Research Article

Association of Serum Adenosine Deaminase Levels in Cytologically Suggested Cases of Tubercular Lymphadenitis: The Experience of a Tertiary Care Centre

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Abstract

Background: Tuberculosis (TB), a communicable disease, caused by Mycobacterium tuberculosis requires a simple, rapid test, which can be easily carried out in a laboratory. Unfortunately, despite a battery of investigations, no definite test is available till date. Adenosine deaminase (ADA), a biochemical marker has been proposed as a useful surrogate marker for TB as its levels can be measured in body fluids.

Methods: A one-and-a-half-year prospective study of 154 cases presenting with lymphadenitis from January 2019 to June 2020 was undertaken. Using cytology, lymphadenitis subjects were divided into two groups: Tubercular (104 patients) as a case group and Reactive (50 patients) as a control group. All cases were followed by serum ADA assay by colorimetric method. Nonparametric tests were performed to compare the two groups.

Results: The mean age of the participants was 28.99 ± 13.26 years with a F:M ratio of 1.81:1. Involvement of cervical lymph nodes was most frequent (89.42% cases). The mean S.ADA level for tubercular and reactive lymphadenitis was 41.71 ± 11.53 U/L and 21.16 ± 4.16 U/L, respectively (P-value < 0.05). The cut-off value calculated was 32.6 U/L. The sensitivity, specificity, PPV, NPV, and accuracy were calculated as 79.81%, 100%, 100%, 70.42%, and 86.36%, respectively.

Conclusion: A statistically significant increase was found in serum ADA levels in tubercular lymphadenitis cases compared to reactive lymphadenitis. Hence, it can be used as an adjunct to FNAC and is a fairly sensitive and specific test. Since it is difficult to always demonstrate AFB in FNAC smears, ADA can be helpful in establishing a definite diagnosis despite smear negativity.

Keywords: adenosine deaminase, lymphadenitis, tuberculosis

1. Introduction

Tuberculosis (TB), a transmittable disease, is amongst the top 10 worldwide causes of death and is the principal cause of fatality from a single infectious vehicle ranking...
over HIV/ AIDS [1]. In 1993, TB was declared as a public health emergency on a global level by WHO [2]. Depending upon the locus of involvement, TB can have two different manifestations clinically: Pulmonary TB, wherein infection is situated in the lungs and Extrapulmonary TB, involving any site other than the lungs, for example, abdomen, lymph nodes, meninges, skin, or genito-urinary tract [3]. The most common manifestation of mycobacterial extra-pulmonary disease is the involvement of peripheral lymph nodes, usually of the cervical area [4]. Several research studies conducted in India have also shown *Mycobacterium tuberculosis* (*M. TB*) to be the pathogen that was commonly isolated from mycobacterial lymphadenitis taking in account most of the cases [5–7].

An expeditious diagnosis is a mainstay for an efficient TB curbing program. A variety of methods for pulmonary TB detection are available but these do not yield sufficient specificity or sensitivity. The sensitivities of TB diagnosis using culture and Ziehl-Neelsen staining are 8–49% and 10–40%, respectively [8]. The gold standard test for the detection of TB is the culture of the causative organism [9]. The technique of choice for extrapulmonary TB detection is histopathological examination. This includes excisional biopsy followed by culture and Ziehl-Neelsen stain. However, being an invasive modality and due to the lack of availability in peripheral centers makes its feasibility limited in low-resource areas [10].

Fine-needle aspiration is an economic, rapid, and simple technique that has high specificity and sensitivity for extrapulmonary TB and can be performed on an outpatient basis. When extrapulmonary TB is considered, Acid Fast Bacilli (AFB) positivity is not always found due to paucibacillary nature particularly for those cases which are associated with immunosuppression. In endemic nations like India, even without AFB positivity, the mere presence of granuloma with/without caseation and with/without giant cells-Langhan's type is taken as and treated for TB [11].

We need an easy, reliable, and rapid test that can easily be executed in the laboratory setting. Therefore, we thought of using serum Adenosine deaminase (ADA) as an adjunctive diagnostic tool considering the fact that fluid ADA levels are already in use for tubercular effusions since 1978. Various articles have found the test to have high sensitivity-92% and specificity-89% for early detection of extrapulmonary TB in cases of tuberculous pericarditis, meningitis, pleuritis, and ascites [12]. Although a good amount of data has validated the yield of ADA in tubercular effusions, we cannot always access these fluids. Instead, serum levels can be easily performed but there is a paucity of literature correlating it to tubercular lymphadenitis. Therefore, we undertook this
study intending to demonstrate the association of serum ADA activity in cytologically suggested tubercular lymphadenitis cases.

2. Material and Methods

This prospective cross-sectional study was carried out in the Department of Pathology of a tertiary care center from January 2019 to June 2020. All peripheral lymph node fine needle aspirations with clinical suspicion of TB during this period were included in this study. A total of 154 subjects were enrolled.

2.1. Inclusion criteria

1. Both genders and all age groups were included.
2. Patients referred for fine needle aspiration cytology of lymph nodes with clinical and cytological suspicion of tubercular lymphadenitis or reactive lymphadenitis.

2.2. Exclusion Criteria

1. All causes of raised serum ADA levels other than TB.
2. Patients who did not give consent for the study.

The study was approved by the Ethical and Research Committee of the Institute. Patients were selected in accordance with predefined inclusion and exclusion criteria of the study. All selected patients were briefed on the nature of the study and a written informed consent for FNAC and serum ADA estimation was obtained. The relevant clinical details, demographic data of the patients such as the name, age, and sex were recorded.

The prepared smears were stained by Giemsa and Ziehl-Neelsen stain. All the stained slides were thoroughly analyzed by two independent trained pathologists with more than eight years’ experience and were examined for the features of tubercular and reactive lymphadenitis. Out of the 154 subjects, 104 with cytologically suggested diagnosis of tubercular lymphadenitis were taken as the case group while 50 age- and sex-matched individuals presenting as reactive lymphadenitis were included as control group.

The tubercular lymphadenitis cases were segregated based on the cytologic patterns described by Dasgupta et al. [13] as follows:
1. Pattern A: Epithelioid granuloma without necrosis,
2. Pattern B: Epithelioid granuloma with necrosis, and
3. Pattern C: Necrosis with/without neutrophilic infiltrate.

A 2-ml blood sample from the patient was then collected into a plain vial and serum ADA estimation using non-Guisti’s method was done (Adenosine deaminase Assay kit, ERBA Mannheim, TransAsia Bio-medicals Ltd.) on semi-automated analyzer. Cytologically suggested cases of TB lymphadenitis were followed-up to assess clinical response to therapy (no evening rise of temperature, improvement in appetite, and weight gain) after one month, and in case of no response, the case was excluded.

2.3. Statistical analysis

The data obtained were analyzed using descriptive statistics, unpaired t-test, one-way ANOVA test, and Chi-square test. SPSS program for Windows, version 25.0 was employed for statistical analysis. *P*-value < 0.05 was considered as statistically significant for all statistical tests. To determine optimal cut-off value of serum ADA levels in tubercular lymphadenitis, a receiver operating characteristics (ROC) analysis was done and area under the curve and its standard deviation (AUC_SD), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

3. Results

Out of the 104 patients that presented with cytological features suggestive of tubercular lymphadenitis, 37 were male and 67 female. Female dominance was present in our study with a F:M ratio of 1.81:1. The age of the patients ranged from 5 to 70 years. The mean age in the case group was 28.99 ± 13.26 years.

The involvement of cervical lymph nodes was most frequent and was observed in 89.42% of cases. On FNAC, pattern B (Figure 1) (69 cases, 66%) was most frequently encountered, followed by pattern A (Figure 2) (25 cases, 25%) and then by pattern C (Figure 3) (9 cases, 9%). Out of the 104 cases of tubercular lymphadenitis, AFB positivity (Figure 4) was seen in 24.04% of the cases. Maximum AFB positivity was observed in cases belonging to pattern C.

The mean serum ADA level in tubercular lymphadenitis was 41.71 ± 11.53 U/L and in reactive lymphadenitis was 21.16 ± 4.16 U/L (Figure 5).
The mean serum ADA level was statistically more significant among tubercular lymphadenitis compared to reactive lymphadenitis ($p$-value < 0.001) (Table 1). No significant difference in the distribution of serum ADA levels between AFB-positive and -negative groups was observed ($p$-value = 0.669) (Table 2). No significant difference was reported in the mean ADA levels among patterns A, B, and C ($p$-value = 0.438) (Table 3).

The optimal cut-off value for serum ADA level was calculated to differentiate cases having tubercular lymphadenitis from reactive lymphadenitis using ROC curve. The optimal cut-off value for identifying tubercular lymphadenitis was found to be 32.6 U/L with resulting sensitivity (79.81%), specificity (100%), PPV (100%), NPV (70.42%), and accuracy for serum ADA level calculated as 86.36% (Figure 6).

**Figure 1:** Photomicrograph showing Epithelioid cell granuloma with a necrotic background (Pattern B); 100x (MGG).

**Table 1:** Comparison of mean serum ADA levels in reactive lymphadenitis (control) and tubercular lymphadenitis (cases) (Department of Pathology, TMU from January 2019 to June 2020) $n = 154$.

|                         | Mean (U/L) | Standard deviation | Mean Difference | $t$-test value | $P$-value |
|-------------------------|------------|--------------------|-----------------|----------------|-----------|
| **Reactive Lymphadenitis** | 21.16      | 4.16               | −20.55          | −12.213        | <0.001*   |
| **Tubercular Lymphadenitis** | 41.71      | 11.53              |                 |                |           |

Unpaired $t$-test; *Significant difference.
4. Discussion

TB has been a public health emergency as well as the chief cause of fatality for a long time. The mainstay for controlling this infection is an early unerring diagnosis followed by an appropriate prompt treatment [1]. In the case of tubercular lymphadenitis, a proper diagnosis will require either an FNA-obtained material or a biopsy which will
Problem rises due to the paucity of demonstrable acid-fast bacilli at such locations thereby impeding a definite diagnosis. In such situations, we require an adjunctive tool that might facilitate a diagnosis [9, 10].

Serum ADA estimation is one such modality which, in addition to being noninvasive, is a simple method for disease detection. ADA can serve as an indicator of cell-mediated
Figure 6: ROC analysis of optimal cut-off value for serum ADA level to differentiate cases of tubercular lymphadenitis from reactive lymphadenitis (Department of Pathology, TMU from January 2019 to June 2020) \( n = 104 \).

The test result variable(s): ADA has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased
1. under the nonparametric assumption and
2. null hypothesis: true area = 0.5.

AUC 0.953 (95% CI, 0.923–0.983, \( p < 0.001^* \)), Sensitivity 79.81% (70.81–87.04%), Specificity 100% (92.89–100.00%), Positive Predictive Value 100%, Accuracy 86.36% (79.91–91.36%), and Negative Predictive Value 70.42% (61.90–77.72%).

TABLE 2: Correlation of serum ADA levels with AFB status (demonstration on Ziehl-Neelsen-stained smear) in cases of tubercular lymphadenitis (Department of Pathology, TMU from January 2019 to June 2020) \( n = 154 \).

| AFB       | Mean (U/L) | Std. deviation | Mean difference | t-test value | P-value |
|-----------|------------|----------------|----------------|-------------|---------|
| Positive  | 40.95      | 10.41          | -1.02          | -0.388      | 0.699†  |
| Negative  | 41.97      | 11.93          |                |             |         |

Unpaired t-test; †Non-significant difference.

TABLE 3: Correlation of serum ADA levels with different cytological patterns of tubercular lymphadenitis (Department of Pathology, TMU from January 2019 to June 2020) \( n = 104 \).

| Pattern | Mean | Standard deviation | F-value | P-value |
|---------|------|--------------------|---------|---------|
| A       | 44.21| 11.42              | 0.833   | 0.438†  |
| B       | 40.98| 11.75              |         |         |
| C       | 40.12| 10.07              |         |         |

One-way ANOVA test; †Non-significant difference.

immunity. Its utility in pleural effusions was first demonstrated by Piras et al. [14]. Many studies [15–17] have established the utility of fluid ADA levels for the diagnosis of tuberculous peritoneal, pleural, pericardial effusions, and even in CSF but they are
not always accessible. Therefore, we thought of using serum levels which we found elevated more in tubercular aetiologies as compared to nontubercular diseases.

In the present study, patients age ranged from 5 to 70 years. The age group most frequently involved among the patients with tubercular lymphadenitis was 21–30 years followed by 11–20 years. In this study, subjects belonging to the case group showed a slight female predominance with a female: male ratio of 1.81:1. Mugulkod et al., Khajuria et al., and Purohit et al. [15–17] exhibited a minor female dominance as well.

Among the sites of involvement, the commonest was the cervical group of lymph nodes which comprised 89.42%. In the study by Mugulkod et al. [15], of the 230 cases of extrapulmonary TB, 184 (80%) were of lymphadenitis. Similar to our study, they also showed the cervical group of lymph nodes as most frequently involved.

The tubercular lymphadenitis cases were segregated based on the cytologic patterns described by Dasgupta et al. [13]. Of the 104 subjects, patterns A, B, and C were observed in 25, 69, and 9 cases, respectively. The commonest pattern in this study was pattern B (66.34% cases) showing epithelioid granuloma with necrosis. The same was observed by Bhattacharya et al. [18] and Khanna et al. [19] in 69.4% and 50.5% cases, respectively.

The presence of epithelioid cells was seen in 91.34% cases. Giant cells were noticed in 35.57% cases. In the present study, AFB positivity (Figure 4) was noted in 24.04% cases which was comparable to the ones reported as 25.65% and 26.1% by Mugulkod et al. [15] and Nassaji et al. [20], respectively.

The mean serum ADA levels in tubercular and reactive lymphadenitis were calculated as 41.71 ± 11.53 U/L and 21.16 ± 4.16 U/L, respectively. Maximum cases, that is, 43.3% showed an ADA value in the range of 41–60 U/L followed by 30.8% cases in the range of 31–40 U/L. The mean serum ADA level was significantly high among tubercular lymphadenitis compared to reactive lymphadenitis.

Results similar to our study were also demonstrated in the studies by Sulakshana et al. [21], Ahmed et al. [22], Abdelsadek et al. [23], Mugulkod et al. [15], and Stevanovic et al. [3]. This was in contrast to the findings of Conde et al. [22]. They concluded that although serum ADA2 levels might help in differentiating pulmonary TB cases from controls, its utility in distinguishing it from other respiratory diseases is limited.

The cut-off value for tubercular lymphadenitis calculated using the ROC curve was 32.6 U/L. At this cut-off, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 79.81%, 100%, 100%, and 70.42%, respectively. In the present study, 20 (19.23%) cases had a serum ADA level below the cut-off value of 32.6 U/L while none of the control group subjects crossed this value. In contrast, Salamanzadeh et al. [24] reported a sensitivity and specificity of 35% and 91%,
respectively, for patients with pulmonary TB. They suggested that due to high specificity, ADA can be employed to rule out TB in suspected patients having a negative culture for AFB. Similarly, Farazi et al. [25] also reported a low sensitivity with a high PPV.

**Limitations**

The small sample size of the study may limit statistical relevance. Only patients with positive clinical response to treatment were included as cases while those who did not respond to treatment like MDR and XDR cases were excluded.

**Recommendations**

Further investigations with appropriate research methodology setup are needed to conclusively opine about the accuracy of serum ADA alone compared to ADA as an adjunct tool to FNAC in making TB diagnosis.

**5. Conclusion**

The raised serum ADA level can be considered as a reliable indicator of tubercular lymphadenitis and is a fairly sensitive and specific test. The use of non-Guisti’s method makes it a rapid test that can be carried out easily even in remote areas with limited facilities. The best cut-off value calculated in our study was 32.6 U/L at which the sensitivity (79.81%) and specificity (100%) were found to be good enough for this test to help distinguish tubercular from non-tubercular aetiologies and also in detecting AFB smear-negative cases. So, in a country like India where TB is endemic, serum ADA can be used as an adjunct to FNAC for a definite diagnosis of tuberculous lymphadenitis especially in difficult situations or diagnostic dilemma. It can also be used as an early guide in subjecting the patient to FNAC considering the cut-off of 32.6 U/L below which we can say with the confidence of 86.36% that it is likely a non-tubercular or tubercular etiology.

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Ethics Considerations

Prior ethical approval was taken from the Institutional Ethics Committee (IEC), Teerthanker Mahaveer Medical College & Research Centre (TMMC&RC), Moradabad vide letter no. TMMC&RC/IEC/18-19/020 dated 27/12/2018.

Competing Interests

The authors declare hereby that there are no conflicts of interest.

Availability of Data and Material

The raw data used during the current study are available from the corresponding author on reasonable request.

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