INTRODUCTION

Among the 22 countries of the world with a high burden of tuberculosis, Pakistan ranks sixth. Tuberculosis is responsible for around 70,000 deaths each year in Pakistan and, according to most recent estimates, has an annual incidence rate of 497 per 100,000 population. Pakistan contributes to almost half of the burden of tuberculosis in the Eastern Mediterranean region (WHO). Traditionally, strategies aimed at early diagnosis and management of tuberculosis have been used to decrease the prevalence as well as incidence of tuberculosis.

Diagnosis of tuberculosis requires identification of Mycobacterium tuberculosis (MTB) in biological samples obtained from a patient who is suspected to have tuberculosis. The national tuberculosis control program Pakistan (NTP) recommends sputum smear microscopy as the first line investigation for diagnosis of tuberculosis in new patients despite its relative insensitivity as a positive sputum smear result requires presence of at least 5000 MTB bacilli per millilitre of sputum. Although newer rapid molecular tests are also available for the diagnosis of tuberculosis, the NTP currently suggests that these tests be reserved for re-treatment cases as well as for patients who are being investigated for multi-drug resistance tuberculosis (MDR-TB). Culture of MTB is still the “gold-standard” for diagnosis of tuberculosis despite the presence of a number of rapid detection tests including the Interferon Gamma Release Assays (IGRAs), because of the inability of these tests to differentiate active tuberculosis from latent tuberculosis. Additionally, the NTP recommends that the IGRAs should not be used to diagnose tuberculosis in wake of insufficient data, lack of quality evidence and increased cost.

Tuberculosis can present in any form and tuberculous pleural effusions constitute around one quarter of cases with tuberculosis. Compared with data from European studies where pleural tuberculosis accounted for 3–5% of cases, in developing countries the incidence of pleural tuberculosis has been documented to be as high as 30%. On the other hand, tuberculous pleural effusions is highly prevalent in Pakistan with an estimated prevalence of 56.6%. When a pleural effusion is identified, the diagnostic work up includes a pleural biopsy in addition to the analysis of pleural fluid. The diagnostic yield of pleural biopsy is variable and it increased to 60–95% in case of pleural effusion. However, traditional pleural biopsy using Abram’s or Copes needle is a blind procedure and is associated with certain limitations such as a lower diagnostic yield as well as a comparatively higher rate of complications when compared with VATS or thoracoscopic biopsy. A number of non-invasive tests have been mentioned in literature that aid in diagnosis of tuberculous pleural effusions.

Keywords: Tuberculosis, tuberculous pleural effusion, adenosin deaminase, pleural biopsy
deaminase or ADA, as it is commonly known, has been the most studied of these markers. Many studies have reported that higher levels of ADA have been found in tuberculous pleural effusions as well as in empyema.\textsuperscript{14,15} Owing to its availability, cost effectiveness and rapidity in terms of test-to-result time lapse, we decided to study the role of ADA as a useful and valid surrogate for pleural biopsy for diagnosing the cause of pleural effusion.

MATERIAL AND METHODS

We conducted this cross sectional validation study at the department of pulmonology, Ayub Teaching Hospital, Abbottabad from 15\textsuperscript{th} January to 31\textsuperscript{st} December 2015. During this period we were enrolled 160 patients with lymphocytic exudative pleural effusion in this study after obtaining an informed consent. The patients were subjected to a detailed physical examination and after pleural effusion was confirmed using imaging, pleural specimen was obtained for histopathology using Abrams’ needle under strict aseptic conditions and appropriate local anaesthesia, following which, pleural fluid specimen was collected for measurement of pleural fluid Adenosine Deaminase levels using the commercially available DAIZYME kit in addition to cytology and microbiology.

RESULTS

Majority of study participants were males (n=104; 65\%). Mean age of study participants was 44.3±0.98 years with a range of 21–64. Tuberculosis was diagnosed on histopathology in 99 (61.88\%) study participants. In the remainder, malignant pleural effusion was found in 25 (15.63\%), empyema in 28 (17.5\%) and 8 (5\%) remained inconclusive. Mean±SD levels of ADA in all patients were 52.18±1.98 U/L. The lowest pleural fluid ADA level was 10 U/L while the highest ADA level recorded in this study was 93 U/L. Among patients diagnosed with tuberculosis, Mean±SD levels of ADA were 52.16±2.4 U/L with a range of 12–93, while the mean±SD level of ADA in patients with pleural effusion due to causes other than tuberculosis was 38.6±3.14 U/L with a range of 20–56.

Further analysis revealed that among patients who had been diagnosed with tuberculosis on biopsy, pleural fluid level of ADA was more than the proposed cut off level of 40 U/L in 88 (88.89\%) patients while only 14 (22.96\%) patients who had been diagnosed otherwise had ADA levels more than 40 U/L. A 2x2 contingency table was constructed in following manner to determine the validity and diagnostic accuracy of pleural fluid adenosine deaminase levels.

Table 1: 2×2 table for calculation of sensitivity specificity, PPV and NPV of pleural fluid ADA

<table>
<thead>
<tr>
<th>Pleural fluid ADA level</th>
<th>Tuberculosis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 40 U/L</td>
<td>a = 88 (88.89%)</td>
<td>b = 14 (22.96%)</td>
</tr>
<tr>
<td>a+b = 102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40 U/L</td>
<td>c = 11 (11.11%)</td>
<td>d = 47 (77.04%)</td>
</tr>
<tr>
<td>a+c = 99</td>
<td>b+d = 61</td>
<td></td>
</tr>
<tr>
<td>a+b+c+d = 160</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following values were calculated:

Sensitivity = a/(a+c) × 100
Specificity = d/(b+d) × 100
Predictive value for a positive test = a/(a+b) × 100
Predictive value for a negative test = d/(c+d) × 100

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ADA were found to be 88.88\%, 77.04\%, 86.28\% and 81.04\% respectively.

DISCUSSION

A high prevalence of tuberculosis in Pakistan means that the threshold for diagnosis of tuberculosis should be kept very low and tuberculosis should be considered as a primary differential in any patient presenting with pleural effusion in association with compatible clinical history.

A search for reliable least invasive diagnostic test for tuberculosis has resulted in identification of many diagnostic tests over the years and these tests range from amplification of mycobacterial DNA via polymerase chain reactions to measurement of C-reactive protein (CRP), pleural viscosity, pleural fluid interferons, interleukins, carcinoembryonic antigen (CEA), tumour necrosis factor (TNF), pleural fluid T-lymphocytes and the vascular endothelial growth factor (VEGF).\textsuperscript{8} However, it must be kept in mind that most of these diagnostic utilities are not available in a developing country like ours.

The ease of performance, cost and time-effectiveness of ADA assays mean that ADA can be used as a reliable marker for diagnosis of tuberculosis.\textsuperscript{16} The levels of Adenosine deaminase which is a catalyst of purine base metabolism and is mostly found in the cell cytoplasm, are ten-times higher inside T-lymphocytes making it useful for diagnosis of immune-related disorders because it is released from T-lymphocytes as well as macrophages during immune responses.\textsuperscript{14}

Over the years, ADA has drawn much attention to itself due to its role in diagnosis of tuberculous pleural effusions. The utility of ADA in diagnosis of tuberculous pleural effusions has been a subject of great research. Ever since ADA was first reported to be of value in diagnosis of tuberculosis in 1978\textsuperscript{17}, many studies have been published with
extremely variable results. In our study, the sensitivity and specificity of ADA in diagnosis of tuberculous pleural effusion were 88.88% and 77.04% respectively. A recently published study from Peshawar reported that the pleural fluid ADA levels were 90.47% sensitive and had a specificity of 76.66% when it came to diagnosis of tuberculous pleural effusions. On the other hand, a study from Lahore reported that pleural fluid ADA levels more than 40 U/L had a sensitivity and specificity of 95.77% and 92.31% respectively in diagnosing tuberculous pleural effusions. Other reports of the sensitivity and specificity of ADA for the diagnosis of tuberculous pleural effusion include 94.29% and 92.16% respectively, from India, 78% and 86% respectively from Turkey and a range of 77–100% and 81–97% respectively from Spain. Some studies have even reported a sensitivity approaching 100% for ADA in diagnosis of tuberculous pleural effusion.

Different cut-off values have been used in the research on utility of ADA in predicting tuberculous pleural effusion. From values as low as 27 U/L to as high as 77 U/L have been used. Using a cut-off value of 35 U/L a recent study from Spain reported that the sensitivity of ADA in diagnosis of tuberculous pleural effusion was 85.7%. Similarly, researchers from Egypt reported that the sensitivity, specificity and diagnostic accuracy of ADA levels in pleural effusion were 80%, 85%, and 83.3% respectively for diagnosis of tuberculous pleural effusion. However, the cut-off value they used was 30 U/L.

It is evident from the above discussion that measurement of ADA level in patients with suspected tuberculous effusion holds promise and we suggest that ADA levels more than 40 U/L should be regarded as associated with tuberculosis until proved otherwise.

CONCLUSION

Despite wide variation in reported sensitivity and specificity, measurement of pleural fluid ADA levels is a useful, rapid and time-effective method for diagnosis of tuberculous pleural effusions. However, more research involving a larger population is needed to validate these findings in our population.

AUTHOR’S CONTRIBUTION

AS proposed the study, collected the data, and contributed to literature review. MK provided valuable input regarding data analysis and study design. MAA, SAA, HK did the literature review, assisted in data collection and analysis in addition to drafting the final manuscript.

REFERENCES


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