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STUDIES

ADENOSINE DEAMINASE ACTIVITY IN SERUM AND LYMPHOCYTES OF MULTIBACILLARY LEPROSY PATIENTS

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Adenosine deaminase (ADA) activity was measured in serum and lymphocytes of 25 untreated patients with multibacillary leprosy. Both serum and lymphocyte ADA levels were significantly high in multibacillary leprosy patients compared to healthy controls. The results obtained did not establish any significant relationship between ADA activity and the type of leprosy.

Key Words : Multibacillary leprosy, Adenosine deaminase, Lymphocyte

Introduction

The spectrum of leprosy is characterised at the tuberculoid pole by development of both T and B cell immunity against Mycobacterium leprae which ultimately kills and clears the bacilli from the tissues. At the lepromatous pole patients exhibit selective T cell unresponsiveness to M leprae with multiplication of organisms in skin and nerves. The exact immunological defect leading to decreased or absent cellular immunity to M leprae in lepromatous leprosy (LL) is not fully understood.

Adenosine deaminase (ADA), an enzyme of purine metabolism, in part regulates the lymphocyte metabolism and is also important for lymphocyte differentiation and growth.[1] It is present in lymphocytes in high concentration.[2] ADA estimation has been used in study of various immune dysfunctions and its activity appears to be necessary for an effective immune response as corroborated by many studies eg, in combined immunodeficiency disease and typhoid fever.[3,4]

In the present report, ADA levels were estimated in serum and lymphocytes of untreated multibacillary leprosy patients.

Materials and Methods

Twenty five untreated multibacillary leprosy (MBL) patients taken randomly from the department of Dermatology, Venereology and Leprosy of Pt B D Sharma Post-graduate Institute of Medical Science, Rohtak and an equal number of healthy subjects, age and sex matched, which served as controls were taken for the present study. The diagnosis of leprosy was confirmed by slit skin smear and skin biopsy. The age of patients varied from 20 to 80 years (mean age 40.7 years). Out of total 25 multibacillary leprosy patients studied, 10 patients had midborderline leprosy (BB), 7 had borderline lepromatous leprosy (BL) and 8 had lepromatous leprosy (LL). Blood samples were collected for both serum ADA and lymphocyte ADA. For the estimation of lymphocyte ADA, heparinized venous blood samples (10 units/ml) were taken and lymphocytes were isolated by

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density gradient method using Ficoll Hypaque medium.[5] Lymphocytes were then homogenised and disrupted with a Potter Elvehjem homogenizer before ADA estimation. ADA activity was estimated in serum and lymphocytes according to the method of Giusti.[6]

Results

The results of ADA activity in serum and lymphocytes of 25 untreated multibacillary leprosy patients and normal healthy controls are shown in Table I. Serum ADA levels of untreated multibacillary leprosy patients were significantly higher than the control group ($p < 0.001$). Lymphocyte ADA activity followed the same pattern as the serum ADA activity ($p < 0.001$).

The results of serum ADA and lymphocyte ADA in the three different subgroups of untreated multibacillary leprosy patients are shown in Table II. It was observed that serum ADA as well as lymphocyte ADA activity progressively increased from LL to BL to BB groups. The differences between the three subgroups were not significant. However, the serum ADA levels were found to be significantly high for all the three subgroups when compared to controls (p value < 0.02, < 0.05 and < 0.05 for LL, BL and BB groups, respectively). Lymphocyte ADA levels showed a similar pattern to serum ADA levels (Table II) and were significantly high in the three subgroups of multibacillary leprosy when compared to the healthy controls (p value < 0.05, < 0.02, < 0.01 for LL, BL and BB groups, respectively).

Table I. Serum and lymphocytes ADA (mean ± SD) multibacillary leprosy patients and healthy controls

	MBL (n=25)	Contorls (n=25)	p - value
Serum-ADA (U/L)	19.18 ± 7.23	12.85 ± 2.92	<0.001
Lymphocyte-ADA ($mU/10^6$ cells)	29.6 ± 11.54	19.6 ± 4.53	<0.001

Discussion

Many hypotheses have attempted to explain the cell mediated defect(s) in lepromatous leprosy but the mechanism of unresponsiveness of lymphocytes is far from clear. For a perfect cell mediated immune response the lymphocytes should not only be present in adequate number but should also display optimal metabolic functional activity. Adenosine deaminase is an important enzyme for lymphocyte differentiation and growth.[1]

Table II. Serum and lymphocytes ADA (mean ± SD) in three different subgroups of multibacillary leprosy patients

	LL (n=8)	BL (n=7)	BB (n=10)	Controls (n=25)
Serum-ADA (U/L)	17.5 ± 5.29	19.34 ± 7.2	20.4 ± 8.86	12.85 ± 2.92
Lymphocyte-ADA ($mU/10^6$ cells)	25.4 ± 7.35	28.15 ± 7.92	35.2 ± 13.6	19.6 ± 4.53

The mean serum and lymphocyte adenosine deaminase (ADA) levels in the multibacillary leprosy group were significantly high as compared to the control group. Increase in ADA levels particularly in lymphocytes has been attributed by various workers to increased lymphocyte proliferation as a result of antigenic stimulation.[4,7] This lymphocyte proliferation in vitro can be measured by lymphocyte transformation test (LTT). Such in vitro studies of LTT have indicated that it is least in LL group and then further increasing significantly from BL to BB groups of patients.[8] However, in the present study the differences observed in the ADA activity of both serum and lymphocytes of various subgroups of multibacillary leprosy were statistically insignificant. This is contrary to the results of LTT reported by other workers along the spectrum of multibacillary leprosy.[8] Thus, the results of the present study do not indicate that ADA levels either in serum or in lymphocytes can be taken as an index of lymphocyte proliferation as suggested in some reports.[7,9] Chaudhary et al[10] have also shown that serum ADA levels may not run exactly parallel with the conventional parameters of cell mediated immunity in leprosy patients.

The results have also not established any statistically significant relationship between ADA levels of different multibacillary leprosy subgroups and their known cell mediated immunity status. This is in contrast to the report of Suribabu et al[11] where it has been shown that serum ADA levels correlated with the immune status in the patients as determined by their type of leprosy. The differences in results could be attributed to many factors such as differences in methodology and patients categorization.

Lack of any correlation between ADA levels and LTT along the spectrum of leprosy can be due to many factors. ADA is also present in monocytes which increases tremendously as monocytes mature into macrophages.[1] Estimation of lymphocyte ADA also incorporates B-lymphocyte ADA which is not at all involved in cell mediated immune response. It has been clearly stated that for correct interpretation of ADA levels with the disease states, one must take into account the isoenzymes of ADA ie, ADA-1 and ADA-2.[12] Therefore, the exact role of ADA can be assessed better by using methodologies which can isolate pure T-lymphocyte populations.

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