

## ORIGINAL ARTICLE

# Cytomorphological Pattern, Evaluation of Serum Adenosine Deaminase (ADA) Level and Clinical Presentation of Peripheral Lymph Node Tuberculosis – An Observational Study

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### Abstract:

**Background:** Lymphadenitis is the most common form of extrapulmonary TB. Fine needle aspiration cytology (FNAC) is the most commonly used diagnostic tool. This study observed the cytomorphological pattern, serum adenosine deaminase (ADA) level and clinical presentation of lymph node TB.

**Methods:** We conducted a cross-section, observational study at the 250-Bedded T.B Hospital, Shyamoli, Dhaka, Bangladesh between January 2019 -January 2020 among the patients with peripheral lymphadenopathy and compatible clinical features favoring tuberculous etiology attending at the outpatient and inpatient department. Patients were categorized in three groups according to the lymph node FNAC pattern. Clinical presentation and radiological findings were evaluated and serum ADA level was estimated. Any association between lymph node cytology and serum ADA level was sought.

**Results:** Total 36 patients were enrolled in the study. Among them 75% were female and the mean age was  $33.5 \pm 14.6$  years. All the patients had cervical lymphadenopathy, and 83.3% had matted lymph nodes. Eighty six percent patients had high serum ADA level above cut-off value. The most common cytomorphological pattern observed in lymph node FNAC was epithelioid granuloma with caseous necrosis (58.3%). There was significant relationship between lymph node cytomorphological pattern and serum ADA level ( $p < 0.05$ ).

**Conclusions:** Tuberculous lymphadenitis is more common among female than male. Most common site of lymph node TB is cervical region, and epithelioid granuloma with caseous necrosis is the most frequently observed cytological pattern. Serum ADA is shown to have a potential role to support the diagnosis of tuberculous lymphadenitis.

**Key words:** Adenosine deaminase (ADA), cytomorphology, fine needle aspiration cytology (FNAC), lymph node tuberculosis (LNTB)

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### Introduction:

Tuberculosis remains a major health burden and is one of the top 10 causes of death worldwide. In

2018, an estimated 10.0 million people were infected with TB, and there were 1.2 million TB deaths among HIV-negative people. It has been the leading

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cause of death from a single infectious disease. Bangladesh ranks 7<sup>th</sup> among the top ten leading countries of global TB burden, with an incidence rate 221/100 000 population.<sup>1</sup>

The main form of the disease is highly transmissible pulmonary TB. Extrapulmonary TB (EPTB) is the infection of sites other than the lungs.<sup>2</sup> Lymph nodes are the most common site of EPTB and accounts for upto 40% of extrapulmonary disease in the USA.<sup>3</sup> A country wise data is not available.

Different studies reported that the peak incidence of lymph node TB (LNTB) is in young adults between 20 and 40 years.<sup>4-6</sup> Female sex, ethnic variety, and poor immune status is associated with the development of LNTB.<sup>7,8</sup> The disease is particularly common in persons with HIV co-infection.<sup>9</sup> Most LNTB involves cervical region and presents with a painless, erythematous, firm mass commonly involving anterior and posterior cervical chain or supraclavicular LN.<sup>10</sup> Progression of the disease is associated with fluctuation and sinus formation.<sup>4, 7</sup>

The diagnosis of LNTB involves fine needle aspiration cytology (FNAC), or excisional biopsy with histopathology, examination of acid-fast bacilli (AFB), and mycobacterial culture or PCR of lymph node aspirates.<sup>10</sup> Epithelioid granuloma with or without caseous necrosis is the prototyped cytological finding. AFB smear is positive in only 25-50% cases and culture in 70-80% cases.<sup>11</sup> Chest X-ray should be done in all patients with suspected TB to evaluate associated pulmonary TB or involvement of mediastinal lymph nodes. Adenosine deaminase (ADA), an enzyme of purine catabolism, is present in an increased concentration within lymphocytes. It has been shown that T lymphocyte population is increased in TB, and hence the activity of ADA is also increased.<sup>12</sup> Measurement of adenosine deaminase activity is very simple. It can be estimated by commercial ADA-MTB kit. The principal is ADA hydrolyses adenosine to ammonia and inosine. The ammonia which is formed further reacts with phenol and hypochlorite in an alkaline medium to form blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of blue colored indophenol complex formed is proportional to the amount of ADA present in the sample.<sup>12</sup>

The validity of serum ADA in the diagnosis of tuberculous pleural and pericardial effusion and tuberculous meningitis is established.<sup>13, 14</sup> But there is paucity of data regarding its role in the diagnosis of LNTB.

This study examined the cytomorphological pattern of LNTB in FNAC, depicted the clinical presentation, and estimated the serum ADA concentration as a supporting biomarker in the diagnosis of tuberculous lymphadenopathy.

## Methods

We conducted a cross-section, observational study at the 250-Bedded T.B Hospital, Shyamoli, Dhaka, Bangladesh, a government referral hospital for the management of tuberculosis patients, over a period from January 2019 to April 2021. Data collection was temporarily aborted from March 2020 to August 2020 due to CoVID-19 situation. Thirty nine patients with peripheral lymphadenopathy and compatible clinical features favoring tuberculous etiology attending at the outpatient and inpatient department of the hospital were preliminarily enrolled in the study. Three patients were discarded, as fine needle aspiration cytology (FNAC) result revealed non-specific lymphadenitis. Thirty six patients with FNAC diagnosis compatible with tuberculous etiology were included in the final analysis. Detailed history, thorough physical examination, relevant hematological investigations, Mantoux test (MT), chest X-Ray, and serum adenosine deaminase (ADA) estimation were carried out. All cases were categorized cytologically into three groups as suggested by Das et al.<sup>15</sup> The cytomorphological patterns were: epithelioid granuloma without caseous necrosis (pattern A), epithelioid granuloma with caseous necrosis (pattern B), and caseous necrosis without epithelioid granuloma with neutrophilic infiltrate (pattern C). A skin induration more than 10 mm in Mantoux test and the serum ADA more than 15 U/L were considered as significant. Statistical analysis was conducted using SPSS v23.0 (IBM Corp. Armonk, NY, USA). Data were presented in both univariate and multivariate tables according to merit. Chi-square test was done to correlate cytomorphological pattern of lymph node FNAC and serum ADA. A 'p value' <0.05 was considered as significant.

**Results:**

The study included 36 patients in total. The demographics and clinical features are summarized in Table I and Table II accordingly. The mean age was  $33.5 \pm 14.6$  years with a range between 15 to 68 years. Lymph node TB had a higher proportion among female (75%) compared to male (25%). Only a few patients (5.6%) had a previous history of TB. More than 90% patients had symptoms persisted for more than four weeks. All the patients had neck swelling, and 5 patients had enlarged lymph node in other locations in addition. Majority of the patients had right cervical adenopathy (55.6%), and 11.1% had bilateral involvement. In most of the cases lymph nodes were matted (83.3%) (Table III). Fever (86.1%), weight loss (66.7%), and dry cough (41.7%) were the other most common presenting features in chronological order. More than eighty percent patients had normal chest X-ray, and 11% had mediastinal lymphadenopathy (Table IV). Twenty five patients (63.9%) had a skin induration more than 10 mm in Mantoux test (Table V). Eighty six percent patients had high serum ADA level above cut-off value (15 U/L), ranging between 6.65-58.5 U/L (Table VI). The most common cytomorphological pattern observed in lymph node FNAC was pattern B, i.e. epithelioid granuloma with caseous necrosis (21 patients, 58.3%) followed by pattern A, i.e. epithelioid granuloma without caseous necrosis (14 patients, 38.9%) (Table VII). Significant association was observed when lymph node cytomorphology pattern was compared with serum ADA level (p value 0.034) (Table VIII).

**Table-I**  
*Demographic characteristics*

Variables	Frequency	Percentage
Age (years)		
≤20	9	25.0
21-30	12	33.3
31-40	3	8.3
41-50	6	16.7
51-60	5	13.9
>60	1	2.8
Mean±SD	33.5	±14.6
Range (min-max)	15.0	-68.0
Sex		
Male	9	25.0
Female	27	75.0
Previous H/O TB		
Yes	2	5.6
No	34	94.4

**Table-II***Clinical presentation of tuberculous lymphadenitis*

Variables	Frequency	Percentage
Duration of symptoms		
<2 weeks	1	2.8
2-4 weeks	2	5.6
>4 weeks	33	91.7
Clinical presentation		
Neck swelling	36	100.0
Fever	31	86.1
Weight loss	24	66.7
Dry cough	15	41.7
Chest pain	5	13.9
Swelling at groin/axillae	4	11.1
Sputum production	4	11.1
Haemoptysis	2	5.6

**Table-III**  
*Lymph node characteristics*

Lymph node	Frequency	Percentage
Site		
Cervical		
Right	20	55.6
Left	12	33.3
Both	4	11.1
Cervical and Axillary	4	11.1
Cervical and submental	1	2.77
Character of LN		
Matted	30	83.3
Discrete	3	8.3
Cold abscess	3	8.3

**Table-IV**  
*Radiological findings*

Radiological findings (Chest X-ray)	Frequency	Percentage
Normal	29	80.6
Mediastinal adenopathy	4	11.1
Consolidation and cavitation	2	5.5
Pleural effusion	2	5.5

**Table-V**  
*Pattern of skin induration in Mantoux test*

Mantoux test (mm)	Frequency	Percentage
<10	11	30.6
10-20	14	38.9
>20	9	25.0
Not done	2	5.6
Mean±SD	14.1	±7.6
Range (min-max)	0.0	-27.0

**Table-VI***Serum adenosine deaminase (ADA) level*

Serum ADA (U/L)	Frequency	Percentage
≤15.0	5	13.9
15.1-30.0	19	52.8
>30.0	12	33.3
Mean±SD	26.1	±11.2
Range (min-max)	6.65	-58.5

**Table-VII***Cytomorphological pattern of tuberculous lymphadenitis*

Cytological pattern	Frequency	Percentage
Epithelioid granuloma without caseous necrosis	14	38.9
Epithelioid granuloma with caseous necrosis	21	58.3
Caseous necrosis without epithelioid granuloma	1	2.8

**Table-VIII***Association between lymph node cytomorphology and serum adenosine deaminase (ADA) level in univariate analysis*

Cytological pattern	Total No. of cases	Serum ADA		P value
		≤15.0 U/L	>15.0 U/L	
Epithelioid granuloma without caseous necrosis	14	1 (7.1%)	13 (92.9%)	0.034 <sup>s</sup>
Epithelioid granuloma with caseous necrosis	21	3 (14.3%)	18 (85.7%)	
Caseous necrosis without epithelioid granuloma	1	1 (100.0%)	0 (0.0%)	

(s= significant, P value reached from chi square test)

**Discussion:**

This study significantly expanded previous observations that lymph node FNAC is a safe, simple and cost-effective outpatient procedure for the diagnosis of peripheral LNTB.<sup>16,17</sup> We categorized FNAC results into three cytomorphological pattern proposed by Das et al.<sup>15</sup> Pattern A – epithelioid granuloma without caseous necrosis, pattern B – epithelioid granuloma with caseous necrosis, and pattern C – caseous necrosis without epithelioid granuloma with neutrophil infiltration. The most common pattern observed in our study was pattern B (58.3%) followed by pattern A (38.9%). Findings show similarities with other studies.<sup>16, 18-20</sup> The formation of granuloma is dependent on good immune response against the organism. Skin induration in Mantoux test is an indirect evidence of immune response against *M. tuberculosis*.

The female to male ratio was 3:1, which is consistent with previous reports.<sup>2, 3, 7, 8, 21-24</sup> The reason for female predominance is not well understood. It is hypothesized that a difference in tumor necrosis factor (TNF) and IL-10 between both sexes may play a role.<sup>32</sup> Other contributing

factors include CD4+ lymphocyte count, endocrine effects, socio-economic and cultural background.<sup>26</sup>

TBLN may occur in any age group. In this study, the peak incidence was seen between 21-30 years and mean incidence 33.5±14.6 years. This finding correlates with other observations.<sup>4-6, 16, 27, 28</sup>

We found that cervical lymph nodes were involved in all patients, and 11% had concomitant axillary lymphadenopathy. Masilamani et al. found 97.2% cervical LN involvement.<sup>10, 29</sup> Similar observations were noted by Chand et al.<sup>30</sup> The organism usually gains access to the cervical LN through the tonsillar LN. More than 80% patients had matted lymph node in our study, which correlates with the studies by Dandapat<sup>31</sup> and Subrahmanyam.<sup>32</sup> Lymph node morphology depends on the stage of presentation. The delayed the presentation, the more complicated the disease. We noted that 91.7% patients presented after 4 weeks of their illness.

Other than lymphadenopathy, fever was the most common clinical presentation (86.11%), followed by loss of body weight (66.7%). Dandapat and Subrahmanyam also found weight loss as the most common feature (85% and 78% accordingly)

followed by fever (40% and 45%).<sup>31,32</sup> Many patients with LNTB present with pyrexia of unknown origin or unexplained weight loss. Thorough physical examination including searching for enlarged lymph node is crucial in TB endemic region to come to a diagnosis.

Raised level of serum ADA was observed in most of the patients (>85%) in this study, with a mean value 26.1±11.2 U/L (cut-off value 15 U/L). The values showed significant correlation (p value 0.034) when compared to the lymph node FNAC results. Bhatta et al.<sup>16</sup> also found raised serum ADA in 64.28% TBLN cases, whereas Ninghot et al.<sup>12</sup> and Mugulkod et al.<sup>32</sup> observed a raised level in 93.3% and 83.3% cases respectively. Serum ADA is an indicator of active cellular immunity. This enzyme causes proliferation and differentiation of T cells and maturation of monocytes to macrophage, hence plays a crucial role in granuloma formation.<sup>33,34</sup> Raised ADA level with strong clinical suspicion with or without a typical cytological feature may help clinicians to reach a confident diagnosis of LNTB.

### Conclusions:

Detection of acid-fast bacilli in representative sample is the gold standard for the diagnosis of tuberculosis. But in extrapulmonary TB, it is not always possible due to paucibacillary nature of the disease. FNAC is a simple, cost-effective, minimally invasive, and relatively safe procedure to diagnose peripheral lymph node tuberculosis. However when cytology report is inconclusive, a supportive biochemical marker may add a great value to the diagnostic yield. This observational study proves the diagnostic potential of serum ADA in peripheral lymph node tuberculosis. Further study with a large sample size and comparison with a control group will improve our understanding of diagnostic efficacy of serum ADA.

### Author contributions

N. K Sarkar and Ashadur Rahman designed the study, wrote manuscript, and takes equal credit (co-first author). N.K Sarkar, Ashadur Rahman, Moumita Roy, Jesmin Akter and Mosharaf Hossain gathered data, analyzed data and revised the manuscript. N. K Sarkar takes full vouch for the content of the manuscript.

### Financial disclosure

The authors disclosed no financial attachment.

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