Global Academic Journal of Medical Sciences

Available online at www.gajrc.com
DOI: 10.36348/gajms.2024.v06i02.004

OPEN ACCESS

ISSN: 2706-9036 (P)
ISSN: 2707-2533 (O)

Original Research Article

The Impact Profile and Adenosine Deaminase of Age and Female Sex on Its Level in Patients with Pleural Effusion Syndrome

Md. Rafiqul Islam[1*]

[1]Senior Consultant (Internal Medicine), 250 Bedded Mohammad Ali Hospital, Bogura, Bangladesh

***Corresponding Author**
Md. Rafiqul Islam
Senior Consultant (Internal Medicine), 250 Beded Mohammad Ali Hospital, Bogura, Bangladesh

Article History
Received: 24.02.2024
Accepted: 01.04.2024
Published: 03.04.2024

Abstract: **Background:** Pleural fluid analysis and closed pleural biopsy are integral parts in the investigative work up of an exudative pleural effusion. For diagnosis of tuberculous pleural effusion (TPE), the yield of pleural fluid culture for mycobacteria is low at about 36%. **Objective:** To evaluate the levels of adenosine deaminase (ADA) in the pleural fluids (P-ADA) of untreated and non-surgically manipulated female and male adult patients with several confirmed causes of PES. **Methods:** This was an observational study conducted at dept. of Internal Medicine, 250 Bedded Mohammad Ali Hospital, Bogura, Bangladesh from January to June 2023. 100 pleural fluid samples from 100 patients with proven causes of PES. The sample size was appropriate according to the study design. Written consent was obtained from all patients. It was precise enough to calculate descriptive and inferential statistics on type I and II errors, effect size, standard deviation, and was not influenced by administrative issues and costs. **Results:** 100 patients summarize the prevalence of the causes of PES and the demographic characteristics. TB was the most prevalent cause of PES (28/100, 28%). Most prevalent male sex was in the lymphoma group (4/0, 100%). None of the five patients with lymphoma were female. When the frequencies were compared for males and females, only adenocarcinoma and lymphoma were significant (chi- square tests, p=0.0021 and p=0.0003, respectively). The causes, prevalence, and median P-ADA (n/%/U/L) were tuberculosis (28/28.0/42.0), adenocarcinoma (24/24.0/9.75), transudate (21/21.0/6.85), simple parapneumonic pleural effusions (PPE; 10/10.0/9.38), complicated PPE/empyema (5/5.0/32.9), lymphoma (4/4.0/401.2), squamous cell carcinoma (4/4.0/13.11), and others (4/4/15.2). For P-ADA, Dunn's post hoc test revealed significance for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma (all P<0.05), and not significant for CPPE/empyema, lymphoma, SCC, and others (all P>0.05). For age, Dunn's post hoc test revealed significance for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinomas (all P<0.05). Sex was not significant in the overall PES group (Chi=0.062, P=0.8028). Kendall's correlation of the relationship between P-ADA and age for pleural tuberculosis (n=26) was significant after 1000 iterations with bootstrap for 95% CI (Tau=-0.213, 95% CI - 0.449-0.0833, P=0.0490). A negative LOESS regression was evident between P-ADA and age >40 years. **Conclusions:** Evaluation of pleural ADA levels is useful for diagnosing pleural tuberculosis, while sex is not. A negative and significant relationship between P-ADA level and age >40 years was evident.

Keywords: Pleural Effusion, Pleural Tuberculosis, Malignancy.

Citation: Md. Rafiqul Islam (2024). The Impact Profile and Adenosine Deaminase of Age and Female Sex on Its Level in Patients with Pleural Effusion Syndrome. *Glob Acad J Med Sci*; Vol-6, Iss-2 pp- 66-72.

INTRODUCTION

Pleural fluid analysis and closed pleural biopsy are integral parts in the investigative work up of an exudative pleural effusion. For diagnosis of tuberculous pleural effusion (TPE), the yield of pleural fluid culture for mycobacteria is low at about 36% [1]. Joint sensitivity of biopsy tissue samples is as high as 90% [1], but closed pleural biopsy is a relatively invasive procedure and involves a long waiting time for mycobacteria culture results. Pleural fluid adenosine deaminase (ADA) has thus become an important diagnostic tool in the evaluation of exudative pleural effusions because it is inexpensive, rapid and has a high accuracy with sensitivity and specificity of up to 100% and 95% respectively for diagnosis of TPE [2]. The causal diagnosis of pleural effusion syndrome (PES) depends on clinical manifestations, imaging findings, and pleural biomarkers in materials withdrawn via surgical procedures performed using different techniques [3]. The analysis of pleural biomarkers is important in clinical practice because the first approach in diagnosing the cause of PES is to correctly classify the pleural fluid as exudate or transudate, according to several published criteria [3-5]. The diagnosis of PTB using traditional methods is challenging due to the low sensitivity of routine TB diagnostic tools [6], viz. sputum smear microscopy (developed over 100 years ago and the most common method for TB diagnosis worldwide). The microbiological diagnosis of pleural TB is difficult to establish because of the paucibacillary nature of the pleural fluid. Therefore, conventional tests, such as bacilloscopy and culture of Mycobacterium sp. in pleural fluids, have low accuracy. Amplification of nucleic acids with a positive result is a presumptive case. However, a negative result cannot exclude a diagnosis. Currently, it is desirable to refer a patient with PES without a causal diagnosis for video-assisted thoracoscopic surgery (VATS) after one or two inconclusive thoracentesis attempts with laboratory analysis of pleural fluid [7]. Total adenosine deaminase enzyme (ADA, E.C. 3.5.4.4) is an accurate biomarker for TB in pleural fluids obtained by thoracentesis. The test does not depend on a human immunodeficiency virus (HIV)- positive serostatus of the patient and tissue from CNPB [7, 8]. In recent years in Brazil, 29% of the total notified cases and relapses of pulmonary TB involved female patients [6]. Evidence for differences between males and females is well documented for a variety of inflammatory conditions [9]. The aim of our research was to evaluate the levels of total ADA in pleural fluids (P-ADA) in female and male adult patients who were untreated and not surgically manipulated, with several confirmed causes of PES.

MATERIALS & METHODS

This was an observational conducted at dept. of Internal Medicine, 250 Bedded Mohammad Ali Hospital, Bogura, Bangladesh from January to June 2023. 100 pleural fluid samples from 100 patients with proven causes of PES. The sample size was appropriate according to the study design. Written consent was obtained from all patients. It was precise enough to calculate descriptive and inferential statistics on type I and II errors, effect size, standard deviation, and was not influenced by administrative issues and costs. The sample size featured an acceptable level of significance, power, expected effect size, and underlying event rate in the population [8].

Inclusion Criteria

The causal diagnosis of PES was confirmed after one or two thoracentesis procedures with laboratory evaluation of the pleural fluid and VATS, if necessary [7, 8]. According to clinical manifestations and imaging findings, pleural TB was confirmed with a positive culture of pleural fluid or tissue. Additionally, confirmation involved the presence of granulomas in the pleural tissue by biopsy and the absence of other pleural granulomatous diseases. All patients had a favorable clinical course after 6–12 months of treatment [7, 8]. Simple parapneumonic pleural effusions (PPEs) have been proven in patients with cough, fever, and lung image examinations with infiltrates or consolidations that disappeared with antibiotic treatment. Patients with empyema were diagnosed with pus in the pleural fluid after thoracentesis and septations on pleural ultrasound.

Exclusion Criteria

The exclusion criteria included absolute contraindications, refusal to accept thoracentesis or other invasive procedures, use of immunosuppressive medications, hemolysis in pleural liquids, renal failure, HIV infection, pleural effusion of an unknown cause, and cases with missing values. According to the ADA dosage kit instructions, patients with biochemical and metabolic disturbances were excluded as these factors interfere with the enzyme level in pleural fluids [8].

ADA Assay: One unit of ADA was defined as the amount of ADA that generated one μmol/min of inosine at 37 °C.

Statistical Analyses

All quantitative and qualitative data from our patients were analyzed using Microsoft Excel, version 2023. The collected data were analyzed using univariate statistical tests. The Shapiro-Wilk

test was used to assess the normality of the data and homogeneity of variances. Non-normal distributions were expressed as median and interquartile range (IQR). Qualitative or categorical variables were expressed as proportions. A chi-squared test was used to compare the proportions. The Mann-Whitney (M-W) non-parametric test was used to compare medians of two independent group data when it was not normally distributed and because logarithmic transformation was not performed. A locally weighted scatterplot smoothing (LOESS or LOWESS) was performed to create a regression line through a scatter plot to determine the relationship between P-ADA and age in the pleural TB group in the correlation analysis.

RESULTS

100 patients summarize the prevalence of the causes of PES and the demographic characteristics. TB was the most prevalent cause of PES (28/100, 28%). Most prevalent male sex was in the lymphoma group (4/0, 100%). None of the five patients with lymphoma were female. When the frequencies were compared for males and females, only adenocarcinoma and lymphoma were significant (chi- square tests, p=0.0021 and p=0.0003, respectively). In male and female sexes, when the prevalence was compared by the Chi-square test, only adenocarcinoma and lymphoma were significant (P=0.0021 and P=0.0003, respectively). The sex differences in the overall pleural effusion syndrome group were not significant according to the chi-squared test (Chi=0.062, P=0.8028). The Shapiro-Wilk test for age (W=0.87) revealed a p-value <0.05 (p=0.0017). The group of patients with older age was the transudate with a median age of 68 years (IQR, 63.0-86.25). The Kruskal-Wallis test for age (H=40.93, p<0.0001) and Dunn's post hoc test (p<0.05) revealed significance for TB vs. transudates, vs. simple PPE, and vs. adenocarcinomas.

Table 1: Demographic characteristics and causes of pleural effusion syndrome in the patients.

Causes	Patient (n)	Prevalence (%)	Age-years medians - IQR (25-75th)	Female (n/%)	Male (n/%)
Tuberculosis	28	28.0	39.0 (29.7-58.2)	15 (55.5)	12 (44.5)
Adenocarcinoma	24	24.0	61.0 (45.0-77.0)	17 (70.8)	7 (29.2)
Transudate	21	21.0	76.0 (63.0-86.25)	10 (50.0)	10 (50.0)
Simple parapneumonic effusion	10	10.0	67.0 (56.0-85.0)	4 (40.0)	6 (60.0)
CPPE and empyema	5	5.0	52.5 (33.5-78.75)	1 (20.0)	4 (80.0)
Lymphoma	4	4.0	53.0 (47.0-63.0)	0 (0.0)	5 (100.0)*
Squamous cell carcinoma	4	4.0	66.0 (55.0-66.0)	2 (40.0)	3 (60.0)
Other exudates	4	4.0	73.0 (53.0-79.0)	2 (50.0)	2 (50.0)
Total	**100**	**100.0**	**58.0 (41.75-73.25)**	**51 (51.0)**	**49(49.0)**

Table 2: Levels of pleural adenosine deaminase evaluated in causes of pleural effusion syndrome confirmed with reference standard diagnostic tests.

Causes	Pleural fluid sample size (n)	P-ADA medians (U/L)	Median interquartile range (25th-75th percentile)
Tuberculosis	28	42.0	32.9-61.9
Adenocarcinoma	24	9.75	6.7-14.9
Transudate	21	6.85	2.67-11.26
Simple parapneumonic effusion	10	9.38	5.68-9.97
CPPE and empyema	5	32.9	16.0-61.7
Lymphoma	4	401.2	11.2-990.5
Squamous cell carcinoma	4	13.11	11.0-28.2
Other exudates	4	15.2	7.4-49.0

Abbreviations:
CPPE (complicated parapneumonic effusions) and P-ADA (pleural adenosine deaminase). The Shapiro-Wilk test for total P-ADA levels (W=0.347) had a P=0.0001.
The Shapiro-Wilk test for ages rejected normality (W=0.87) with a P-value <0.05 (P=0.0017).

The P-ADA levels of the 100 patients are shown in Table 2. The Shapiro-Wilk test for total P-ADA levels (W=0.347) revealed a P-value <0.05 (P=0.0001). The median values were statistically significant using the Kruskal-Wallis test (H=81.34, P<0.0001). When Dunn's post hoc test was used for pairwise comparison of subgroups, significant results (P<0.05) were obtained for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma.

Results were not significant (P>0.05) for CPPE and empyema, lymphoma, SCC, and other exudates.

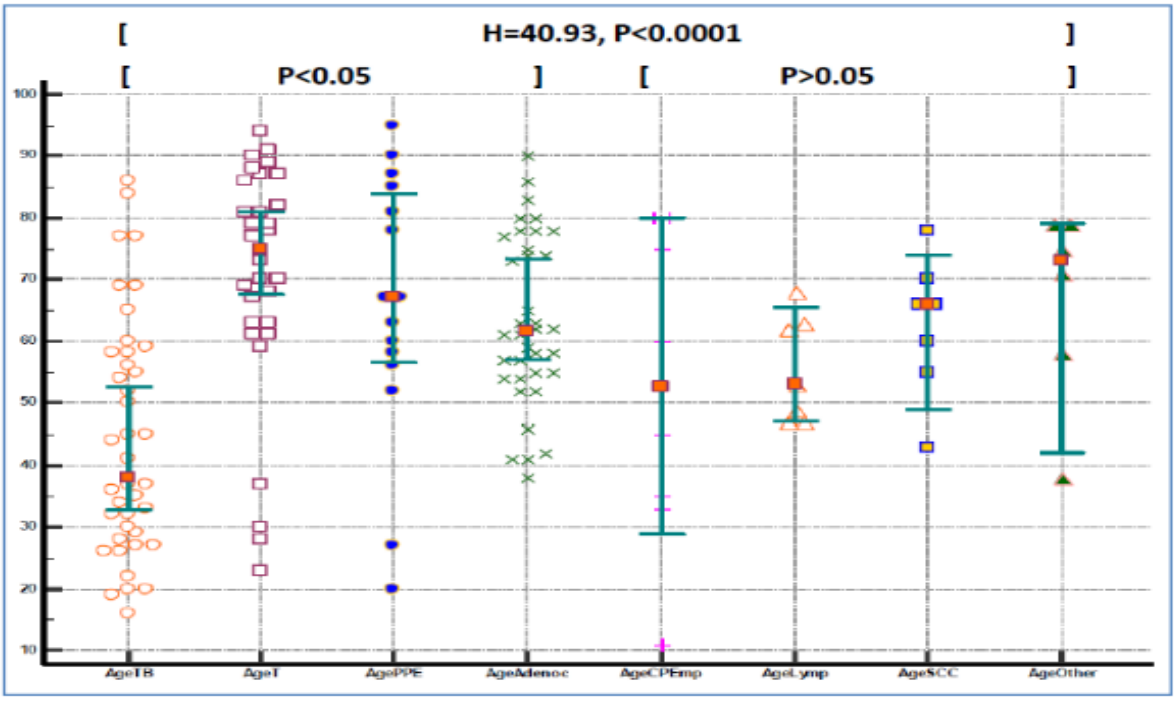

Fig. 1: Age statistical analysis in several causes of pleural effusion syndrome from Table 1. Kruskal-Wallis test for ages was significant (H=40.93, P<0.0001). Dunn's post hoc test with P<0.05 was significant for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinomas. Abbreviations: CPPE, complicated parapneumonic effusions; TB, pleural tuberculosis; Lymp., lymphoma; SCC, squamous cells carcinoma; PPE, parapneumonic pleural effusion.

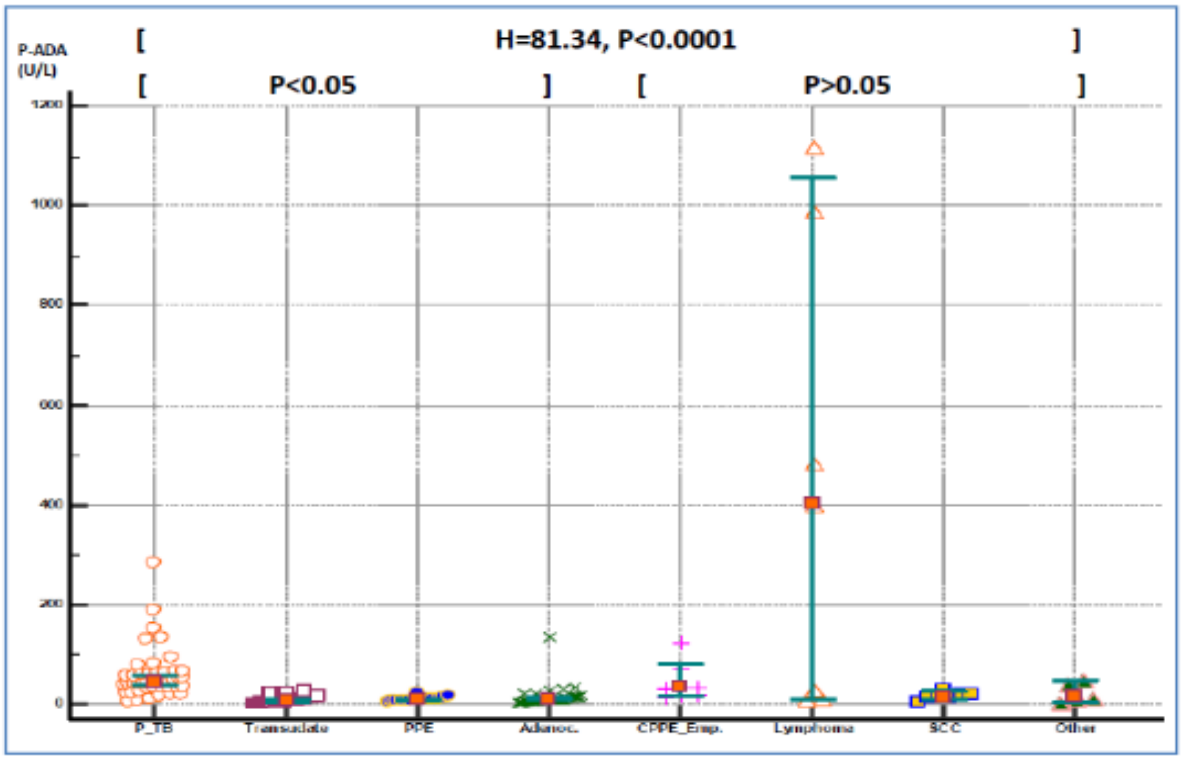

Fig. 2: Pleural adenosine deaminase levels (P-ADA, U/L) in different diagnostic groups of pleural effusion syndrome. Kruskal-Wallis test, H=81.34, P<0.0001. Dunn's post -hoc test, P<0.05 for tuberculosis vs. transudates, vs. simple PPE, and vs. adenocarcinoma, and P>0.05 for CPPE and empyema, lymphomas, squamous cell carcinoma, and other exudates.

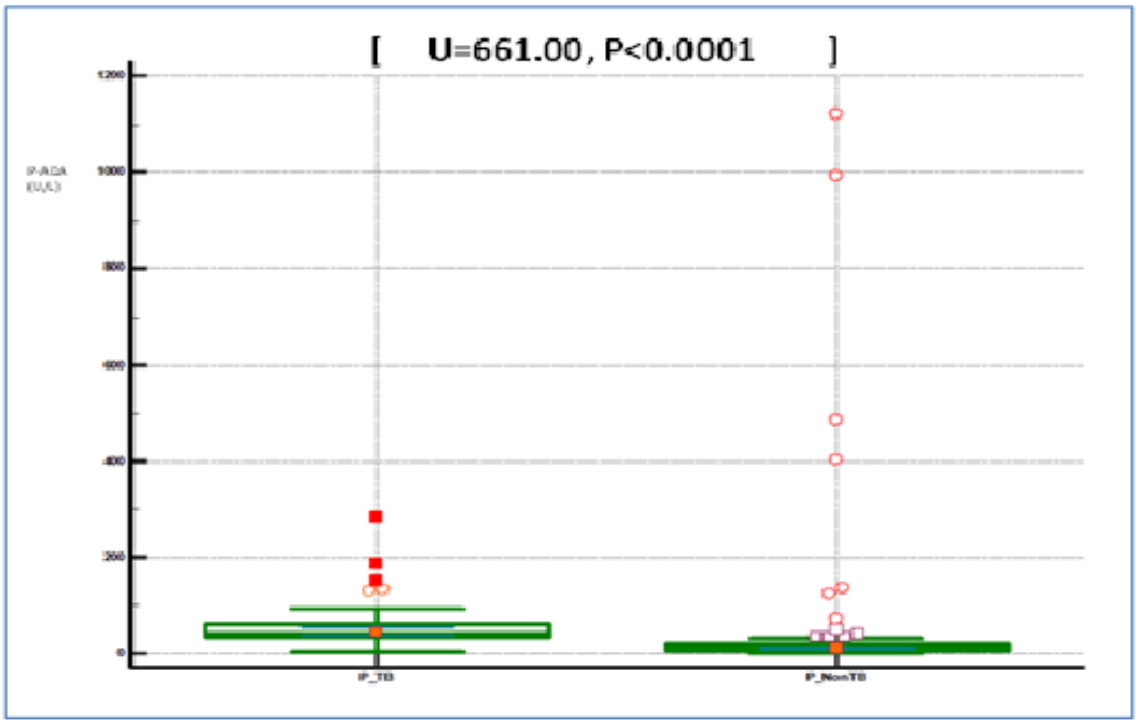

Fig. 3: Median values of pleural adenosine deaminase levels (P-ADA, U/L) summarized in two groups of pleural effusion syndrome, tuberculosis (P-TB, median=42.03, IQR, 32.92-60.20) and non-tuberculosis (P-nonTB, median=9.87, IQR, 5.99- 16.90). Mann-Whitney test (U=661.00; P<0.0001).

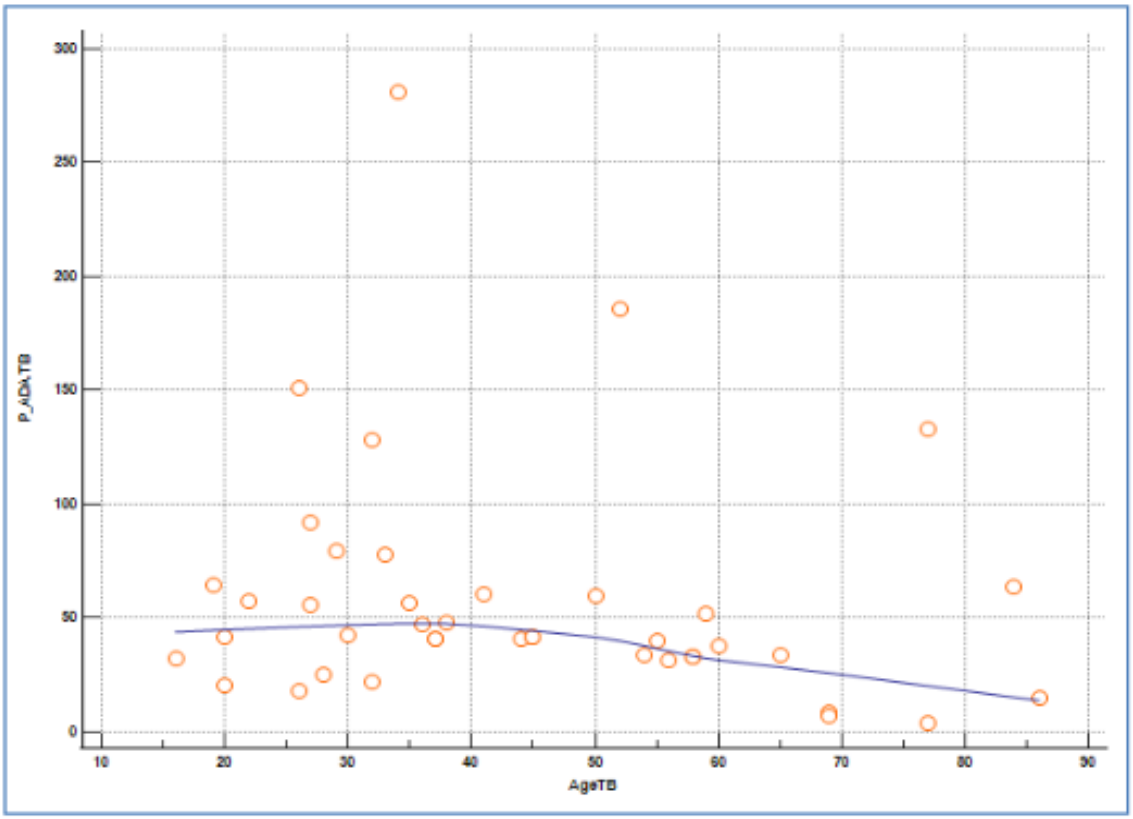

Figure 4: shows the degree of the relationship between the variables, P-ADA and age, for pleural TB in a sample size of 26 cases with LOESS regression line and statistical notes. Spearman coefficient of rank correlation (rho= -0.261, 95% CI, -0.526-0.0508, P=0.0994). The Kendall's correlation was negative and significant after 1000 iterations with bootstrap for 95% CI (Tau=-0.213, 95%CI, - 0.449-0.0833, P=0.0490).

DISCUSSION

Pleural fluid characteristics need to be considered during interpretation of pleural fluid ADA as it decreases with age and increases with pleural fluid protein. Few studies have looked at the factors affecting pleural ADA. Parapneumonic effusions were diagnosed based on clinical and radiographic features that were consistent with an acute pulmonary infection and exclusion of other causes of pleural effusion. Effusions were attributed to congestive cardiac failure (CCF) if the effusion was a transudate with consistent clinical features. The profiles of adenosine deaminase, age, and sex were investigated in 100 patients with PES. The study cohort excluded children and adolescents. TB was the most prevalent cause of exudative pleural effusion in our cohort. Female sex was most prevalent in adenocarcinoma, transudates, SCC, and other exudates. However, when the prevalence rates were compared between males and females by the Chi-square test, only adenocarcinoma and lymphoma were significant (P=0.0021 and P=0.0003, respectively). In addition, the sex differences in the overall PES group were not significant according to the chi-squared test (Chi=0.062, P=0.8028). TB was the cause of PES, with a minor median age. The Kruskal-Wallis test for age was significant (H=40.93, P<0.0001). Dunn's post hoc test (P<0.05) was significant for TB vs. transudates, vs. simple PPE, and vs. adenocarcinomas. The sexes and median ages were similar to those reported in other studies published by our group [10, 11]. The Kruskal-Wallis test for P-ADA levels revealed significance (H=81.34, P<0.0001; Table 2). Dunn's post hoc test revealed significance (P<0.05) for TB vs. transudates, vs. simple PPE, and vs. adenocarcinoma, but was not significant (P>0.05) for CPPE and empyema, lymphoma, SCC, and other exudates. The median levels of P-ADA evaluated in the two groups of PES, TB (P-TB) and non-TB (P-non-TB). When compared using the Mann- Whitney test, the level of P-ADA was significant (U=661.00, P<0.0001). A Spanish study with 4,147 patients concluded that total P-ADA had 93% sensitivity, 92% specificity, positive likelihood ratio of 12, negative likelihood ratio of 0.08, and an area under the curve of 0.968 for TB pleural effusion [12]. Developing countries, such as Bangladesh, India, Pakistan, and others, should implement ADA testing as the first option for pleural TB diagnosis [13]. P-ADA levels may be falsely low in elderly patients and falsely elevated in complicated parapneumonic effusions, empyema, rheumatoid arthritis, and lymphomas [7,14, 15]. The Spearman's rank correlation coefficient was negative and not significant (rho= - 0.261, 95% CI, -0.526-0.0508, P=0.0994). However, Kendall's correlation after 1000 iterations from bootstrap for 95% CI was negative and significant (Tau=-0.213, 95% CI, -0.449-0.0833, P=0.0490). The LOESS regression line clearly showed a negative relationship between P-ADA levels and age >40 years. This statistical finding is new and important for the pleural ADA study. Finally, some considerations in this study must be mentioned. First, for clinical applications in PES, a profile of P-ADA, age, and sex was shown with appropriate statistical approaches, such as the design of the study, distribution of the data, and nature of the observations (paired/unpaired). Second, as a future perspective for pleural TB diagnosis, different cut-off point values for ADA must be calculated in relation to the age of patients. Third, the limitations of this study were that diagnostic accuracy measures, specifically the rates of sensitivity, specificity, predictive values, likelihood ratios, and odds ratio from P-ADA levels were not calculate as a secondary objective.

CONCLUSION

In conclusion, evaluation of pleural ADA levels is useful for pleural TB. Sex was not found to be significant for differential diagnosis. However, a negative and significant relationship was evident between P-ADA level and age >40 years.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2013. Geneva: WHO, 2012. http://www.who.int/tb/publications/global_report/en/ (accessed 7 April 2013).
2. Western Cape Government. World TB Day, March 2012. 23 March 2012. http://www.westerncape.gov.2/news/world-tb-day-24-march-2012 (accessed 7 April 2013).
3. Frye, M. D., Pozsik, C. J., & Sahn, S. A. (1997). Tuberculous pleurisy is more common in AIDS than in non-AIDS patients with tuberculosis. Chest, 112(2), 393-397. http://dx.doi.org/10.1378/chest.112.2.393
4. Kataria, Y. P., & Khurshid, I. (2001). Adenosine deaminase in the diagnosis of tuberculous pleural effusion. Chest, 120(2), 334-336. http://dx.doi.org/10.1378/chest.120.2.334.
5. Sandeesha, V., Kiran, C. V. R., Ushakiran, P., Sulemani, M. D., & Lakshmanakumar, N. (2020). A comparative study of serum effusion albumin gradient and Light's criteria to differentiate exudative and transudative pleural effusion. Journal of Family Medicine and Primary Care, 9(9), 4847-4852.
6. Behrsin, R. F., da Silva Junior, C. T., Cardoso, G. P., Barillo, J. L., de Souza, J. B. S., & de Araújo, E. G. (2015). Combined evaluation of adenosine deaminase level and histopathological findings from pleural biopsy with Cope's needle for the diagnosis of tuberculous pleurisy. International

journal of clinical and experimental pathology, 8(6), 7239.
7. Da Silva Jr, C. T., Behrsin, R. F., Cardoso, G. P., & De Araújo, E. G. (2013). Evaluation of adenosine deaminase activity for the diagnosis of pleural TB in lymphocytic pleural effusions. Biomarkers in medicine, 7(1), 113-118.
8. Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. Nature Reviews Immunology, 16(10), 626-638.
9. Moons, K. G., Altman, D. G., Reitsma, J. B., Ioannidis, J. P., Macaskill, P., Steyerberg, E. W., ... & Collins, G. S. (2015). Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Annals of internal medicine, 162(1), W1-W73.
10. Von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., & Vandenbroucke, J. P. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. The lancet, 370(9596), 1453-1457.
11. Kadam, P., & Bhalerao, S. (2010). Sample size calculation. International journal of Ayurveda research, 1(1), 55.
12. Villena, V., López-Encuentra, A., García-Luján, R., Echave-Sustaeta, J., & Martínez, C. J. Á. (2004). Clinical implications of appearance of pleural fluid at thoracentesis. Chest, 125(1), 156-159.
13. Porcel, J. M. (2018). Biomarkers in the diagnosis of pleural diseases: a 2018 update. Therapeutic advances in respiratory disease, 12, 1753466618808660.
14. Delacour, H., Sauvanet, C., Ceppa, F., & Burnat, P. (2010). Analytical performances of the Diazyme ADA assay on the Cobas® 6000 system. Clinical biochemistry, 43(18), 1468-1471.
15. Khan, R. N., Ahmed, S. I., Kausar, S. F., Saba, F., Din, S., Deen, Z. U., & Shah, A. (2019). Lymphocytic Pleural Effusion and an Enzyme Involved in Purine Metabolism: A Tertiary Care Experience in Karachi, Pakistan. Cureus, 11(2).