ADENOSINE DEAMINASE ACTIVITY IN TYPHOID FEVER

O.P. Mishra
B.L. Gupta
Z. Ali
G. Nath
L. Chandra

ABSTRACT

Serum adenosine deaminase (ADA) activity was determined in 41 patients of typhoid fever and 15 normal controls. The mean ADA activity was significantly raised in typhoid fever patients as compared to controls ($p < 0.001$). The peak enzymatic activity was observed in the first week of illness. Complicated patients had lower mean ADA activity at diagnosis as compared to uncomplicated group and they showed a rise in enzyme level during defervescence, repeated in a few cases. A significant correlation between serum ADA activity and lymphocyte percentage was found ($r = 0.4245$, $p < 0.001$). It is concluded that ADA activity in typhoid fever patients not only indicates immunity but also has a prognostic value.

Key words: *Adenosine deaminase, Typhoid.*

From the Department of Pediatrics, Biochemistry and Microbiology, Institute of Medical Science, Banaras Hindu University, Varanasi 221 005.

Reprint requests: Dr. O.P. Mishra, 6, Rashminagar Colony, P.O. Banaras Hindu University, Varanasi 221 005.

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Recently, multidrug resistant cases of typhoid fever have been reported from different parts of the country(l,4). Immunity of the host and virulence of the organism alter the clinical spectrum. Cell mediated immunity plays a major role in recovery and is often impaired and delayed in patients developing complications(5,6).

Adenosine deaminase (ADA) enzyme is required for lymphocyte proliferation and differentiation(7). Its main biological activity is detected in T-lymphocytes. Low lymphocyte ADA has been observed in diseases causing impaired immune response(8-10). Raised levels of enzyme have been found in tubercular pleural, peritoneal, pericardial fluids and cerebrospinal fluid of patients with tuberculous meningitis(ll-13). Its raised activity has also been reported in adult patients of typhoid fever by some workers(14,15).

Thus, the increasing severity of disease, impaired -Immunity and possible relationship between ADA and immune response prompted us to undertake the present study: *(i)* to estimate ADA activity in typhoid fever patients at diagnosis and normal controls; *(ii)* to observe the enzymatic activity in complicated and uncomplicated cases of typhoid fever; and *(iii)* to find out the correlation, if any, between serum ADA activity and lymphocyte percentage in study subjects.

Subjects and Methods

Forty one patients of typhoid fever in the age group of 9 months to 13 years, diagnosed by isolation of *Salmonella typhi* from blood and/or positive Widal test and 15 normal children in the same age group were selected for the study. The latter served as controls.

Blood samples were collected through

venepuncture taking aseptic measures. Serum was separated into clean, dry sterile vials, stored at -10°C and ADA activity was assayed within a week. The samples were centrifuged at 3000 rpm for 10 min and the supernatant was used for assay.

Serum ADA activity was measured spectrophotometrically (UV-1201 Shimadzu spectrophotometer) at 265 nm in an assay mixture containing 0.1 mM adenosine, 15 mM potassium phosphate buffer (pH 7.4), 1.25% glycerol and 0.05 ml of serum. The optical density of adenosine solution at this wavelength is directly proportional to the concentration of adenosine. Hence, the rate of disappearance of adenosine is taken as an index of ADA activity and is followed by the rate of decrease in optical density at 265 nm(16). One unit of ADA activity represents the deamination of one micromole of adenosine per min at 37°C and enzyme activity was expressed in IU/L.

Statistical analysis was performed by the Student's 't' test and correlation and regression coefficients were also calculated.

Results

The serum adenosine deaminase activity in 15 normal control and 41 patients of typhoid fever at diagnosis, are presented in *Table I*. The overall mean serum ADA activity in typhoid fever was significantly higher as compared to controls ($p < 0.001$). The mean serum ADA levels in patients presenting in different weeks of illness were also significantly higher in comparison to controls ($p < 0.001$). The peak level of mean serum ADA activity was observed in the first week of illness. Thereafter, there was a

TABLE I—*Serum Adenosine Deaminase (ADA) Activity in Control and Patients with Typhoid Fever at Diagnosis (Mean ± SD)*

Group	n	Serum ADA activity (IU/L)
I. Control	15	34.20 ± 10.96
II. Typhoid fever	41	181.53* ± 45.60
(*A*) 5-7 days from onset	5	193.25* ± 43.36
(*B*) 8-14 days from onset	20	184.04* ± 48.96
(*C*) 15-21 days from onset	7	179.85* ± 32.97
(*D*) >21 days from onset	9	171.77* ± 51.85

* Test of significance as compared to control: $p < 0.001$;
Significance levels (p) between: A *vs* B = NS; A *vs* C = NS; A *vs* D = NS.

fall in the enzymatic activity in patients presenting in second, third and fourth weeks of disease, but these levels did not differs significantly as compared to the value of the first week.

Typhoid fever patients were further analyzed on the basis of presence or absence of complications during the course of disease. It was observed that patients with complications showed a lower mean ADA activity as compared to uncomplicated cases but the difference was not significant *(Table II).*

The data of the 10 patients of typhoid fever, where repeat ADA activity during defervescence could be done, are depicted in *Table III.* The parameter was analyzed by separating into uncomplicated arid complicated groups (comprising of 4 cases of encephalopathy with paralytic ilcus/bronchopneumonia/petechiae and one patient of gastrointestinal tract hemorrhage). There was a significantly lower mean ADA activity at diagnosis in patients having complications in comparison to uncomplicated children ($0.01 < p < 0.02$). The enzymatic activity increased during defervescence in these cases and became comparable to that of uncomplicated patients.

The correlation between serum ADA activity and lymphocyte (%) in study subjects (15 controls + 41 typhoid fever) was significant ($r = 0.4245$, $p < 0.001$). A linear relationship *(Fig. 1)* was observed between these two parameters and regression equation was derived ($Y = 1.72 + 2.997 X$).

Discussion

Adenosine deaminase is an enzyme that catalyses the irreversible hydrolytic deamination of adenosine into inosine and ammonia. It is considered as marker of cell mediated immunity, with an increase in its level in different diseases(ll,17). The raised level of ADA activity under antigenic stimulation shows the importance of this enzyme in rapid proliferation of cells in order to prevent the accumulation of toxic metabolites(17).

In the present study, a significantly raised level of ADA activity was found in typhoid fever patients as compared to controls ($p < 0.001$). The peak enzyme activity

TABLE II— *Serum Adenosine Deaminase (ADA) Activity at Diagnosis in Uncomplicated and Complicated Patients of Typhoid Fever (Mean ± SD)*

Group	n	Serum ADA activity (IU/L)
Uncomplicated	30	185.66 ±45.88
Complicated	11	161.18* ±43.88

* p = NS.

TABLE III- *Serum Adenosine Deaminase (ADA) Activity in Uncomplicated and Complicated Patients of Typhoid Fever (Mean ± SD)*

Groups	n	Serum ADA activity (IU/L)	
		At diagnosis	At defervescence
Uncomplicated	5	205.0 ±36.99	188.4 ± 34.63
Complicated	5	137.6* ±30.64	189.2** ±72.04

*$0.01 < p < 0.02$;**p = NS.

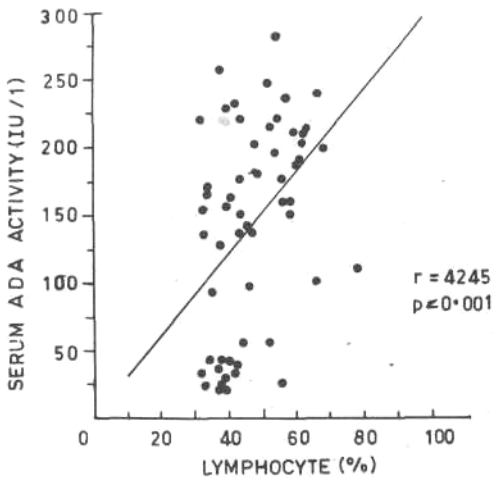

Fig. 1. Correlation between serum adenosine deaminase activity and lymphocyte percentage in study subjects.

was observed in the later part of the first week and it persisted in cases presenting in subsequent weeks also. Similar findings have also been reported by previous authors(14,18), but they observed peak level of the enzyme in the second week of illness. The raised ADA level indicates good immune response in these patients as immunity is cell mediated in typhoid fever. Cell mediated immune response to the infection, as detected by leucocyte migration inhibition test, is positive in the second week of illness(19) but the raised serum ADA level already present in the first week indicates development of immunity right from the first week of illness. Although there was fall in the enzymatic activity in subsequent weeks, it was not significant, hence, pointing towards persistence of cell mediated immunity in typhoid fever over ensuing weeks. The raised ADA activity in peripheral lymphocytes (L-ADA) in patients with typhoid fever has also been observed by some workers(14,15). However, Galanti *et* a/.(14) showed that raised L-ADA activity was detected in the second week of illness and remained elevated upto four weeks.

Further, it was found that complicated patients had a lower mean serum ADA activity at diagnosis in comparison to uncomplicated cases. The lower ADA levels in complicated patients indicate depressed immunity. This fact is further supported by the known negativity of leucocyte migration inhibition test in complicated patients of typhoid fever(19).

The analysis of 10 patients of typhoid fever, where repeat ADA levels were done during defervescence period, demonstrated that complicated patients had significantly lower mean ADA activity at diagnosis as compared to the uncomplicated group. It showed recovery in enzymatic activity after therapy during defervescence and became comparable to that of uncomplicated patients. From this observation, it is clear that recovery from typhoid fever is associated with development of cell mediated immunity and patients with complications have a poor or delayed cell mediated immunity. Kliosla *et al*.(15) have also observed similar findings in lymphocyte ADA activity in adult patients with typhoid fever. The depressed cell mediated immunity in this disease may be due to severe endotoxemia as the lipopoly-saccharide component of Gram negative bacteria is known to have a suppressive effect on T-cell activity(20,21). Within the constraints of a small sample size, this observation shows that low levels of ADA at diagnosis in typhoid fever have prognostic significance as these patients tend to develop complications and treatment should be given more energetically.

The correlation between serum ADA

activity and lymphocyte percentage in blood was significant ($r = 0.4245$; $p < 0.001$) and both the parameters had linear relationship. Baganha *et al.* (17) observed a highly significant correlation ($r = 0.612$; $p < 0.001$) between the levels of ADA activity and the percentage of CD_4 T-cells in pleural exudates of tubercular etiology and the authors concluded that ADA activity could be a new marker of cell mediated immunity.

Thus, die raised ADA values in serum of typhoid fever patients indicate cellular immunity against the bacterium. The decreased enzyme activity at diagnosis in patients showing complications reflects a depressed immune response. Therefore, its activity can also be helpful in assessing the severity of disease process.

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