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Study of Adenosine Deaminase Levels in Patients of Pulmonary Tuberculosis with and Without Pleural Effusion

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Abstract: *Tuberculosis (TB) is one of the oldest and commonest infectious diseases also known as "master of death" or "Captain of death". Pulmonary TB(PTB) and TB with pleural effusion remains a diagnostic challenge. Adenosine deaminase(ADA) is an enzyme of purine catabolism which is an inexpensive and easy test in early routine evaluation of patients with pleural effusion and helps to avoid invasive test like biopsy. Therefore, this study was aimed to determine the exact role of ADA in TB patients with and without pleural effusion and in non-tuberculosis pleural effusion and to provide a clear picture of ADA for early diagnosis & management of TB.*

Materials and method: *Study comprised of 132 subjects of which 33 are healthy controls and 33 are confirmed cases of pulmonary TB without effusion, 33 are PTB with effusion and 33 are non-TB effusion patients. Age group range was from 20- 70 years. Estimation of serum and pleural fluid Adenosine deaminase by Guisti and Galanti method of enzymatic analysis.*

Results: *Serum ADA levels in pulmonary tuberculosis (55.09 $\pm$ 11.02) patients and pulmonary tuberculosis with pleural effusion (44.01 $\pm$ 7.82) were significantly higher ($p < 0.001$) when compared with healthy controls (18.11 $\pm$ 6.13). Pleural fluid ADA levels were significantly higher ($p < 0.0001$) in pulmonary tuberculosis with pleural effusion (82.61$\pm$ 12.03) than in non tuberculosis pleural effusion (27.72 $\pm$7.80).*

In the present study, the mean pleural fluid ADA were significantly higher as compared to mean serum ADA in pulmonary tuberculosis with pleural effusion ($p < 0.0001$) and in non tuberculosis pleural effusion ($p < 0.0008$).

Conclusion: *ADA level in serum as well as in pleural fluid in the diagnosis of pulmonary TB with or without pleural effusion is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.*

Keywords: *Adenosine deaminase, pulmonary TB, pulmonary TB with pleural effusion, non-tuberculosis pleural effusion.*

I. INTRODUCTION

TB is one of the oldest and commonest infectious diseases also known as "*master of death" or "Captain of death*" [(1)]. It is still a global burning problem and now the world's seventh leading cause of death [(2)]. The severity of the disease can be judged by the fact that it affects all ages, irrespective of the sex. No other disease has so much socio-economic health significance as TB in a country like India. TB, a bacterial disease is chronic granulomatous infection, caused by *Mycobacterium tuberculosis* and occasionally by *Mycobacterium africanum*. TB is categorised as pulmonary tuberculosis and extra-pulmonary tuberculosis. The main symptoms of TB are chronic cough, low grade fever – evening rise of temperature, haemoptysis, chest pain, dyspnoea ,loss of weight ,unresolved pneumonia [(3)]. Its complications being massive haemoptysis, cor pulmonale, fibrosis/emphysema , calcification, obstructive airway disease, bronchiectasis, bronchopleural fistula. Pleural TB is one of the most common extra-pulmonary manifestations of the disease and may represent up to 10% of all cases [(4)]. PTB and TB with pleural effusion remains a diagnostic challenge. TB can be diagnosed by Mantoux, Acid fast bacilli (AFB) staining, sputum culture, X-ray chest and newer advances like polymerase chain reaction (PCR). Directly observed treatment (DOTS) has helped in curing tuberculosis to some extent but unless and until a proper diagnosis is made, TB will remain a major health problem. The above mentioned diagnostic methods are very useful for the diagnosis of TB but have a low yield. Direct analysis of pleural fluid

for detection of acid-fast bacilli (AFB) by the Ziehl-Neelsen or similar method is positive in less than 5% of cases, and the culture on Lowenstein-Jensen medium takes more than four weeks and does not surpass a 40% positivity rate [(5)].

Adenosine deaminase (EC 3.5.4.4), called ADA by Spencer et al [(6)], is an enzyme of purine catabolism which catalyses the pathway from adenosine to inosine [(7)]. Its distribution in the human organism is ubiquitous [(8)], but its physiologic role is especially important in lymphoid tissue. Its level is ten times higher in lymphocytes than in erythrocytes [(9)], and particularly in T-lymphocytes with variations according to cellular differentiation [(10)]. Estimation of serum ADA activity is a simple, rapid, non-invasive and relatively less expensive method, so it should find a place in routine laboratory investigation [(11, 12)]. It also helps in early diagnosis and treatment of the patient and prevents the spread of disease in the community [(13)].

Therefore, this study was planned to determine the exact role of ADA in TB patients with and without pleural effusion.The results of this study will help to provide a clear picture of ADA for diagnosis & prognosis of TB, & hence future plans for TB can be better executed.

II. AIM OF THE STUDY

To determine the mean values of serum ADA in the patients of PTB with and without pleural effusion and in non-tuberculosis pleural effusion

III. OBJECTIVES OF THE STUDY

1. To assess & compare the serum Adenosine deaminase (ADA) level in pulmonary TB, pulmonary TB with pleural effusion and non-tuberculosis pleural effusion patients and compare with the healthy controls.
2. To compare value of Adenosine deaminase in serum and pleural fluid in patients of pulmonary TB with pleural effusion and with those in patients with non TB pleural effusion

IV. MATERIALS AND METHODS

1. Study design: This is a prospective, non-randomised, single centric, non-interventional, open labelled study.
2. Study duration: It was carried out in a span of 1 year from June 2012 to May 2013.
3. Study population with sample size: Study comprised of 132 subjects of which 33 are clinically, radiologically and microscopically confirmed cases of pulmonary TB without effusion(group A), 33 are PTB with effusion(group B), 33 are non-TB effusion patients(group C) and 33 are healthy controls (group D). Age group range was from 20- 70 years. The controls and patients voluntarily participated in the study. Informed consent was taken from controls and cases before collecting the blood and pleural fluid samples.
   TABLE 1: Categorisation of study population in different groups (n = 132).
4. Study site: Patients coming to pulmonary medicine department were taken for the study and samples were analysed in central laboratory of biochemistry department of the J.J hospital, Mumbai.
5. Ethical committee approval -The study was approved by the ethical committee of the institute.
6. Inclusion criteria: Healthy controls.
   - Participants of either gender with age >18 years.
   - Microbiologically and radiological confirmed cases of pulmonary TB, pulmonary TB with pleural effusion and nontubercular effusion
   - Participants willing to sign informed consent form.
7. Exclusion criteria:
   - Participants who were suffering from other chronic illness in which ADA levels are also affected like –Extra-pulmonary TB, Enteric fever, Viral hepatitis, Diabetes mellitus, Nephrotic syndrome, Leprosy, Infectious mononucleosis, HIV, Chronic malnutrition.
   - Pregnant and lactating women.
   - Participants on Drugs which affect ADA values like interferon alpha, deoxycoformycin, ribavirin and viramidine.
   - Participants not ready to give written consent.
- Participants suffering with emergency fatal conditions
8. Equipment: Semi auto analyser (Erba Mannheim Chem-5 Plus V_2).
9. Collection of blood sample: Under all aseptic precautions about 2 ml of venous blood was drawn. Serum was separated immediately by centrifugation (2500 rpm for 10 mins) and was kept in deep freezer (-70^0C) till further assay and maximum 2 freezing and thawing were allowed.
10. Collection of pleural fluid: Under all aseptic precautions, tapping was done. The recommended location varied depending upon the source. The preferred site was the mid-axillary line, in the sixth, seventh, or eighth intercostals spaces. In case of little effusion procedure was performed under ultrasound guidance.

11. Method of estimation of serum and pleural fluid Adenosine deaminase (ADA): Guisti and Galanti method of enzymatic analysis [(14)], using ADA-MTB[(R)] kit from Microxpress a division of Tulip diagnostics (P) Ltd [(15)].
12. Principle: Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenols complex formed is directly proportional to the amount of ADA present in the sample.(1)
13. Normal range: in serum and pleural fluid.
    Normal - < 30 U/L
    Suspect – 30 - 40 U/L
    Strong suspect - > 40 - 60 U/L
    Positive - > 60 U/L
14. Linearity: The procedure was linear up to 150 U/L if values exceed, the sample was diluted with distilled water and the assay was repeated.

V. STATISTICAL ANALYSIS

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements were presented on Mean ± SD and results on categorical measurements in Number. Unpaired t test was used to find the significance of study parameters on continuous scale between two groups (Inter group analysis).one way ANOVA test was applied to compare different parameters in between different groups along with post hoc Tuckey's test to compare parameters within group. Diagnostic statistics viz. Sensitivity, Specificity, Positive predictive value and Negative predictive value was computed to find the correlation of ADA for diagnosis with tuberculosis patients. For statistical analysis the "Graphpad instat 3, San Diego, California" software was used. Microsoft word and excel have been used to generate tables and graphs.

VI. RESULTS AND OBSERVATIONS

Comparison of mean values of ADA (u/l) in serum in different groups:

TABLE 2.

Fig 1.

Serum ADA levels in group A (55.09 $\pm$ 11.02) patients were significantly higher ($p < 0.001$) when compared with group D (18.11 $\pm$ 6.13). At the same time significant difference was found when compared with groups B and C ($p < 0.001$).

Serum ADA levels in group B (44.01 $\pm$ 7.82) patients were significantly higher (p value < 0.001) when compared with group D (18.11 $\pm$ 6.13). Also, significant difference was found when compared with group A and C ($p < 0.001$).

Serum ADA levels in group C (21.92 $\pm$ 5.33) patients were higher but not statistically significant ($p > 0.05$) when compared with group D (18.11 $\pm$ 6.13).

The sensitivity, specificity, positive predictive value and negative predictive value of the serum ADA in pulmonary tuberculosis group by taking cut off value of 33.3 U/L came to be 96.69%,96.69%, 96.69% and 96.69% respectively. Similarly, the sensitivity, specificity, positive predictive value and negative predictive value of the serum ADA in pulmonary tuberculosis with effusion group by taking the same cut off value of 33.3 U/L were 93.93%, 96.69%, 96.87% and 94.11%.

Mean value of ADA (u/l) in pleural fluid in groups b &c:

TABLE 3.

Fig 2.

In the present study, the mean pleural fluid ADA (82.61 $\pm$12.03) was higher as compared to mean serum ADA (44.01$\pm$ 7.82) in group B. This was highly significant ($p < 0.0001$).

Similarly, the mean pleural fluid ADA (27.72 $\pm$7.80) was higher than mean serum ADA (21.92 $\pm$ 5.33) in group C and was highly significant p-value < 0.0008.

But, the values of ADA in serum and pleural fluid in group B samples were higher as compared to group C samples

Comparison of mean serum ADA and pleural fluid ada within groups b and c:

TABLE 4.

Fig 3.

VII. Discussion

In this context, the exact role of ADA in TB patients with and without pleural effusion and its usefulness in diagnosing tuberculosis is tried to justify.

Adenosine deaminase is an enzyme in the purine salvage pathway required for converting adenosine to inosine. Its levels are ten times higher in lymphocytes than in erythrocytes [16] and particularly so in T-lymphocytes. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes and T-lymphocyte blastogenesis can be inhibited by inhibitors of ADA [17,18]. Likewise, a deficiency of ADA is associated with severe defects in the cell mediated and the humoral arms of the immune system, predisposing the patient to opportunistic infections like pulmonary tuberculosis.

In the present study there was statistically significant increase ($p<0.001$) in mean serum ADA levels in pulmonary TB (55.09 $\pm$ 11.02), pulmonary TB with effusion cases (44.01 $\pm$ 7.82) as compared to healthy controls (18.11 $\pm$ 6.13) and non-TB pleural effusion patients (21.92 $\pm$ 5.33). These findings are in accordance with the studies made by -

Lakshmi et al, in 1992 found the average serum ADA values in 61 active pulmonary tuberculosis patients and 25 healthy controls. In their study they stated that serum ADA level was more in TB patients than controls which helps for diagnosis [19]. Saeed Amniafshar et al, in 2004 evaluated 51 cases of active pulmonary tuberculosis (21 females and 30 males aged 47.7 ± 19 years), 50 (14 female and 36 male aged 48.4 ± 11 years) of healthy controls. Mean serum ADA level in pulmonary tuberculosis (42.4 ± 21.5 IU/L) and was significantly more than controls (26.6 ± 8.21 IU/L) ($p< 0.0001$) [20]. Meena Verma et al, in 2004 studied 100 patients of which 53 were suffering from pulmonary tuberculosis and 35 normal healthy control subjects. The mean serum ADA activity in pulmonary TB patients was 35.5 ± 6.93 U/L as 52 compared to 16.20 ± 2.85 U/L in control group, showing highly significant ($P<0.001$) difference. ADA activity was highest in tuberculosis than compared to controls [21]. Jhamaria JP et al, in 1988 estimated serum ADA level in 20 healthy controls, 102 cases of pulmonary tuberculosis, 20 cases of suppurative lung diseases (lung abscess and bronchiectasis) and 18 cases of lung malignancy. The mean serum ADA in healthy controls was 10.09 ± 2.99 U/L, in pulmonary TB 42.47 ± 3.34 U/L were observed. They concluded that ADA activity is highest in pulmonary tuberculosis patients than compared to controls [22]. K.Srinivasa Rao et al in 2010 found that in diagnosis of pulmonary TB, serum ADA showed high percent positivity of 88% followed by chest X-ray 76%, ESR 72%, Sputum AFB 63%, Mantoux 61%. He also reported high serum ADA levels in pulmonary TB as compared to non tubercular pulmonary diseases and sputum AFB negative pulmonary tuberculosis cases showed elevated level of serum ADA at par with sputum AFB positive cases [23].

Significantly increased ADA activity ($p<0.001$) in the serum of pulmonary TB and tubercular pleural effusion patients compared to healthy controls and non-TB pleural effusion is due to activation of cell mediated immunity. In tuberculosis there are increased numbers of T-lymphocytes and macrophages in pleural fluid which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes. In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T-cells may cause a high serum ADA activity in patients with pulmonary disease [24].

Also, in the present study serum ADA levels in non tubercular pleural effusion were higher as compared to healthy controls but not statistically significant ($p>0.05$).

In this study, findings seem to confirm that ADA activity is a useful parameter for the diagnosis of tuberculosis and tubercular effusion. The mean levels of pleural fluid ADA in tubercular pleural effusion (82.61 $\pm$ 12.03) were higher significantly ($p<0.0001$) as compared to pleural fluid ADA levels in non tubercular pleural effusion (27.72 $\pm$ 7.80). This is in accordance with the studies of Y.C.Gary Lee et al, who in 2001 studied 106 cases of lymphocytic pleural effusion origin of different etiologies and concluded saying that ADA levels in TB pleural fluid exceeds than that in other non tuberculosis lymphocytic pleural fluid [25]. Moreys Casagrande Kaisemann et al, in 2004 concluded that ADA determination in pleural fluid is a sensitive and specific method for diagnosis of pleural TB and its use can preclude need for pleural biopsy in initial workup of pleural effusion of patients [4]. Bharat Kumar Gupta et al, in 2010 determined ADA activity in 96 pleural fluid samples comprising of tubercular and non tubercular pleural fluid samples and found that pleural fluid ADA levels were significantly higher in TB pleural fluid as compared with non TB pleural fluid [26].

So, pleural fluid ADA level is very helpful test to rule out a tubercular aetiology of pleural effusion. Cell-mediated response is the predominant form of immune response to tubercular infection, while both cell-mediated and humoral immune responses are elicited by most non-tubercular infections in human body. Thus ADA activity, a marker of T-cell activation and cell-mediated immune response can help differentiate tubercular aetiology from non-tubercular. Piras et al were first to report high ADA in tubercular pleural effusion [27].

Present study showed that levels of pleural fluid ADA were significantly higher than serum ADA levels in both tuberculous ($p< 0.0001$) and non-tuberculous ($p<0.0008$) pleural effusions, suggesting a localized intra-pleural production of ADA .This is in accordance with the study of S K Sharma et al, in 2001 who stated

that using 100 IU/L as the cut-off, it is possible to avoid pleural biopsy in as much as 40% of patients as certain diagnosis of TB. And also, concluded that ADA estimation is a useful test for the diagnosis of TB with pleural effusion which is adequately sensitive and specific and at same time inexpensive and easy to perform [28].

Most of the authors suggest ADA assay as the routine screening test, a sensitive marker and an inexpensive test. The present study evaluates the usefulness of ADA with a cut-off value of 33.3 U/L in serum to diagnose pulmonary tuberculosis patients efficiently. Statistically analysed data shows significance of ADA in diagnosis of PTB without effusion with sensitivity, specificity of 96.69% and 96.69 % respectively, with positive predictive and negative predictive values of 96.69 % and 96.69 % respectively.

The usefulness of ADA was evaluated with a cut-off value of 33.3 U/L in serum to diagnose pulmonary tuberculosis with pleural effusion patients. The sensitivity, specificity, positive predictive value and negative predictive value of 93.93%, 96.69%, 96.87% and 94.11% was found respectively. Thus, ADA estimation in serum of pulmonary tuberculosis without pleural effusion may be of more diagnostic importance than ADA estimation in serum of pulmonary tuberculosis with pleural effusion patients.

In the present study, a few cases with pulmonary tuberculosis showed elevated ADA activity in spite of sputum negative for tuberculosis. This suggests that ADA activity in pleural fluid samples from patients suffering from the same is a sensitive marker for the diagnosis and patients can be started on Empirical treatment while waiting for other test reports to be positive. This is in accordance with the study made by Mukesh Kumar Agarwal et al, in 1991 .They studied serum ADA levels in 38 healthy controls, 36 cases of smear negative, culture positive patients of pulmonary TB, 34 cases of non-TB respiratory diseases. They found that mean serum ADA levels compared with healthy controls and non TB cases were higher in smear negative, culture positive patients of pulmonary TB [29].

Thus, from present study results, it is observed that serum & pleural fluid ADA measurement plays a very important role in diagnosis of PTB without pleural effusion & with effusion respectively but, current study has certain limitations like small sample size which may be because of the knowledge or attitude of the patients with their condition. Major limiting factor is about the cut off value of ADA which was taken in the study, as it may vary at different places but considering the values given on ADA kit different values were tried & decided for this value which may or may not be accurate but in given circumstances with the patients values this cut off value gave a significant result. Therefore, it is recommended that a large sample size study in which different groups of PTB patients with their different categories like new cases, defaulters, relapse or failure, MDR should be taken & ADA values should be assessed which will give the exact role of ADA not just only in diagnosis of PTB but also in the prognosis of the disease. Measurement of ADA values is definitely having a very important role in the diagnosis of PTB patients with and without pleural effusion if other factors which may also affect ADA levels are excluded thus giving a sensitive, specific, reliable, inexpensive, rapid, non invasive and easily available investigation in a disease like TB for which whole nation is deeply concerned.

VIII. INDENTATIONS AND EQUATIONS

(1)

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{Hypochlorite} \xrightarrow{\text{Alk medium}} \text{Blue Indophenol complex}$$

IX. FIGURES AND TABLES

Fig 1 COMPARISON OF MEAN VALUES OF ADA (U/L) IN SERUM IN DIFFERENT GROUPS:

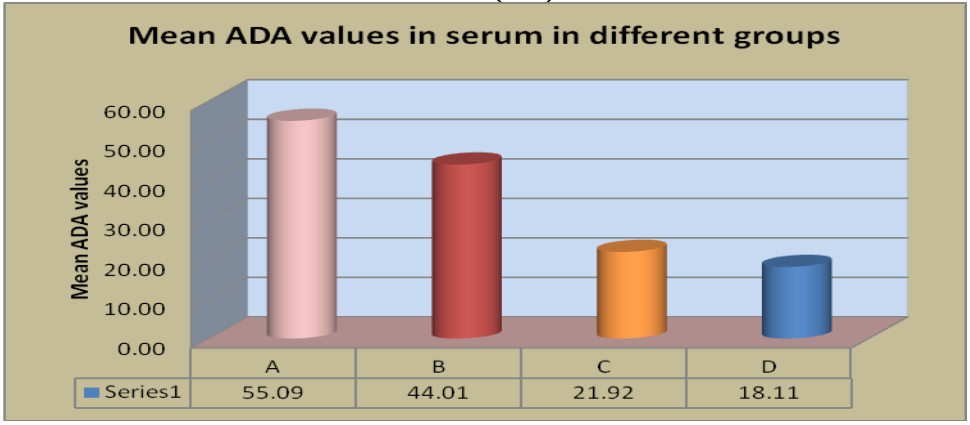

Fig 2. MEAN VALUE OF ADA (U/L) IN PLEURAL FLUID IN GROUPS B &C:

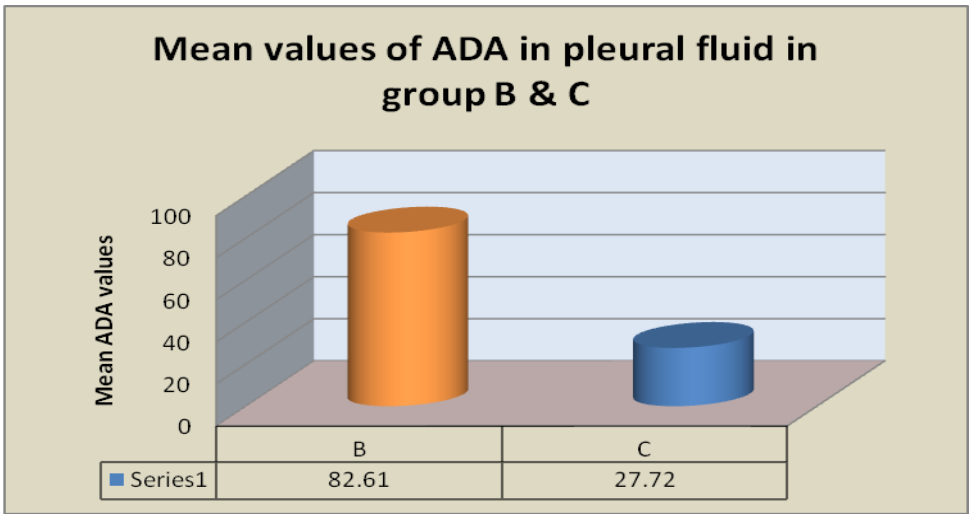

Fig 3. COMPARISON OF MEAN SERUM ADA AND PLEURAL FLUID ADA WITHIN GROUPS B AND C:

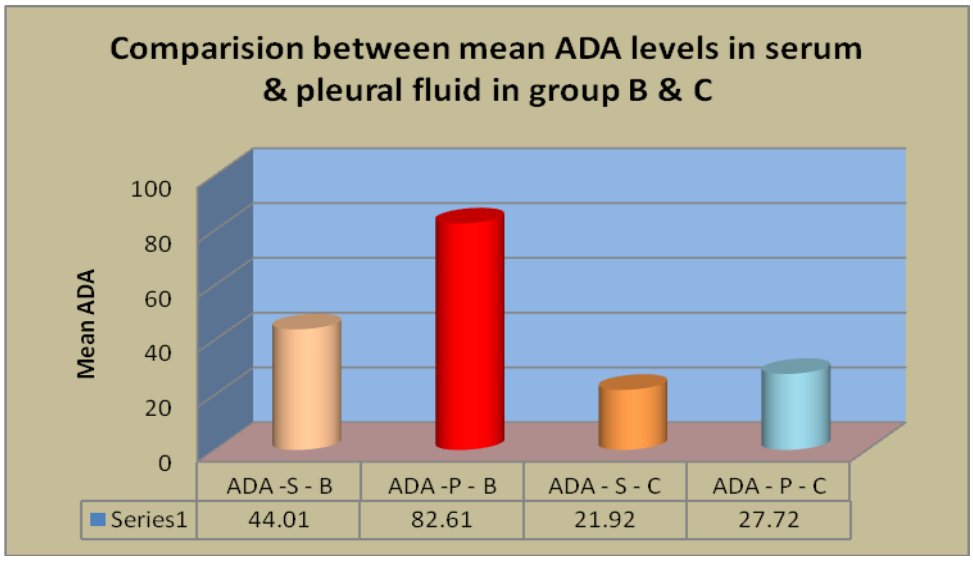

TABLE 1: CATEGORISATION OF STUDY POPULATION IN DIFFERENT GROUPS (N = 132).

Study group	No. of patients	Nature of group
A	**33**	**Pulmonary TB**
B	**33**	**Pulmonary TB with pleural effusion**
C	**33**	**Non TB with pleural effusion**
D	**33**	**Healthy controls**

TABLE 2: COMPARISON OF MEAN VALUES OF ADA (U/L) IN SERUM IN DIFFERENT GROUPS:

GROUPS	MEAN ADA ± SD	P value
A	**55.09 ± 11.02**	**<0.0001***
B	**44.01 ± 7.82**	
C	**21.92 ± 5.33**	
D	**18.11 ± 6.13**	

Footnote – all values are expressed as mean ± SD.
For comparison of P value in all groups one way ANOVA test was applied.
*P <0.05 was considered to be significant**.**

TABLE 3: MEAN VALUE OF ADA (U/L) IN PLEURAL FLUID IN GROUPS B &C:

GROUPS	MEAN ADA ± SD	P-VALUE
B	**82.61 ± 12.03**	**<0.0001***
C	**27.72 ± 7.80**	

Footnote: Unpaired t test, *p-value <0.0001, highly significant.

TABLE 4: COMPARISON OF MEAN SERUM ADA AND PLEURAL FLUID ADA WITHIN GROUPS B AND C:

GROUPS	PARAMETER	MEAN ADA ± SD	P – VALUE
B	ADA serum	44.01± 7.82	< 0.0001*
B	ADA pleural fluid	82.61 ±12.03	
C	ADA serum	21.92 ± 5.33	< 0.0008*
C	ADA pleural fluid	27.72 ±7.80	

Footnote: All values are expressed as mean ± SD *'p' value < 0.05 considered significant

X. CONCLUSION

Serum ADA was significantly higher in pulmonary TB patients and pulmonary TB with pleural effusion patients compared to healthy controls and non TB pleural effusion. Pleural fluid ADA was significantly higher in pulmonary TB with pleural effusion patients compared to non-TB with pleural effusion patients. Pleural fluid ADA was significantly higher than serum ADA levels in pulmonary TB with pleural effusion and in non-TB with pleural effusion patients.

Thus serum ADA and pleural fluid ADA plays a very important role as an investigation in the diagnosis of PTB with or without effusion. Serum and pleural fluid ADA level measurement in the diagnosis of pulmonary TB without pleural effusion and pulmonary TB with pleural effusion respectively is a very sensitive, specific, inexpensive, rapid, easily available & reliable investigation.

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