ORIGINAL ARTICLE

DIAGNOSTIC UTILITY OF PLEURAL FLUID ADENOSINE DEAMINASE LEVEL IN TUBERCULOUS PLEURAL EFFUSION

Amir Suleman, Muhammad Kamal*, Munir Ahmad Abbasi, Syed Abbas Anwar, Haroon Khan**

Department of Pulmonology, Ayub Teaching Hospital, Abbottabad, *Department of Community Medicine, Poonch Medical College, Rawalakot-AJK, **Department of Biochemistry, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad-Pakistan

Background: Early diagnosis and management of tuberculosis is essential for decreasing the disease burden. Pakistan is one of the few countries of world with a very high burden of tuberculosis. Many diagnostic tests are available for detection of tuberculosis but each is fraught with certain limitations of its own. **Methods**: This study was a cross sectional validation study that sought to determine the validity of pleural fluid adenosine deaminase levels for diagnosis of tuberculous pleural effusion. **Results**: A total of 160 patients with exudative lymphocytic pleural effusions were enrolled in this study. The mean pleural fluid ADA level was 52.18±1.98 U/L. The mean pleural fluid ADA level in patients diagnosed to have tuberculosis on pleural biopsy/histopathology was higher as compared to patients who did not have tuberculous pleural effusion 52.16±2.4 U/L vs 38.6±3.14 U/L. The difference was found to be statistically significant between the two groups ($p<0.05$). The sensitivity, specificity, ppv and npv of pleural fluid ADA level were 88.88%, 77.04%, 86.28% and 81.04% respectively. **Conclusion**: Despite wide variations in the reported sensitivity and specificity of pleural fluid ADA level, it can be used as a surrogate for pleural biopsy when the latter is not feasible.

Keywords: Tuberculosis, tuberculous pleural effusion, adenosin deaminase, pleural biopsy

INTRODUCTION

Among the 22 countries of the world with a high burden of tuberculosis, Pakistan ranks sixth.[1] 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.[1,2] 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.[3]

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.[4] 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).[4] 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.[4]

Tuberculosis can present in any form and tuberculous pleural effusions constitute around one quarter of cases with tuberculosis.[5] 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%.[6,7] On the other hand, tuberculous pleural effusions is highly prevalent in Pakistan with an estimated prevalence of 56.6%.[8]

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 tuberculous pleural effusions.[8] 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.[9,10]

A number of non-invasive tests have been mentioned in literature that aid in diagnosis of tuberculous pleural effusions.[11–14] Adenosine

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.[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 15th January to 31st 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

Pleural fluid ADA level	Biopsy result		
	Tuberculosis	Other	
> 40 U/L	a 88 (88.89%)	b 14(22.96%)	a+b =102
< 40 U/L	c 11 (11.11%)	d 47 (77.04%)	c+d = 58
	a+c =99	b+d = 61	a+b+c+d =160

The following values were calculated:
Sensitivity=a/a+c x 100
Specificity=d/b+d x 100
Predictive value for a positive test=a/a+b x100
Predictive value for a negative test=d/c+d x100
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).[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.[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.[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[17], many studies have been published with

extremely variable results.[8] 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.[8] 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.[14] 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[15], 78% and 86% respectively from Turkey[18] and a range of 77–100% and 81–97% respectively from Spain.[19] Some studies have even reported a sensitivity approaching 100% for ADA in diagnosis of tuberculous pleural effusion.[20–22]

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.[8] 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%.[23] 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.[24]

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

1. Qasim M. TB kills over 70,000 people every year in Pakistan. The News. Islamabad. Islamabad; 2016 Mar 24.
2. Fatima R, Harris RJ, Enarson DA, Hinderaker SG, Qadeer E, Ali K, *et al*. Estimating tuberculosis burden and case detection in Pakistan. Int J Tuberc Lung Dis 2014;18(1):55–60.
3. Kamal M, Ali S, Hussain SJ, Abbasi MA. Demographics of tuberculosis in district mansehra. J Ayub Med Coll Abbottabad 2015;27(4):890–3.
4. National TB Control Program Pakistan. National Guidelines for the Control of Tuberculosis in Pakistan [Internet]. National TB Control Program Pakistan; 2015 [cited 2016 Apr 27]. Available from: /http://ntp.gov.pk/uploads/NATIONAL_GUIDELINE_ON_TB_CASE_MANAGEMENT_REV_JAN_2015.pdf
5. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest 2007;131(3):880–9.
6. Light RW. Pleural diseases. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. P.427.
7. Frank W. Tuberculous Pleural Effusion. In: Mahboub B, editor. Tuberculosis - Current Issues in Diagnosis and Management [Internet]. InTech; 2013 [cited 2016 Apr 27]. Available from: http://www.intechopen.com/books/tuberculosis-current-issues-in-diagnosis-and-management/tuberculous-pleural-effusion
8. Ullah Z, Ali S, Rafiq M, Khan S, Basit A, Iqbal Z, *et al*. Diagnostic accuracy of pleural fluid adenosine deaminase in patients with tuberculous pleural effusion keeping closed pleural biopsy as a gold standard. Pak J Chest Med 2015;21(2):47–53.
9. Bibby AC, Maskell NA. Pleural biopsies in undiagnosed pleural effusions; Abrams vs image-guided vs thoracoscopic biopsies. Curr Opin Pulm Med 2016;22(4):392–8.
10. Wiewiorski M, Hiebinger A, Hoechel S, Müller-Gerbl M, Barg A, Valderrabano V, *et al*. Transcutaneous pleural biopsy with a retrograde forceps: a novel approach. Surg Endosc 2016;30(1):396–400.
11. Verea Hernando HR, Masa Jimenez JF, Dominguez Juncal L, Perez Garcia-Buela J, Martin Egaña MT, Fontan Bueso J. Meaning and diagnostic value of determining the lysozyme level of pleural fluid. Chest 1987;91(3):342–5.
12. Ribera E, Ocaña I, Martinez-Vazquez JM, Rossell M, Español T, Ruibal A. High level of interferon gamma in tuberculous pleural effusion. Chest 1988;93(2):308–11.
13. Caminero JA, Rodriguez de Castro F, Carrillo T, Diaz F, Rodriguez Bermejo JC, Cabrera P. Diagnosis of pleural tuberculosis by detection of specific IgG anti-antigen 60 in serum and pleural fluid. Respiration 1993;60(1):58–62.
14. Ashraf M, Hussain M, Chima KK, Ayyaz S. Diagnostic Role of Adenosine Deaminase level in Exudative Lymphocytic Pleural Effusions. Pak J Chest Med 2016;22(1):03–7.
15. Gupta BK, Bharat V, Bandyopadhyay D. Sensitivity, specificity, negative and positive predictive values of adenosine deaminase in patients of tubercular and non-tubercular serosal effusion in India. J Clin Med Res 2010;2(3):121–6.
16. Burgess LJ, Swanepoel CG, Taljaard JJ. The use of adenosine deaminase as a diagnostic tool for peritoneal tuberculosis. Tuberculosis (Edinb) 2001;81(3):243–8.
17. Piras MA, Gakis C, Budroni M, Andreoni G. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br Med J 1978;2(6154):1751–2.
18. Ciledag A, Kaya A, Erol S, Sen E, Celik G, Cesur S, *et al*. The comparison of pleural fluid TNF-alpha and IL-10 levels with ADA in tuberculous pleural effusion. Curr Med Chem 2010;17(19):2096–100.

19. Pérez-Rodriguez E, Jiménez Castro D. The use of adenosine deaminase and adenosine deaminase isoenzymes in the diagnosis of tuberculous pleuritis. Curr Opin Pulm Med 2000;6(4):259–66.
20. Valdés L, San José E, Alvarez D, Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role, and relevance to the origin of increased ADA in tuberculous pleurisy. Eur Respir J 1996;9(4):747–51.
21. Ocaña I, Martinez-Vazquez JM, Segura RM, Fernandez-De-Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluids. Test for diagnosis of tuberculous pleural effusion. Chest 1983;84(1):51–3.
22. Valdés L, San José E, Alvarez D, Sarandeses A, Pose A, Chomón B, *et al*. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. Chest 1993;103(2):458–65.
23. Arnold DT, Bhatnagar R, Fairbanks LD, Zahan-Evans N, Clive AO, Morley AJ, *et al*. Pleural Fluid Adenosine Deaminase (PFADA) in the Diagnosis of Tuberculous Effusions in a Low Incidence Population. PLoS One 2015;10(2):e0113047.
24. Helmy NA, Eissa SA, Masoud HH, Elessawy AF, Ahmed RI. Diagnostic value of adenosine deaminase in tuberculous and malignant pleural effusion. Egypt J Chest Dis Tubercl 2012;61(4):413–7.

Address for Correspondence:

Amir Suleman, Assistant Professor & Head, Department of Pulmonology, Ayub Teaching Hospital, Abbottabad-Pakistan
Cell: +92 301 892 5009
Email: amirsuleman2013@gmail.com