

Original Article

Diagnostic Value of Serum Adenosine Deaminase (ADA) in Tuberculous Lymphadenitis

Mihir Kanti Adhikari¹, Shohael Mahmud Arafat², Mehruba Alam Ananna³, Md Atikur Rahman⁴, Hasan Imam⁵, Shah Mohammad Mohaimenul Haq⁶

Background: Tuberculosis is one of the major infectious disease in Bangladesh and also a re-emergent disease in the developed world. Extra-Pulmonary Tuberculosis (EPTB) comprises a significant share of all cases of tuberculosis and tuberculous lymphadenitis is the commonest form. It often requires FNAC or biopsy of lymph node to reach a final diagnosis of tuberculous lymphadenitis, which is invasive, expensive, and needs an advanced setting. Estimation of ADA in different body fluid and serum has been suggested as a quick, cheap, and reliable test for tuberculosis.

Materials and Methods: This was a cross-sectional observational study, conducted in Bangabandhu Sheikh Mujib Medical University Hospital over a two year period and included 68 participants, divided equally into two group e.g. tuberculous lymphadenitis and a healthy comparison group. As tuberculous lymphadenitis, only newly diagnosed cases, labeled on the basis of histopathological findings of epithelioid granuloma with caseation necrosis were included. Serum ADA concentrations were estimated by enzymatic method and compared between groups.

Results: The mean serum ADA concentration was found to be 25.52 ± 7.11 U/L in tuberculous lymphadenitis group, which was significantly higher ($p=0.000$) than that of the healthy comparison group (14.82 ± 3.85 U/L). Three different cut-off values of serum ADA (18.25 U/L, 20.45 U/L, 22.15 U/L) were used to determine the best predictive value for the diagnosis of tuberculous lymphadenitis. Sensitivity and specificity of these cut off values were 91.2% and 82.4%, 79.4% and 88.2%, 67.6% and 97.1% respectively.

Conclusions: It is clearly denoted by the study that, tuberculous lymphadenitis patients has significant higher serum ADA then the healthy comparison group. This result indicates that serum ADA can be used as a useful adjunct in the diagnosis of tuberculous lymphadenitis with conventional investigations.

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Key words : Serum ADA, Adenosine deaminase, Tuberculous lymphadenitis, Socio-demography of Tuberculous Lymphadenitis, Sensitivity and specificity of serum ADA.

Tuberculosis (TB) is one of the oldest diseases that is affecting human over centuries. It is a virulent infectious disease worldwide and a re-emergent disease in the current developed world. TB lymphadenitis constitutes about 35 percent of extra-pulmonary tuberculosis (EPTB) and it is 15 to 20 percent of all cases of tuberculosis¹. It is almost exclusively caused by Mycobacterium tuberculosis in Bangladesh.² Non-tuberculous mycobacterial (NTM) infection is rare here and also uncommonly reported from India¹.

Editor's Comment :

- Tuberculous lymphadenitis (LNTB) is the most common form of EPTB.
- Diagnosis of LNTB needs invasive procedure and technically demanding histopathological study.
- Estimation of ADA in body fluids has been established as a diagnostic tool for tuberculosis.
- This study has shown that estimation of serum ADA is also useful in diagnosis of LNTB.

The diagnosis of tuberculous lymphadenitis is usually based on clinical presentation, radiological findings, and positive tuberculin or Boston Consulting Group (BCG) tests. If Fine Needle Aspiration Cytology (FNAC) or biopsy of enlarged lymph node reveals granulomatous lesion with caseous necrosis along with positive mycobacterial stain and culture, it helps to confirm the diagnosis. In Bangladesh, AFB positivity has a high association with caseation necrosis³. Conventional Acid-Fast Bacillus (AFB) smears have low sensitivity and it requires a long time for M tuberculosis to become evident in culture. Waiting for culture result to start treatment was found to be associated with higher mortality in tuberculous peritonitis⁴. So, the finding of granuloma with caseation necrosis is often considered final for the diagnosis of tuberculous lymphadenitis and treatment initiated. In many other cases, the difficulties regarding obtaining biopsy specimen and histopathological study leads to empirical anti-tuberculous therapy. It is, therefore becomes

¹MBBS, MD (Medicine), Junior Consultant in Medicine, Two-fifty Bedded District Hospital, Habiganj, Bangladesh

²MBBS, FCPS (Medicine), MRCP (UK), FRCP (Edinburgh), FICP (Hon), Professor, Department of Internal Medicine, Bangabandhu Sheikh Mujib Medical University Hospital, Dhaka, Bangladesh and Corresponding Author

³MBBS, FCPS (Medicine), CCD, Associate Professor (Nephrology), Associate Professor (Nephrology), Consultant in Medicine, BIRDEM General Hospital and Ibrahim Medical College, Bangladesh

⁴MBBS, MD (Medicine), Chief Resident, Department of Internal Medicine, BSMMU, Dhaka, Bangladesh

⁵MBBS, MD (Medicine), Assistant Professor, Department of Internal Medicine, BSMMU, Dhaka, Bangladesh

⁶MBBS, MD (Medicine), Medical Officer, Kuwait Bangladesh Friendship Government Hospital, Dhaka, Bangladesh

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imperative to find out some rapid and useful tests for the diagnosis of tuberculosis.

Adenosine deaminase (ADA) is associated with purine catabolism. It controls proliferation and differentiation of T lymphocytes. The most important isoforms of ADA, are ADA-1 and ADA-2, which are located on different gene loci⁵. ADA-2 appears to be found only in monocyte ADA1 in all cells⁶. The sensitivity and specificity of ADA depend on the prevalence of tuberculosis in the population. In the regions with a high incidence of tuberculosis, ADA appears to be a useful marker for early diagnosis of tuberculosis. ADA-2 found to be the predominant isoforms in tuberculosis accounting for 80-90% of the activity. The isoenzyme ADA-1 is elevated in the presence of empyema and para-pneumonic effusions⁷.

The value of ADA as a diagnostic marker of tuberculosis has been established in numerous studies, especially in tuberculous pleural effusion, ascites, and pericardial effusion. An elevated pleural fluid ADA level predicts tuberculous pleuritis with the sensitivity of 90-100% and the specificity of 89-100%⁸. Dwivedi *et al* (1990) described the sensitivity of 100% and specificity of 96.6% at an ADA level >33 U/L for early diagnosis of tuberculous peritonitis, another study by Gupta *et al* (1992) shown 95% sensitivity and 94.1% specificity at an ADA level >30 U/L^{9,10}. In South Africa, a prospective study showed an ADA cut-off level of 40 U/L resulted in a test sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of 84%, 80%, 91%, 66%, and 83%, respectively in tuberculous pericarditis¹¹. Koh *et al* (1994) studied pericardial fluid ADA level along with histopathology of pericardial biopsy and found a cut-off level of 40 U/L has the sensitivity of 93% and the specificity of 97% in the diagnosis of tubercular pericardial effusion, which is similar to the study by Mathur *et al* (2006) that showed 100% sensitivity and 83.3% specificity^{12,13}.

Bangladesh is a country with a high tuberculosis burden. FNAC from lymph node and biopsy is only available in the advanced hospitals that lack in number. Furthermore, tuberculous lymphadenitis involving internal structures like mediastinal or intra-abdominal lymph nodes, CT guided or laparoscopic approach is required, which is technically demanding and difficult even in advanced centers. With such limited resources, an ideal test for tuberculosis should be economic, minimally invasive, of high accuracy and quick to perform. ADA is a simple and inexpensive colorimetric test that can be done easily with limited resource and short training. The study was intended to assess the usefulness of serum ADA in the diagnosis of tuberculous lymphadenitis.

MATERIALS AND METHODS

This cross-sectional observational study was conducted in the Department of Internal Medicine BSMMU from March, 2015 to February, 2017. The study included 34 patients of tuberculous lymphadenitis over 18 years and older, of both gender. Another age and gender match 34 healthy individuals were added as a comparison group. Convenient sampling technique was adopted

and subjects were chosen as per inclusion and exclusion criteria. Patients presented with constitutional features of tuberculosis, like fever, weight loss, anorexia with lymphadenopathy were considered for lymph node FNAC or biopsy. Epithelioid granuloma with caseous necrosis was labeled as tuberculous lymphadenitis. Participants who had pulmonary tuberculosis or extra-pulmonary tuberculosis along with tuberculous lymphadenitis were excluded as well as metabolic derangements like hepatic, renal or electrolyte abnormality, organic or inorganic psychosis, or current pregnancy. Before starting anti-tuberculous chemotherapy, serum ADA was measured by adenosine deaminase assay kit supplied by Diazyme laboratories, CA, USA in the laboratory of the Department of Microbiology of BSMMU. Ethical clearance was obtained from the institutional review board of BSMMU and informed written consent was taken from all patients. Data were compiled and analyzed by Statistical Package for the Social Sciences (SPSS). Continuous variables were analyzed using Student t-test and one way ANOVA, when and where appropriate, categorical variables were compared using the chi-square test. A p-value <0.05 was considered statistically significant. A Receiver Operating Characteristic (ROC) curve was used to determine the sensitivity and specificity of serum ADA for the diagnosis of tuberculous lymphadenitis.

RESULTS

In this study, the age range of the tuberculous lymphadenitis as well as healthy controls ranged from 18 to 60 years, the mean (\pm SD) age was 29.94 (\pm 12.13) years and 30.24 (\pm 10.21) years respectively. Among the respondents, in both of the groups' majority were female (70.6%) (Table 1). In tuberculous lymphadenitis group, majority of the respondents (94.10%) had fever, other predominate symptoms were loss of appetite (79.4%), weight loss (82.4%), and pain in lymph nodes (20.6%). Symptoms like cough, nausea and vomiting were present in 11.8% of patients. In this group, 8 (23.5%) of 34 participants had a history of contact with smear positive pulmonary TB patients, while 1 (2.9%) of them had a previous

Table 1 — Socio-demographic Characteristics of Participants (n=64)

Demographic characteristics	Tuberculous lymphadenitis (n=34) Frequency (%)	Healthy control (n=34) Frequency (%)
Age (Years) :		
18-20	10 (29.4%)	10 (29.4%)
21-40	17 (50.0%)	17 (50.0%)
41-60	7 (20.6%)	7 (20.6%)
Mean age (\pm SD)	29.94 (\pm 12.13)	30.24 (\pm 10.21)
Gender :		
Male	10 (29.4%)	10 (29.4%)
Female	24 (70.6%)	24 (70.6%)
Residence :		
Rural	15 (44.1%)	19 (55.9%)
Urban	19 (55.9%)	15 (44.1%)

history of tuberculosis. In this group, most of the patients (73.5%) presented with single regional lymphadenitis and cervical lymph nodes were mostly affected (95.8%). The largest lymph nodes were >2cm in 50% of patients. Consistency was firm in the majority (85.3%) of patients, 44.1% of them had matted lymphadenitis. Cold abscess with discharging sinus was found in 14.7% of cases.

In tuberculous lymphadenitis group, mean serum ADA concentration was found to be 25.52 (± 7.11) U/L and in the healthy comparison group it was 14.82 (± 3.85) U/L (Table 2). In the male participants, mean serum ADA was 26.23 (± 10.12) U/L and in the female, it was 25.22 (± 5.66) U/L.

The receivers operating characteristic (ROC) curve was utilized. The area under the curve (AUC) was significant (0.935). The sensitivity and specificity of serum ADA in the diagnosis of tuberculous lymphadenitis for different cut off values were estimated (Table 3 and Fig 1).

DISCUSSION

Tuberculous lymphadenitis is the most prevalent form of extrapulmonary tuberculosis. The final diagnosis needs FNAC or biopsy, and is difficult due to the invasiveness of the procedure and its unavailability in the low resource setting. Estimation of ADA in different body fluids is a simple method of investigation and is proved to be diagnostic. Previous studies have also shown the usefulness of serum ADA in pulmonary tuberculosis and in limited cases of Extrapulmonary Tuberculosis (EPTB).

Among the respondent, in tuberculous lymphadenitis group, most were females (70.6%), such female predilection has also been reported by previous studies^{14,15}. Most (79.4%) of respondents of this group was within 18 to 40 years of age. Many previous studies also reported its predominance in children and young adults^{14,15}. Majority of the respondents (94.10%) of this group had fever, but objective evidence of raised body temperature was found in 55.90% of cases. It can be explained by the natural

Table 2 — Comparison of Serum ADA Concentrations (U/L) between Study Groups (n=68)

Group	Mean (\pm SD)	Min	Max	P value	t
Tuberculous lymphadenitis	25.52(± 7.11)	14.10	41.30	0.000 ^a	7.714
Healthy group	14.82(± 3.85)	8.30	23.90		

a = Independent sample t test was done.

Table 3 — Sensitivity and Specificity of Serum ADA (U/L) at Different Cut Off Values in Tuberculous Lymphadenitis

Cut of value of serum ADA	Sensitivity	Specificity
18.25 (U/L)	91.2	82.4
20.45 (U/L)	79.4	88.2
22.15 (U/L)	67.6	97.1

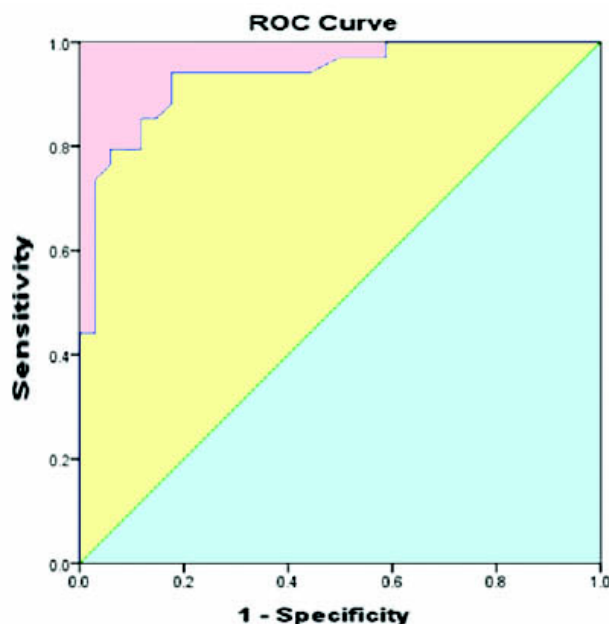


Fig 1 — Receiver operating characteristic (ROC) curves showing the sensitivity and specificity of serum ADA level

history of disease; tuberculosis causes evening rise of temperature but as most of the cases was taken from outdoor at morning, elevated temperature couldn't be demonstrated. In tuberculous lymphadenitis group, most of the patients (73.5%), presented with single regional lymph node enlargement, among them cervical lymph node involvement was most common (95.8%), which is consistent with previous studies^{15,16}.

In this study, mean serum ADA concentration was found to be significantly higher in tuberculous lymphadenitis group (25.52 \pm 7.11 U/L) than the healthy comparison group (14.82 \pm 3.85 U/L) ($p=0.000$). This finding is consistent with a previous study who found mean serum ADA concentration as 20.2 (± 3.64) U/L and 14.95 (± 3.22) U/L in tuberculous lymphadenitis and healthy control respectively.¹⁷ Giusti (1984) have also shown serum ADA in healthy population as 17.05 (± 3.75) U/L¹⁸. Another study in Belgrade, Serbia, Stevanovic et al. (2011) found serum ADA concentration 31 (± 11) U/L in a group of patients of extrapulmonary tuberculosis, of whom 27% was tuberculous lymphadenitis¹⁹. A recent study described elevation of serum ADA level to a range of 31-40 U/L in 65% of cases and 41-60 U/L in 20% of cases in a group consisting of 30 cases of tuberculous lymphadenitis²⁰. These concentrations were much higher than the findings of the current study.

Three different cut-off values of serum ADA (18.25 U/L, 20.45 U/L, 22.15 U/L) were used to determine the best predictive value for the diagnosis of tuberculous lymphadenitis in the current study. Sensitivity and specificity of these cut off values were 91.2% and 82.4%, 79.4% and 88.2%, 67.6% and 97.1% respectively. Receivers operating characteristic (ROC) curve shown area under the curve (AUC) was significant (0.935) (Fig 1).

We admit few limitations of our study like small sample size and

convenient sampling technique. For the diagnosis of tuberculous lymphadenitis, the case definition of the presence of granuloma with caseation necrosis in FNAC or biopsy was used. Serum total ADA was measured in this study, which is less specific than ADA 2 for the diagnosis of tuberculous lymphadenitis.

CONCLUSIONS

This study clearly demonstrated significant elevation of serum ADA concentration in the tuberculous lymphadenitis patients in comparison to the healthy control. This result indicates that serum ADA can be a useful adjunct in the diagnosis of tuberculous lymphadenitis with conventional investigations. To overcome the limitations of this study a large scale multicenter study with random sampling and using culture positivity of *Mycobacterium tuberculosis* from FNA or biopsy material of lymph nodes as gold standard can be done. Serum ADA 2 concentration, which is more specific to tuberculosis, can also be measured.

Contributions :

Adhikari MK,¹ designed the study, data collection tools, collected, compiled, cleaned and analyzed the data, and drafted and revised the paper. Arafat SM,² designed the study, and revised the paper. Ananna MA,³ analyzed the data and revised the draft. Rahman MA,⁴ collected and compiled the data, and drafted the paper. Imam H,⁵ collected and compiled the data. Haq SM,⁶ collected, compiled, cleaned and analyzed the data.

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Competing interests : None declared.

Ethical Clearance : Ethical clearance was obtained from the Institutional Ethics Committee.

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