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ORIGINAL ARTICLE

Detection of Sensitivity and Specificity of Serum Adenosine Deaminase as A Diagnostic Marker of Extra Pulmonary Tuberculosis

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Abstract

Background: *The diagnosis of Extra Pulmonary Tuberculosis(EPTB) is difficult due to its nonspecific signs and symptoms and presence of few organisms in the involved site. Diagnosis of extra pulmonary TB is usually done by direct or indirect methods. Direct methods includes TB bacilli found by microscopy, culture or molecular methods. Indirect methods involve detection of humoral or cell mediated immune response of host to mycobacterial antigen or detection of biomarkers like Adenosine deaminase (ADA), Interferon ã (IFN ã) etc. By considering the importance of rapid and accurate diagnosis in TB treatment and control, the present study is planned to investigate the sensitivity and specificity of serum adenosine deaminase for diagnosis of EPTB.*

Objectives: *The main objectives of the study was to detect sensitivity and specificity of serum ADA level in the diagnosis of extra pulmonary tuberculosis.*

Methods: *This cross-sectional observational study was conducted in the Respiratory Medicine Department, NIDCH, Mohakhali, Dhaka in between October 2018-March 2020. Patients of clinically presumptive EPTB were taken(65 patients) according to selection criteria. Biopsy taken from EPTB involved site (pleura, Lymph node, Skin). Based on histopathological study, all of the patients were divided into two groups: Group A is histopathologically confirmed extra pulmonary tuberculosis and Group B is histopathologically not confirmed extra pulmonary tuberculosis.Blood sample was collected from all participants and send for serum ADA measurement by ADA-MTB KIT and compared with both groups. Data were analyzed using appropriate statistical formula.*

Results: *In this study, Most of the study subjects were in age group between 30–39 years (Group A-36.2%, Group B -27.8%) and 40-49 years (Group A -29.8%, Group B -22.2%). Among Group A, 20 (42.6%) were male and 27 (57.4%) were female and among Group B (histopathologically not confirmed extra pulmonary tuberculosis), 10 (55.6%) were male and 8 (44.4%) were female. Pleural effusion(55.3%), lymphadenopathy(38.3%) were observed higher in Group A than Group B. according to histopathological study, majority (55.3%) of Group A*

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patients had pleural TB, 40.4% had lymph node TB and 4.3% had skin TB. In group B patients, 50.0% had pleural TB, 38.9% had lymph node TB and 11.1% had skin TB. Serum ADA had sensitivity 91.5%, specificity 83.3%, PPV 93.5%, NPV 78.9% and accuracy 89.2% respectively in diagnosis of extra pulmonary TB at the cut- off value of 43U/L.

Conclusion: *This study revealed that serum ADA is significantly higher in histopathologically confirmed extra pulmonary tuberculosis patients than histopathologically not confirmed extra pulmonary tuberculosis patients.Serum ADA estimation are a useful tool for diagnosis of extra pulmonary tuberculosis particularly pleural TB, lymph node TB and skin TB.It could be used as a supporting tool for diagnosis of all presumptive EPTB patients.*

Keywords: *Serum ADA,extra pulmonary tuberculosis,sensitivity,specificity etc.*

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Introduction:

Tuberculosis (TB) remains a major global problem and a public health issue of considerable magnitude. It is one of the leading causes of death from infectious diseases worldwide usually caused by the bacterium Mycobacterium tuberculosis[1]. Presently, one-third of the world's population is thought to be infected with TB. New infections occur in about 1% of the population each year. There are two types of clinical manifestation of tuberculosis (TB) includes pulmonary TB (PTB) and extra pulmonary TB (EPTB). EPTB is the TB involving organs other than the lungs such as pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges. EPTB constituted about 20% of all cases of TB. In HIV-positive patients, EPTB accounts for more than 50% of all cases of TB[2]. Extra pulmonary TB (EPTB) results from the hematogenous and lymphatic spread of M. tuberculosis bacilli. Patients with EPTB may manifest constitutional symptoms such as fever, anorexia, weight loss, malaise and fatigue. Patients with EPTB especially may present with pyrexia of unknown origin (PUO) which may arise the diagnostic clue for EPTB[3]. Tuberculous lymphadenopathy is the most common form of EPTB, constitutes 35% of all cases of EPTB. Cervical lymph nodes the most common site of tuberculous lymphadenopathy constitute about 60%–90% of cases, classically termed as "scrofula[4]. TPE predominates in men, with an overall male-to-female ratio of 2:1. The gold standard for the diagnosis of tuberculous pleural effusion (TPE) remains the detection of mycobacterium tuberculosis in pleural fluid or pleural biopsy specimens either by microscopy and/or culture or the histological demonstration of caseating granulomas in the pleura along with acid fast bacilli [5]. Cutaneous tuberculosis is a relatively uncommon, comprising 1-1.5% of all extra pulmonary tuberculosis manifestations, which manifests only in 8.4-13.7% of all tuberculosis cases. The main etiological agent of the cutaneous tuberculosis is mycobacterium tuberculosis occasionally M. bovis or BCG vaccine[6]. The diagnosis of EPTB is difficult due to its nonspecific signs and symptoms and presence of few organisms in the involved site[7]. Diagnosis of extra pulmonary TB is usually done by direct or indirect methods. Direct methods includes TB bacilli found by microscopy, culture or molecular methods. Indirect methods involve detection of humoral or cell mediated immune response of host to mycobacterial antigen or detection of biomarkers like Adenosine deaminase (ADA), Interferon ã (IFN ã) etc. Low sensitivity of microscopy and staining(0-40%), prolonged diagnostic time(6-8 weeks)of culture method (gold standard) and invasiveness of histological techniques makes EPTB diagnosis more problematic. Nucleic acid amplification techniques (NAAT) are costly and not available everywhere. Antibody based tests and cell mediated immunity based tests gives only supportive evidence. So, it is essential to develop a test which is rapid, cost effective, non invasive, easy to perform in a resource poor country[8]. ADA(Adenosine deaminase) is an enzyme that catalyze the deamination reaction from adenosine to inosine. It is a indicator of active cellular immunity[9]. ADA helps in the differentiation of lymphoid cells and the maturation of monocytes

to macrophages[10]. The ADA found more in lymphocyte than erythrocyte [11]. ADA estimation in serum is an indirect biochemical test. Population of T lymphocyte increases in tuberculosis due to antigenic stimulation. Measurement of ADA level is very simple. It is a rapid test for early diagnosis of tuberculosis[12]. By considering the importance of rapid and accurate diagnosis in TB treatment and control, the present study is planned to investigate the sensitivity and specificity of serum adenosine deaminase for diagnosis of EPTB.

Materials and methods:

This cross-sectional observational study was conducted in the Respiratory Medicine Department, NIDCH, Mohakhali, Dhaka in between October 2018-March 2020. Patients of clinically presumptive EPTB were taken (65 patients) by history, clinical examination and appropriate investigations according to selection criteria. Written informed consent was obtained from eligible participants. Biopsy taken from EPTB involved site (pleura, Lymph node, Skin). Based on histopathological study, all of the patients were divided into two groups: Group A is histopathologically confirmed extra pulmonary tuberculosis and Group B is histopathologically not confirmed extra pulmonary tuberculosis.Blood sample was collected from all participants and send for serum ADA measurement by ADA-MTB KIT and compared with both groups. Data were analyzed using appropriate statistical formula. All statistical tests were performed at 5% levels of significance and level of $p < 0.05$ were considered significant. The summarize data were present in the table and chart.

Results:

In this study, Most of the study subjects were in age group between 30–39 years (Group A-36.2%, Group B -27.8%) and 40-49 years (Group A -29.8%, Group B -22.2%) (Table I). Among Group A, 20 (42.6%) were male and 27 (57.4%) were female and among Group B (histopathologically not confirmed extra pulmonary tuberculosis), 10 (55.6%) were male and 8 (44.4%) were female (Figure 1). Pleural effusion(55.3%), lymphadenopathy(38.3%)and skin TB (4.1%) were observed higher in Group A than Group B(Figure 2). According to histopathological study, majority (55.3%) of Group A patients had pleural TB, 40.4% had lymph node TB and 4.3% had skin TB. In group B patients, 50.0% had pleural TB, 38.9% had lymph node TB and 11.1% had skin TB (Table II). According to the ROC curve, a cut-off value 43U/L of serum ADA was found which can differentiate EPTB patients with 91.5% sensitivity and 83.3% specificity(Figure 3).

Table-I

Distribution of study population of both groups according to age (N=65)

Age (years)	Group A (n=47)	Group B (n=18)	p-value
18 - 29	7 (14.9)	2 (11.1)	
30 - 39	17 (36.2)	5 (27.8)	
40 - 49	14 (29.8)	4 (22.2)	
50 and above	9 (19.1)	7 (38.9)	
Mean±SD	31.49±11.54	36.50±14.26	0.148 n.s

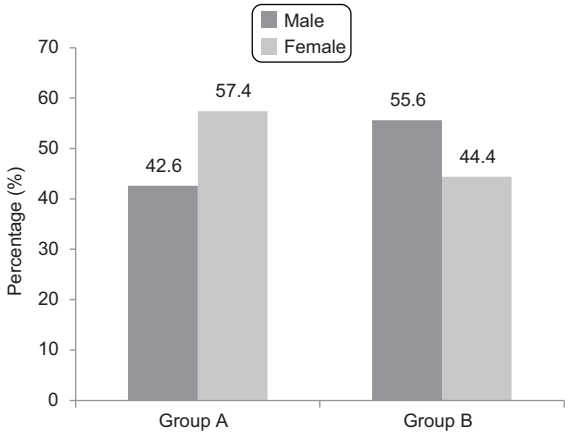

Fig.-1: *Distribution of study population of both groups according to gender (N=65)*

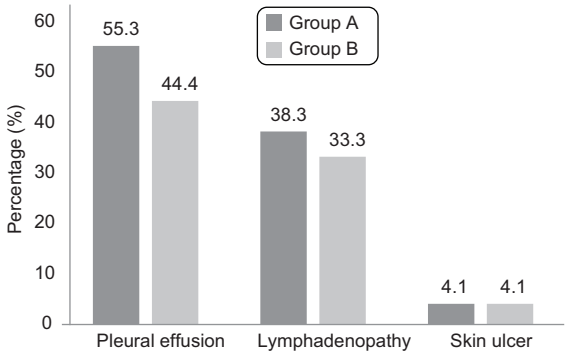

Fig.-2: *Clinical presentation of both groups (N=65)*

Considering 43U/L as the cut-off value of serum ADA, e™ 43 U/L was considered test positive and <43 U/L was considered test negative. Screening results of extra pulmonary tuberculosis among 65 patients, true positive were 43 cases and false positive were 3 cases, false negative were 4 cases and true negative were 15 cases (Table IV). Serum ADA had sensitivity 91.5%, specificity 83.3%, PPV 93.5%, NPV 78.9% and accuracy 89.2% respectively in diagnosis of extra pulmonary TB at the cut- off value of 43U/L (Table V).

Table-II

Distribution of study population according to histopathological study (N=65)

	Group A (n=47)	Group B (n=18)
Pleural TB	26 (55.3)	9 (50.0)
Lymph node TB	19 (40.4)	7 (38.9)
Skin TB	2 (4.3)	2 (11.1)

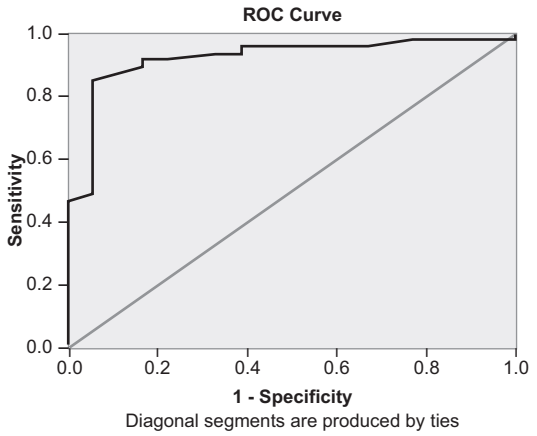

Fig.-3: *Receiver operating characteristic curve showing performance of serum ADA in differentiating EPTB patients (N=65)*

Table-IV

Screening results of extra pulmonary tuberculosis (N=65)

Screening for ADA	Group A (n=47)	Group B (n=18)	Total
≥43 U/L	43	3	46
<43 U/L	4	15	19
Total	47	18	65

Table-V

Serum ADA for diagnosing extra pulmonary TB (N=65)

Serum ADA	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ADA (≥43 U/L)	91.5	83.3	93.5	78.9	89.2

Discussion:

In this cross-sectional observational study total 65 patients who were clinically presumptive as extra pulmonary tuberculosis were included. Majority of Group A patients had pleural TB (55.3%) and among rest, 40.4% had lymph node TB & 4.3% had skin TB. Among Group B patients 50.0% had pleural TB, 38.9% had lymph node TB and 11.1% had skin TB. Most studies found that lymph nodes, pleura, abdomen, bones, joints and genitourinary system as most common sites of involvement by EPTB[13]. One study found pleural TB as the most common form of extra pulmonary TB in adults though other study observed lymph node as most common site of involvement by EPTB and skin as the least common site of involvement[14,15].

In our study, pleural TB is the most common form of EPTB may be because our study was conducted in a tertiary level hospital, specialized for TB and most patients were referred from primary health care center for respiratory related diseases. Though there is no age predilection for EPTB and it is known to affect all age groups, majority of the patients of our study belonged to age group 30-49 years (61.5%) with a mean 32.88±12.45 years of age. Female preponderancies generally noticed by various studies including this study (53.8%) and other study owing to illiteracy, social exclusion, malnutrition and economic dependency with little access to health care[16,17]. There was no significant difference in age and gender distribution of our study patients between Group A and Group B as p-value >0.05 which is consistent with other studies[18].

The meta-analysis of 11 studies that included 2,251 patients and used different cut-off values of ADA activity showed a sensitivity of 99% and a specificity of 89%[19]. Recently published 3 meta-analyses showed a sensitivity of 91.8% to 92% and a specificity of 88.4% to 90%[20]. In our study Receiver Operating Characteristic Curve analysis found highly significant cut-off value (43U/L) of serum

ADA with sensitivity 91.5%, specificity 83.3%, PPV 93.5%, NPV 78.9% and accuracy 89.2% respectively in diagnosis of extra pulmonary TB.

ADA was equal to or greater than 47U/L in 100% of patients with TB pleurisy, but only in 5% of the patients with non-TB pleurisy (sensitivity 100% and specificity 89%)[21]. Serum ADA levels were done for 54 cases in the study of Antin et al, of which 45 (83.33%) cases showed elevated levels with the cut off value 50U/L[22]. In our sample, we had a relatively low but satisfactory resulting sensitivity and specificity. The negative predictive value of this test is high and this gives it a place as a widely usable screening test to exclude EPTB.

Although histopathology is an inexpensive and reliable tool for detecting extra-pulmonary TB cases in resource limited settings, studies have highlighted the limitations of associating specific histopathological features with TB. Furthermore, histopathology can be invaluable in arriving at specific tissue diagnosis in diseases clinically mimicking TB such as lymphomas. Although the conventional methods (smear microscopy/culture) were used as a reference standard, these methods are not sufficient to detect all EPTB . Besides, regarding our limitation that we could not determine whether a higher value of adenosine deaminase correlated with particular localization, as we included relatively small number of individual cases in our study. Nevertheless, the present study suggests to use serum ADA estimation as the biochemical marker in the diagnosis of EPTB highlighting it as simple, rapid, cheaper and accurate diagnostic test.

Conclusion:

This study revealed that serum ADA is significantly higher in histopathologically confirmed extra pulmonary tuberculosis patients than histopathologically not confirmed extra pulmonary tuberculosis patients. Overall sensitivity, specificity, PPV, NPV and accuracy for detecting EPTB of serum ADA were 91.5%, 83.3%, 93.5%, 78.9% and 89.2% respectively at the cut off value of 43U/L. Serum ADA estimation are a useful tool for diagnosis of extra pulmonary tuberculosis particularly pleural TB, lymph node TB and skin TB.

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