

Research Article

STUDY OF SERUM ADENOSINE DEAMINASE LEVELS IN FNAC CONFIRMED CASES OF TUBERCULOUS LYMPHADENITIS

^{1,*}Sulakshana, M. S., ¹Siddiq M Ahmed and ³Jayakumar, C. K.

¹Tutor, Department of Pathology, Bangalore Medical College and Research Institute, Fort, K R Road, Bangalore-560002

²Professor, Department of Pathology, Bangalore Medical College and Research Institute, Fort, K R Road, Bangalore-560002

³Assistant Professor, Department of Pathology, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur

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ABSTRACT

Background: Tuberculosis has emerged as one of the most lethal diseases man has ever faced. India accounts for nearly one third of global burden of tuberculosis. Tubercular lymphadenitis is the most common type of extra pulmonary tuberculosis. This study was done to assess the diagnostic significance of serum adenosine deaminase levels in FNAC confirmed cases of tuberculous lymphadenitis and to determine its sensitivity and specificity; and to rule out early tuberculosis in cases reported by FNAC as reactive lymphadenitis.

Methods: A two year prospective study of 35 cases presenting with lymphadenitis from October 2010 to October 2012 was done. The study subjects were first confirmed by fine needle aspiration cytology and AFB staining. This was followed by the measurement of serum adenosine deaminase levels.

Results: The mean age of the patients in the present study was 28 years with no significant difference in the gender distribution. Under reactive lymphadenitis, abscess, and suppurative lymphadenitis groups, reactive lymphadenitis was more common with 60% of cases. Under granulomatous lymphadenitis group, granulomatous lymphadenitis was more common with 80% of cases. Numbers of granulomas/20 HPF were significantly more associated with Tubercular lymphadenitis with $p=0.008^{**}$. Number of giant cells /20 HPF were statistically similar in two groups with $P=1.000$. The most common significant range of serum adenosine deaminase levels was 31 to 40 U/L.

Conclusion: Serum adenosine deaminase levels are an adjunct to FNAC in the diagnosis of tuberculous lymphadenitis. As the number of granulomas increase, the serum adenosine deaminase levels also increases.

INTRODUCTION

Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes *Mycobacterium tuberculosis*. Globally, approximately 16 million people are suffering from active TB, with an estimated 8.5 million developing active TB each year resulting in approximately 2 million deaths yearly. Tuberculosis (TB) is one of the leading causes of mortality and morbidity in developing countries (WHO Reports, 2003). India is the highest TB burden country with World Health Organisation (WHO) statistics for 2011 giving an estimated incidence Figure of 2.2 million cases of TB for India out of a global incidence of 8.7 million cases. The estimated TB prevalence figure for 2011 is given as 3.1 million.

It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB (WHO Global Tuberculosis control, 2012). In developing countries, TB is one of the most common opportunistic infections in people who are seropositive for HIV-1. The clinical manifestations of tuberculosis are dependent on the cellular immune responses to the tubercle bacilli, characterized by the accumulation of monocytes/macrophages, lymphocytes and polymorphonuclear leukocytes in tuberculosis lesions. These responses are initiated on sensitization of T-lymphocytes by the bacterial antigen with the release of cytokines which regulate macrophage function (Lamsal and Gautham, 2007). Tuberculosis usually affects the lungs, but extra pulmonary tuberculosis is of equal importance of which tuberculous lymphadenitis is the most common.

*Corresponding author: Sulakshana, M. S.

Department of Pathology, Bangalore Medical College and Research Institute, Fort, K R Road, Bangalore-560002

The diagnosis of tuberculous lymphadenitis is confirmed routinely by fine needle aspiration cytology and AFB staining.

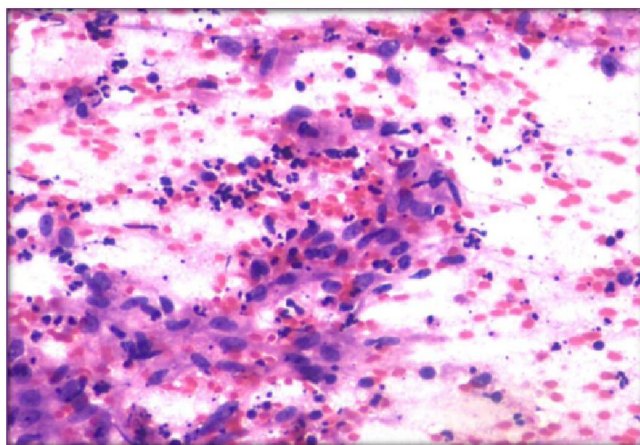


Fig. 1. Photomicrograph showing epithelioid granuloma; 40x (H and E)

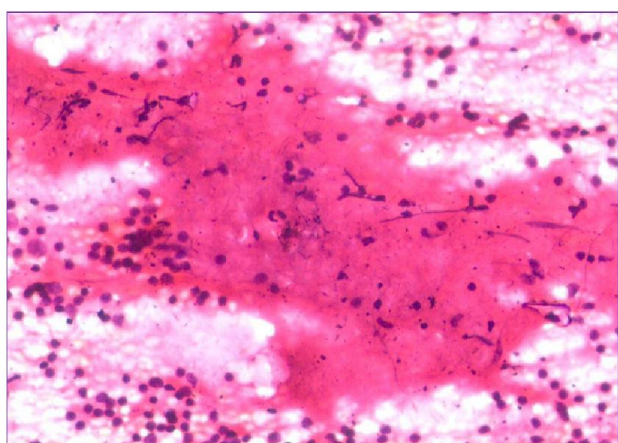


Fig. 2. Photomicrograph showing caseous necrosis; 40x (H and E)

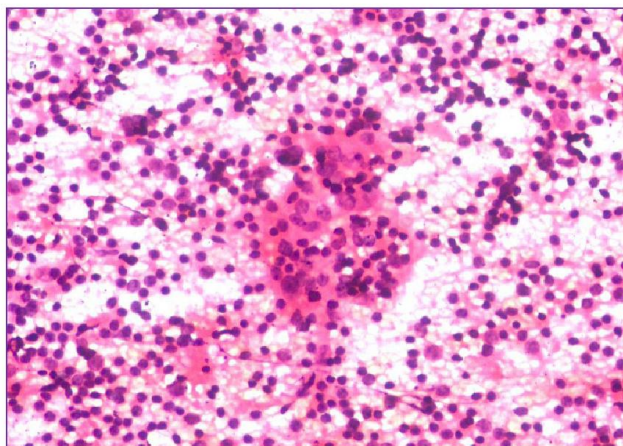


Fig. 3. Photomicrograph showing histiocytic aggregate with lymphocytes in background; 40x (H and E)

Recent studies showed extensive delay in TB diagnosis (Storla and Yimer *et al.*, 2008). There are different diagnostic methods but they have some drawbacks. To prepare mycobacterium culture, which is the golden standard for TB diagnosis, it may take 8 weeks. Finding acid-fast bacilli is the quick screening method for pulmonary TB diagnosis; nevertheless, its sensitivity is low. The polymerase chain reaction (PCR) test for TB diagnosis is expensive and it requires skilled personnel and lot of equipment.

Therefore, in recent years, there has been a great demand for finding new microbiological, genetic, immunological, and biomedical diagnostic methods to diagnosis TB quickly and accurately.

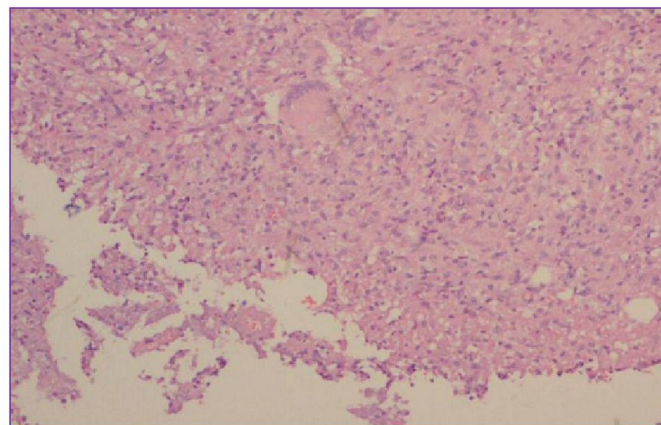


Fig. 4. Histopathology of Koch's lymphadenitis showing epithelioid granuloma and Langhan's giant cell 10x (H and E)

Measuring of adenosine deaminase (ADA) activity is a biomedical method. ADA is an enzyme which contributes in purine metabolism. ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity and previous studies have proved its value in TB diagnosis, even for assessing TB effusions. Activity of this enzyme increases in TB patients. Previous studies used effusion fluids and a very limited number of studies used patients' serum. It is not always possible to access effusion liquids everywhere in pulmonary and extrapulmonary TB; therefore, it would be helpful to take advantage of serum levels (Afrasiabian and Mohsenpour, 2013). Hence this study was done to assess the diagnostic significance of serum adenosine deaminase levels in cases of tuberculous lymphadenitis confirmed by fine needle aspiration cytology; to determine sensitivity and specificity of serum ADA levels in tuberculous lymphadenitis and to detect early tuberculosis by serum ADA levels in cases reported as reactive lymphadenitis by fine needle aspiration cytology and confirmed by histopathological examination.

MATERIALS AND METHODS

The patients with clinically suspected tuberculous lymphadenitis and reactive lymphadenitis underwent fine needle aspiration cytology in the department of Pathology, Bangalore Medical College and Research Institute. After obtaining approval and clearance from the institution ethical committee, patients were included for the study.

Methodology

The diagnoses were first confirmed by fine needle aspiration cytology and AFB staining. This was followed by the measurement of serum adenosine deaminase levels by colorimetric method. The patients whose serum ADA levels were high and their lymph node FNA reported as reactive lymphadenitis were subjected to lymph node biopsy for histopathological examination.

Statistical Methods

Descriptive and inferential statistical analyses were carried out in the present study. Significance was assessed at 5 % level of significance. Student t test (two tailed, independent) and Chi-square/ Fisher Exact test have been applied.

RESULTS

The most common age group affected was between 21-30 years followed by 11-20 years (Table 1). Males were more frequently affected (54.3%) than females (45.7%). Results of the Fine needle aspiration study of the lymph nodes were as follows:

Table 1. Age distribution of patients studied

Age in years	Reactive lymphadenitis	Tubercular lymphadenitis	Total
1-10	0(0%)	4(13.3%)	4(11.4%)
11-20	3(60.0%)	5(16.7%)	8(22.9%)
21-30	1(20.0%)	10(33.3%)	11(31.4%)
31-40	0(0%)	6(20.0%)	6(17.1%)
41-50	0(0%)	2(6.7%)	2(5.7%)
>50	1(20.0%)	3(10.0%)	4(11.4%)
Total	5(100%)	30(100%)	35(100%)
Mean \pm SD	28.60 \pm 20.29	28.60 \pm 16.09	28.60 \pm 16.41

Reactive lymphadenitis was more frequent compared to abscess and suppurative lesion. Granulomatous lymphadenitis was a more frequent diagnosis compared to cold abscess, caseous necrosis and early granulomatous lymphadenitis. Number of granulomas/20 HPF were significantly more associated with Tubercular lymphadenitis with $p=0.008^{**}$ (Table 2). Number of giant cells /20 HPF were statistically similar in the two groups with $P=1.000$ (Table 3).

Table 2. Distribution of number of granulomas /20 HPF

Number of granulomas/20 (5sqmm) HPF	Reactive lymphadenitis	Tubercular lymphadenitis	Total
Nil	5(100%)	6(20.0%)	11(31.4%)
1-2	0(0%)	15(50.0%)	15(42.9%)
3-4	0(0%)	7(23.3%)	7(20.0%)
>4	0(0%)	2(6.7%)	2(5.7%)
Total	5(100%)	30(100%)	35(100%)

Table 3. Distribution of number of giant cells/20 HPF

Number of giant cells/20 HPF	Reactive lymphadenitis	Tubercular lymphadenitis	Total
Nil	5(100%)	25(83.3%)	30(85.7%)
1	0(0%)	5(16.7%)	5(14.3%)
Total	5(100%)	30(100%)	35(100%)

Adenosine deaminase levels were more frequently in the range of 31-40 U/L with 65% of cases followed by 41-60 U/L in 20% of cases (Table 4).

Table 4. Adenosine deaminase levels U/L in the patients studied

Adenosine deaminase levels U/L	Reactive lymphadenitis	Tubercular lymphadenitis	Total
<30	1(20.0%)	1(3.3%)	1(2.9%)
31-40	3(60.0%)	20(66.7%)	23(65.7%)
41-60	1(20.0%)	6(20.0%)	7(20.0%)
>60	1(20.0%)	3(10.0%)	4(11.4%)
Total	5(100%)	30(100%)	35(100%)

DISCUSSION

The sensitivities of ZN staining and culture are 10-40% and 8-49%, respectively in the diagnosis of TB infection (Jay, 1985). Definitive diagnosis of TB requires culture of the suspected organism. Because Mycobacterium tuberculosis grows very slowly, it can take up to six weeks to isolate it in culture. Because of these problems in exact diagnosis of tuberculosis, numerous additional tests are in use with the intention to facilitate the diagnosis. In the recent use are among others, tests who determine the biological (biochemical) markers of tuberculosis infection, such as levels of adenosine deaminase concentration (ADA) or the level of interferon gamma. Adenosine deaminase is an enzyme required for converting adenosine to inosine in the purine salvage pathway. Its activity is involved in the differentiation and proliferation of lymphocytes and activation of macrophages. This enzyme is important in the rapid proliferation of cells to prevent the accumulation of toxic metabolite. Adenosine deaminase activity (ADA) increases during cellular activation to detoxify toxic metabolite (Piras and Gakis *et al.*, 1978). There is restriction of lymphocytic blastogenesis following the activation of ADA inhibitors through biologic and nonclarified mechanisms, possibly connected with the conversion of dioxadenosine into dioxy-ATP in lymphocytic cells, which could cause its destruction by inhibition of DNA synthesis (Carson and Seegmiller, 1976).

This is an important enzyme in T-lymphocytes where it is 10 times higher concentration than in erythrocytes. Its activity increased during the reproduction and response to antigenic stimulation of lymphocytes (Sharma and Suresh *et al.*, 2001). Therefore, its increased concentration may be found in all fluids accumulated in the zones of tuberculosis serositis, which is used in diagnosis (Mathur and Tiwari *et al.*, 2006). Piras *et al.*, (Piras and Gakis, 1978) reported an increase in ADA level in TB pleural effusion; other studies have also confirmed such an increase in TB pericardial effusions, peritoneum, and central nervous system (CNS) (Afrasiabian and Mohsenpour, 2013).

In Agarwal *et al.*'s study, ADA level was 15.3 (± 0.23) in healthy people, 19 (± 0.68) in non-pulmonary TB cases, and 38.48 (± 1.56) in pulmonary TB patients (Agarwal and Mukerji *et al.*, 1991). In Jhamaria *et al.*, 's study, the average of serum ADA level was 19.9 U/L (± 2.99) in control group, 43.95 U/L (± 2.48) in sputum smear-positive people with typical or progressive disease, and 42.09 U/L (± 1.46) and 40.02 U/L (± 2.58) in negative sputum patients with mild or typical disease. In their study, in the cut-off point of 33 U/L, sensitivity and specificity were 98% and 100%, respectively (Jhamaria and Jenaw *et al.*, 1998). It seems that as the disease progresses, ADA levels increase.

Serum ADA activity and lysozyme levels have been noted to be significantly elevated in children with different forms of tuberculosis in comparison to controls (Mishra and Yusaf *et al.*, 2000). Some researchers have studied ADA activity in sputum and serum of PTB patients and found higher ADA activity in those patients with respect to other lung pathology (Dilmac and Ucoluk *et al.*, 2002). High levels of ADA activity have been observed in pleural fluid and serum of patients with tuberculous effusion compared to neoplastic effusion (Baganha and Pego *et al.*, 1990).

A previous study of serum ADA in pulmonary tuberculosis, malignancy and non-tubercular respiratory diseases showed significantly higher levels in pulmonary tuberculosis patients than other groups (Bansal and Singh *et al.*, 1991). It has been reported that with an ADA activity cutoff value of 54 IU/L, the sensitivity is 82% and specificity is 97% for the diagnosis of tuberculosis. Reported cutoff values for ADA (total) vary from 47 to 60 U/L (Valdes and San Jose *et al.*, 1993; Perez-Rodriguez and Perez Walton *et al.*, 1999). Burgess L.J. showed ADA activity in tuberculous effusion was higher than in any other diagnostic group. At a level of 50U/L the sensitivity and specificity for the identification of tuberculosis was 90% and 89% respectively (Burgess, 1995). Strankinga W.F. investigated 10 patients with tuberculosis pleurisy and 76 patients with pleural effusions of other etiology. The ADA activity in the tuberculous patients was significantly higher than in the other groups while the exception of those with empyema. Specificity 87% and sensitivity 100% of this test for tuberculosis are high when a reference limit of more than 53 U/L is taken (Strankinga, 1987).

There are two principal isoenzymes of ADA, ADA-1 and ADA-2. ADA-2 is released by monocyte macrophages when they are stimulated by the presence of live microorganisms in their interior (Gakis and Calia *et al.*, 1989). An increase in total ADA activity may be characterized by M. tuberculosis infecting the macrophages. The raised ADA activity under antigenic stimulation is found in infections, such as tuberculosis and typhoid fever, where cell-mediated immunity (CMI) is stimulated (Mishra *et al.*, 1994). Other earlier studies have also shown increased levels of serum ADA in a number of diseases where CMI is stimulated like Behcet's disease (Kose *et al.*, 2001), typhoid (Ungerer *et al.*, 1996), tuberculosis (Sharma *et al.*, 2001), acute nephrotic syndrome (Mishra *et al.*, 1997) and cancers (Ergoly *et al.*, 1984).

Although many sensitive tests involving molecular diagnostics are available for the rapid diagnosis of TB, such technology is not available in developing countries. The colorimetric method for the measurement of total ADA described by Guisti and Galanti in 1984 has an advantage over other methods because of its low cost, simplicity of technique and rapid turnaround time (Guisti and Galanti, 1984). The estimation of ADA activity in body fluid therefore serves as a reasonable tool in the diagnosis of TB pleural effusion, especially when other clinical laboratory tests are negative. The present study showed that there is significant rise in ADA levels in tubercular lymphadenitis. It is an important adjunct to FNAC in the diagnosis of tubercular lymphadenitis. ADA estimation in serum is simple, inexpensive and a rapid method to help the clinician make diagnosis of tubercular lymphadenitis in adjunct to FNAC.

To the best of our knowledge, this is one of the first studies to correlate serum ADA levels with FNAC in diagnosing TB. In the present study we have calculated an ADA cut off value of 35U/L in serum for the diagnosis of TB lymphadenitis. Using this cut off value, we have found sensitivity and specificity to be 84% and 83% respectively, for tubercular lymphadenitis patients diagnosed on the basis of FNAC. The method of ADA estimation is easy, simple and does not require expensive equipment or elaborate laboratory arrangement except a simple colorimeter.

It takes only 2 hours and it is also plasma activity is high in diseases where cellular immunity is stimulated such as tuberculosis. As determination of ADA is not costly or time consuming, it should be done routinely, particularly if the diagnosis of tuberculosis is in doubt. In our study, the most significant range of adenosine deaminase levels was 31 to 40 U/L. Five cases reported as reactive lymphadenitis which were subjected to histopathological examination revealed epithelioid granulomas with spotty caseous necrosis, which otherwise showed high serum ADA level. Thus these cases were proved to be significant for further study.

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