Comparing the efficacy of ADA and PCR in diagnosing tuberculosis in pleural effusion

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ABSTRACT

BACKGROUND

Pleural effusion due to TB is currently the most common location for extrapulmonary TB and cases of pleural TB can be expected to increase with the increasing incidence of TB Worldwide. Historically, pleural biopsy was found to be more sensitive in diagnosing TB in pleural effusion as it is more invasive and hazardous than thoracentesis, alternative diagnostic approaches have been extensively evaluated. Adenosine Deaminase (ADA) has been developed and widely used for the diagnosis of TB due to its simplicity.

The aim of the study is to assess and compare the validity of ADA and PCR by calculating the sensitivity and specificity in diagnosing tuberculosis among the patients with pleural effusion.

MATERIALS AND METHODS

A cross-sectional study was undertaken at our hospital for a period of 2 years between January 2014 to January 2016. 124 patients were admitted during that period and after getting the informed consent signed by the study population. They were included in our study. ADA activity was measured by the standard method as suggested by Guisti. Two mL of pleural fluid was collected in sterile container and was either immediately analysed or refrigerated at 4°C and analysed within 2 days. An ADA value >40 U/L was taken as the cutoff for calculating sensitivity and specificity. Polymerase chain reaction for M. tuberculosis 65 kDa gene was performed on pleural fluid specimens as previously described. The data were entered and analysed by using SPSS version 20.

RESULTS

Among 124 patients, 97 patients had been confirmed TB. So far, our analysis, we took 97 patients in which 79 were confirmed TB through HPE, 17 through pleural fluid culture and one patient was diagnosed by pleural fluid AFB smear. In our study, the ADA cutoff was assigned as 40 IU/L. The mean ADA levels among the patients with TB pleural effusion were found to be 53.5 IU/L. The sensitivity was 94%, whereas the specificity and the positive predictive value was found to be 100% and the negative predictive value was 82%. The sensitivity of ADA in diagnosing TB in pleural effusion was much higher than the sensitivity of HPE and PCR and the difference was found to be statistically significant, whereas identifying the true negatives (specificity) was almost similar in HPE and ADA and it was 92.5% in PCR, but the difference was not statistically significant and a similar pattern of results was also seen with positive predictive value. Negative predictive value was higher in ADA when compared to HPE and PCR and the difference was found to be statistically significant.

CONCLUSION

Adding ADA to the pleural fluid workup in high prevalence areas could reduce costs, morbidity and time to diagnosis. According to the current study results, PCR still need refinement before they significantly add to the evaluation of pleural diseases.

KEYWORDS

Pleural Effusion, Tuberculosis, ADA, PCR, HPE.

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BACKGROUND

Tuberculosis (TB) remains a public health challenge with nearly 2 billion persons (~29% of the world’s population) exposed to Mycobacterium tuberculosis annually and 8 million new cases of TB diagnosed each year resulting in 2 million deaths. India is the country with the highest burden of TB. The World Health Organisation (WHO) TB statistics for India for 2015 had given an estimated incidence of 2.5 million cases of TB in India out of a global incidence of 9.6 million.1 Although, most persons present with pulmonary
symptoms, presentation with extrapulmonary symptoms is also possible. About 10-15% of patients who are immunocompetent and 50-70% of patients with AIDS present with extrapulmonary symptoms. Pleural effusion due to TB is currently the most common location for extrapulmonary TB and cases of pleural TB can be expected to increase with the increasing incidence of TB worldwide. Due to the specific pathogenesis of the pleural TB effusions, which is related more to an immune response than to a direct mycobacterial involvement, the diagnosis is still difficult and controversial.\(^{1,2}\) Proving the TB aetiology of a pleural effusion is very important since in the absence of a correct diagnosis, there will be no adequate treatment leading eventually to relapse and development of severe complications, some of them requiring prolonged treatments or even major and/or mutilating surgical procedures.\(^{9-11}\)

Although, we have many methods for the diagnosis of pulmonary TB; for example, Ziehl-Neelsen (ZN) staining, Polymerase Chain Reaction (PCR) and culture. These methods do not provide enough sensitivity and specificity. The sensitivities of ZN staining and culture are 10-40% and 8-49% respectively in the diagnosis of TB infection.\(^{12}\) The definitive diagnosis of EPTB depends on the demonstration of Mycobacterium tuberculosis in the specimens like pleural fluid, ascitic fluid, pericardial fluid, Cerebrospinal Fluid (CSF) or pleural biopsy specimen and can also be established with reasonable certainty by demonstration of granuloma in the parietal pleura, peritoneum, pericardium, etc.\(^{13}\) Although, mycobacterial culture is the gold standard in diagnosing TB, Mycobacterium spp. grows very slowly and it can take up to six weeks to isolate it in culture. Determination of susceptibility to drugs can add another three to six weeks to the process.

Thoracoscopy in diagnosing tubercular serositis offers a near 100% positive diagnostic yield on histology and 76% positive on culture.\(^{4}\) However, historically, since pleural biopsy is more invasive and hazardous than thoracocentesis, alternative diagnostic approaches have been extensively evaluated.\(^{5}\) Adenosine Deaminase (ADA) has been developed and widely used for the diagnosis of TB due to its simplicity, low cost and quickly available results. Many studies have confirmed the high sensitivity and specificity of ADA (sensitivity 92% and specificity 89%) for early diagnosis of EPTB such as tuberculous pleuritis, pericarditis, ascites and meningitis.\(^{13}\) ADA activity is usually measured with a colorimetric method, which is fast, inexpensive, reproducible and easy to perform.\(^{14}\) However, the predictive value of ADA activity depends on the local prevalence of TB.\(^{15}\) Moreover, ADA activity can be increased in other diseases that present with pleural effusion such as lymphoma, collagen vascular diseases and bacterial empyema and it must be used with care in countries with a low incidence of TB.\(^{16}\) Owing to the specific amplification of M. tuberculosis DNA and the high reported sensitivity, PCR could be useful in paucibacillary TB. However, in previous studies, no consistent advantage of PCR has been observed. However, in countries like India where TB is the most common infectious disease, much studies were not conducted in comparing the efficacy between ADA and PCR in diagnosing TB in pleural effusion, so this study was undertaken to compare the validity of ADA and PCR among the patients with pleural effusion caused due to TB.

**Aim**- To assess and compare the validity of ADA and PCR by calculating the sensitivity and specificity in diagnosing tuberculosis among the patients with pleural effusion.

**MATERIALS AND METHODS**

A cross-sectional study was undertaken at our hospital for a period of 2 years between January 2014 to January 2016. All patients admitted with pleural effusion during that period were included in our study. 124 patients were admitted during that period and after getting the informed consent signed by the study population, they were included in our study. The study was carried out after getting the clearance from the institutional ethical committee.

A positive AFB staining of pleural fluid or sputum smears, a positive Lowenstein-Jensen (LJ) medium culture of sputum, pleural fluid or tissue followed by biochemical identification or the finding of caseous granuloma in pleural tissue were considered as the criteria for diagnosing TB in the patients. Patients were submitted to blood and pleural fluid tests for Lactic Dehydrogenase (LDH), glucose, cholesterol, amylase, protein and albumin concentration. Blood was tested for Human Immunodeficiency Virus (HIV) infection by two different ELISA methods in the same blood sample only in patients who specifically consented to this. Pleural fluid was also tested for total and differential cell count, Gram staining, AFB staining and LJ culture. The pleural fluid was considered exudative when the pleural fluid/serum protein ratio was superior to 0.5 and lymphocytic when it contained more than 50% lymphocytes.

ADA activity was measured by the standard method as suggested by Guisti.\(^{14}\) Two mL of pleural fluid was collected in sterile container and was either immediately analysed or refrigerated at 4°C and analysed within two days. An ADA value >40 U/L was taken as the cutoff for calculating sensitivity and specificity. Polymerase chain reaction for M. tuberculosis 65 KDa gene was performed on pleural fluid specimens as previously described. The primers used were-forward primer- 5’-GAGATCGAGCTGGAGATCC-3’, and reverse primer- 5’-AGCTGAGCCCAAAGGTGGT TTA-3’. The PCR conditions were an initial denaturation step at 94°C for 7 min. and 35 cycles of 94°C for one minute, 60°C for two minutes and 72°C for two minutes. Final extension was done at 72°C for 10 minutes. PCR product was visualised under Ultraviolet (UV) light after electrophoresis on 1.2 percent agarose gel with ethidium bromide.

The data were entered and analysed by using SPSS version 20. The accuracy of ADA and PCR tests was evaluated by determining the sensitivity and specificity as compared to the reference standard- combined histopathologic, microbiologic and clinical criteria. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for both ADA and PCR using...
the standard formulas and their statistical significance was calculated by using McNemar test.

RESULTS

A total of 124 patients with pleural effusion were subjected for further investigations. Among them, 97 patients were confirmed TB, and for 18 patients, the pleural fluid was found to be transudate, and for the remaining 9 patients, there was no adequate pleural fluid or tissue for examination. So far, our analysis, we took 97 patients, in which 79 were confirmed TB through HPE, 17 through pleural fluid culture and 1 patient was diagnosed by pleural fluid AFB smear.

Table 1 shows the age and gender wise distribution of the study population. It is seen from the table that majority of the study subjects were males with a sex ration of 1.85:1 (M:F). The minimum age group affected with TB in our study was 28 years and the maximum was 63 years, but most of the study subjects were in the age group between 30-50 years with a mean age of 43.6 years among males and 41.8 years among females. The validity of the individual test was analysed for 97 patients with TB pleural effusion. The remaining 18 patients who had pleural effusion were found to have transudate causes. Among the various transudate causes in our patients, we found majority of them having cirrhosis (33.3%) followed by congestive cardiac failure (27.7%), cancer (16.6%), lymphoma (16.6%) and CRF (11.1%). The validity of HPE showed 81% sensitivity and 100% specificity in diagnosing TB in pleural effusion and the positive predictive value was found to be 100% and the negative predictive value was 60% (Table 2).

In our study, the ADA cutoff was assigned as 40 IU/L. The mean ADA levels among the patients with TB pleural effusion were found to be 53.5 IU/L. The sensitivity was 94%, whereas the specificity and the positive predictive value was found to be 100% and the negative predictive value was 82% (Table 3).

The validity of PCR was shown in Table 4. The sensitivity was 85.5% and the positive predictive value was 98%, whereas the specificity was found to be lower than the ADA and HPE with 92.5% and the negative predictive value was 64%.

The comparison of three different tests to assess the statistical significance was shown in Table 5. McNemar test was used to assess the statistical significance. The sensitivity of ADA in diagnosing TB in pleural effusion was much higher than the sensitivity of HPE and PCR and the difference was found to be statistically significant, whereas identifying the true negatives (specificity) was almost similar in HPE and ADA and it was 92.5% in PCR, but the difference was not statistically significant and a similar pattern of results was also seen with positive predictive value. Negative predictive value was higher in ADA when compared to HPE and PCR and the difference was found to be statistically significant.

DISCUSSION

In the present, out of 124 patients with pleural effusion, 97 patients were confirmed with TB. The average age of TB pleurisy was found to be more common among the middle-aged population between 30-50 years. The mean age group in our study subjects was 43 years among males and 41 years among females and these results were consistent with the study done by Mohd Arif Kelam and Berger HW. Exudative lymphocytic pleural effusions commonly

encountered in clinical practice often constitute difficult diagnostic problems. The two most common causes are malignancy and tuberculous effusions. For tuberculosis, the limitations of diagnostic tests include few positive staining and culture from pleural fluid as well as time consumption for identification.

In our study, most of the patients presented with fever and productive cough as their chief complaints and the similar complaints were also quoted in the study done by Berger HW10 and Moudgil et al.19 In the present study, the mean ADA levels was 53.5 IU/L ± 11.6 and it was almost in par with the study done by Pinal C Shah13 where he found the mean ADA levels was 62 IU. In our study, we fixed the cutoff value for ADA >40 for diagnosing it as TB as quoted in the previous studies done by Gupta V K et al21 and Bhargava D K et al.22

The present study shows the sensitivity and specificity for ADA in diagnosing TB was 94% and 100% and it is almost in par with the other studies Ocana et al.23 studied specificity of 97% and sensitivity of 100% of the test in tuberculosis at cutoff 45 U/L, Inma Ocana et al.24 sensitivity of and specificity of 0.97 at cutoff 50 U/L, Jose Banales et al.25 studied 98% sensitivity and 96% specificity at cutoff 70 U/L for the diagnosis of tuberculosis. Esther San Jose et al.26 found sensitivity 100% and specificity 95% at cutoff 47 U/L. Y. Aoki et al.27 found sensitivity 100% and specificity 95% at cutoff 42 U/L. Luis Valdes et al.27 found ADA sensitivity and specificity were 81% and 89% at a cutoff 45 U/L. Lesley Burgess et al.29 found at cutoff 47 U/L, 100% sensitivity and 87.5% specificity. SK Sharma et al.30 studied at a cutoff 35 U/L, sensitivity 83.3% and specificity of 66.7%. Danielle M. Lima et al.31 studied ADA activity and PCR on pleural fluid of tuberculosis and nontuberculous patients. They found that cutoff 40 U/L sensitivity and specificity of ADA were 68.8% and 72.4%, where combined use of ADA and PCR improves sensitivity (87.5%) and specificity (72.4%). Wu-Huei Hsu et al.32 found mean ADA 137.9 ± 30.7 U/L in immunocompetent tuberculosis patients, 65.8 ± 49.9 U/L in immunocompromised tuberculous patients and 36.9 ± 25.6 U/L in nontuberculous patients and concluded that diagnostic value of ADA in immunocompromised hosts with tuberculous pleural effusion is not as significant as in immunocompetent hosts.

PCR in the detection of M. tuberculosis DNA is a potentially useful diagnostic method. It has been well studied in different clinical specimens including pleural fluid, but its use is approved only for AFB-positive respiratory samples (sputum). In the present study, PCR had a high sensitivity, but not significantly superior to the histopathologic examination or ELISA. In addition, implementation of this technology is expensive and contamination is a major concern. Two false-positive results were observed in our patients, none of whom had a previous history of TB. A high PCR specificity was achieved by including additional steps in the procedure, but at the cost of increasing its worktime.31 Another limitation with PCR tests in pTB is the relatively high false-negative rate possibly a consequence of the presence of inhibitory substances in the sample. Serial sample washing stages can minimise this inhibition, but again they are time consuming.34 It is clear that new procedures to optimise the performance of PCR testing in pleural fluid samples are still necessary.

CONCLUSION
Estimation of adenosine deaminase in pleural fluid is a simple, rapid and less expensive laboratory investigation for the diagnosis of tuberculous pleural effusion when the diagnosis is uncertain by other investigations. ADA proved reliable both in confirming and in ruling out pTB as it had a very high sensitivity and specificity and it also proved superior to the histopathologic results and PCR. Adding ADA to the pleural fluid workup in high prevalence areas could reduce costs, morbidity and time to diagnosis. According to the current study results, PCR still need refinement before they significantly add to the evaluation of pleural diseases.

REFERENCES


