrifuged at 10000 rpm for 10 minutes and the aqueous phase was collected in another fresh eppendorf tube. An equal volume of chloroform: isoamyl alcohol C:I (24:1) was added to the aqueous phase and vortexed briefly for 15 seconds. Then again centrifuged at 10000rpm for 10 minutes to have aqueous phase. An equal volume of isopropanol was added to precipitate DNA. A 70% ethanol was used to remove the traces of isopropanol. The extracted DNA Was air dried and dissolved in TE buffer for further workup. it and kept at room temperature for 5 minutes.

2.2. PCR amplification of target gene

Mtp40 gene of *Mycobacterium tuberculosis* was amplified by using the specific primers. For amplification, a nested PCR was executed. The primary PCR cycle in a volume of 25 μ l, using Forward 5'CGGCAACGCGCCGTCG-GTGG3' and reverse primer 5'CCCCCACGGCAC-CGCCGGG3' with following thermal conditions: initial denaturation of 95°C for 3 minutes followed by 35 cycle of denaturation 94°C for 1 min, annealing at 65°C for 1 minute and extension at 72°C for 45 seconds and final extension of 10 minutes at 72°C was executed. PCR secondary cycle is subjected to initial denaturation of 95°C for 3 minutes followed by 35 cycle of denaturation 94°C for 1 min, annealing at 65°C for 1 minute and extension at 72°C for 45 seconds and final extension of 10 minutes at 72°C. Primers were 5'CGTTCGGGATGCACTGCG3' and reverse primer 5'CACCCGGCGAATTCGTCAC3' by using 1 μ l of amplicon of primary cycle in a final volume of 25 μ l.

3. Results

Total of 200 patients of pleural effusion were included in the present study

We observed Mtp40 specific amplification in a total of 19 cases of the pleural effusion which is Tubercular etiology. Sensitivity 19%, Specificity 100%, Positive predictive value 100%, Negative predictive value 55.25%, Diagnostic Accuracy 59.5%

Table 1: Baseline characteristics

Age group	Male	Female	Total
15-25yr	27	15	42
26-35yrs	18	7	25
36-45yrs	23	5	28
46-55yrs	26	12	38
56-65yrs	25	12	37
66-75yrs	11	5	16
>75yrs	12	2	14

Table 2: Final diagnosis of patients

Characteristics	Non Tubercular Pleural effusion	Tubercular Pleural effusion
Number	100	100
Male	72 (72%)	71 (71%)
Female	28 (28%)	29 (29%)
Age (Mean \pm SD)	50.97 \pm 17.56 yrs	41.7 \pm 19.72 yrs

Table 3: Pleural fluid characteristics

Parameters	Non tubercular pleural effusion	Tubercular pleural effusion
Total count	4826 \pm 23	26593 \pm 87
Lymphocyte %	23.5 \pm 32	71.5 \pm 28
Neutrophil %	71.89 \pm 32	17.2 \pm 25
Sugar (mg/dl)	112.4	84.4
Protein (gm/dl)	3.59	4.92
ADA (IU/L)	28.8	106.16

Table 4: Result of TB - PCR

TB - PCR	Non Tubercular pleural effusion	Tubercular pleural effusion	P value
Positive	0	19	0.00
Negative	100	81	

4. Discussion

Molecular methods are important tools for conforming Pleural Tuberculosis due to their high specificity >90%. They are non-invasive and low risk. Sensitivity of PCR varies from 37% - 77% different studies. This variation is because of different target used in different studies⁶ and this is much lower than in other body fluids. The low sensitivity of PCR in pleural fluid was explained by the low bacilli load, presence of substances which inhibit amplification in pleural fluid.⁷

Some of the previous studies indicated that PCR techniques using MTP40 are fast, specific methods for diagnosis if TB.⁷ High sensitivity up to 100% has been detected by nested.

PCR based on MTP40 target of *M.Tuberculosis* in genitourinary TB.⁸ And MTP40 PCR is more sensitive method for detection of TB from pleural fluid than AFB and culture.⁹

In our study the sensitivity of TB PCR using Mtp40 target to detect Tuberculous pleural effusion is 19% and specificity is 100%. This is lower than previous studies. In a study by LM Montenegro et al, found that nested PCR had sensitivity of 33.3% in pleural fluid and specificity of 94.4%.⁹ Lima et al¹⁰ studied 45 patients (16 had pleural TB) and reported that sensitivity of 31.3% and a specificity of 96.6%. Much higher sensitivity detected in other studies. Liu et al.¹¹ reported a sensitivity of 43.3% and a specificity of 95.5%, Kumar et al.¹² reported a sensitivity of 51.7% and a specificity of 100% in the nested nPCR results. This may be due to the use of IS1610 target used in most of the studies which is not tested in our study.

There are high number of false negative results seen in our study. The reason could be the pleural effusion in TB patients may be due to the hypersensitivity reaction rather than pleural invasion.¹³ Another reason could be the specific organism may not be present in the samples due to its uneven distribution in pleural effusion or the particular strain MTP40 might be absent in the organism. Because MTP40 might not be present in all M. Tuberculosis strain.¹⁴

5. Conclusion

Nested PCR – Mtp40 Target can be used for rapid diagnosis of Tubercular pleural effusion on high suspicion. But negative results doesn't rule out tuberculosis.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Cite this article: Rajak BK, Mishra JK, Gupta MK, Anbarasan . Utility of MTP40 nested PCR in diagnosis of tubercular pleural effusion. *J Community Health Manag* 2020;7(2):61-63.