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Adenosine Deaminase activity in Plasma of Children with Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Acute Lymphoblastic Leukemia is stem cell malignant disorder characterized by overproduction of Lymphoblast in Bone marrow that eventually spill into circulation. Adenosine Deaminase activity has been shown to increase in most chronic conditions especially lymphocytic proliferation and other malignancies. The objective of the study was to determine the concentration of plasma Adenosine Deaminase in children suffering from Acute Lymphoblastic Leukemia.

Methods: Adenosine Deaminase activity in plasma of ALL and normal children under 14 years of age visiting was measured. Total Leukocyte count and absolute lymphocyte count were performed in hematology laboratory of Kanti Children's Hospital.

Results: Adenosine Deaminase activity in plasma of children suffering from Acute Lymphoblastic Leukemia was higher than non- ALL children with leukocytosis and lymphocytosis and healthy Control groups without leukocytosis. The plasma ADA level was 48.85 ±2.42 IU/L in ALL patients, which was significantly higher by student's two-tailed t-test (P=0.002) than plasma ADA level in control subject (mean ADA=39.72 IU/L Std. error mean =1.27 IU/L). Increased plasma ADA level was found in non-ALL with leukocytosis and lymphocytosis and ALL patients.

Conclusions: Adenosine Deaminase activity in plasma of children suffering from Acute Lymphoblastic Leukemia was increased. Hence plasma ADA can serve as another serum biomarker in addition to other haematological markers and clinical characteristics of acute lymphoblastic leukemia (ALL). It is easy to perform in small laboratories and will be beneficial as a non interventional prognostic marker.

Key words: acute lymphoblastic leukemia, absolute lymphocyte count, adenosine deaminase.

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is stem cell malignant disorder characterized by overproduction of lymphoblast in Bone marrow that eventually spill into circulation. This mutation protrudes stem cell to produce impair normal proliferation of stem cell and loss of differentiation of beyond blast stage and reduced apoptosis death.[1,2] ALL primarily affects children and exhibits the best response to standard chemotherapy as compared to Acute Myeloblastic Leukemia (AML). ALL is most common form of childhood cancer and accounts for one-fourth of all childhood cancer and three fourth of all newly diagnosed Leukemia. Adenosine Deaminase is an enzyme of purine Salvage pathway that catalysis the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine. Adenosine Deaminase Activity (ADA) activity is found to be increased in most chronic diseases and haematological malignancies. It exist in at least three molecular forms ADA1, ADA1+cp ADA2

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in Human. Adenosine Deaminase activity in plasma of children suffering from Acute Lymphoblastic Leukemia was higher than non- ALL children in researches done in this regards by other researchers.[4-9] The study was carried out to determine the concentration of plasma Adenosine Deaminase (ADA) in Nepalese children suffering from Acute Lymphoblastic Leukemia (ALL).

METHODS

A Cross sectional, analytical with control group. Study was carried out at department of biochemistry, Kanti Children Hospital, Mahrajgunj, Kathmandu, Nepal from October 2007 to March 2008. Ethical approval from Kanti Children Hospital and children guardian's consent were taken. The subjects were taken from ward and out patient department of Kanti Children's Hospital Maharajgunj, Kathmandu, Nepal. The children suffering from Acute Lymphoblastic Leukemia diagnosed by bone marrow examination were taken as study group and serum sample from the patients were taken. The study comprises of following groups:

Control group - The subject consisted of patients without leucocytosis and lymphocytosis. The subjects had total leukocyte count - less than 11000/cumm and lymphocyte not more than 40%. (N=33);

Non- Acute Lymphoblastic Leukemia group - The subjects consisted of patient having total leukocyte count- more than 11000/cu mm and lymphocytosis. These were the patients suffering from any disease, which had leucocytosis with lymphocytosis (N=34).

Acute Lymphoblastic Leukemia group- The subject consisted of the hematologically diagnosed acute lymphoid leukemia patients (N=35).

Children below age fourteen years of age were included in the study and parents of the Children who refused to give consent to participate in the study were excluded from the study. Adenosine Deaminase activity in plasma of subjects were determined according to method based on the method of Guisti & Galanti based Burtholet reaction that is the formation of colored indophenols complex from ammonia liberated from adenosine and measured in biochemical analyzer.[3] The biochemical analyzer used was State Fax; USA made semiautomatic biochemistry analyzer. Total Leukocyte count and absolute lymphocyte count were performed in Hematology Laboratory of Kanti Children's Hospital.

The data obtained was analyzed using statistical package for social sciences version 11.5 for windows. Comparisons were done between controls and the ALL group; between the control group and the NON-ALL group; and also between the ALL group and NON-ALL group.

RESULTS

High plasma ADA level (mean= 49.19 IU/L, SD mean = 2.18 IU/L) was found in Non-ALL with leukocytosis and lymphocytosis as compared to control group (mean= 39.72 IU/L, SD error =1.27 IU/L). The plasma ADA level in Non-ALL with leukocytosis and lymphocytosis was significantly higher than control subject. There was a significant increase in plasma ADA level in children suffering from acute lymphoblastic leukemia (ALL) as compared to Normal control subject. The plasma ADA level was 48.85 ±2.42 IU/L in ALL patients, which was significantly higher by student's two-tailed t-test (P=0.002) than plasma ADA level in control subject (mean ADA=39.72 IU/L SD error mean =1.27 IU/L). Increased plasma ADA level was found in non-ALL with leukocytosis and lymphocytosis and ALL patients. Here no significant increase in non-ALL with leukocytosis and lymphocytosis (P=0.0918, Z=1.04) was found in comparison to ALL groups.

Peripheral blood total leukocyte count was lower in ALL patients (mean TLC=3308.57/cumm, SD mean=400.64/cumm) as compared to Non-ALL subjects with leukocytosis and lymphocytosis (mean TLC=12705.88/cumm, SD error=374.81/cumm). Peripheral blood total leukocyte count was found lower in ALL patient (mean= 3308.57± 400.64/ cumm) as compared to control group (mean =7836.36± 220.27/cumm). The peripheral blood total leukocyte count was found to be higher in Non-ALL patient with leukocytosis and lymphocytosis (mean TLC=12705.88/cumm, SD=374.81/cumm) in comparison to control subject (mean =7836.36/cumm, SD =220.17/cumm).

The above table shows that both plasma level of ADA and Absolute lymphocyte count are higher in Non-ALL and lower in ALL groups. However plasma ADA level in ALL is higher than control group.

DISCUSSION

We found increased ADA level in plasma of ALL patients and result were well correlated with the researches done in this regards by other researchers.[4-9] However, plasma ADA level was also raised in patients with non-ALL like leukocytosis and lymphocytosis as compared to ALL and control groups. We found that ADA activity of plasma is expressed in ALL patients and Non-ALL patients at increased level. In contrast normal children expressed low level of ADA. The mean plasma ADA activity in control children was 39.72 IU/L. ADA activity is generally much higher in ALL patients than other leukemia and control groups.[10]

Table 1. Plasma concentration of ADA and absolute lymphocyte count in control and non-ALL with leukocytosis and lymphocytosis

Marker	Types of Groups	N	Mean	SD	SD Mean
ADA	CONTROL	33	39.72	7.30	1.27
	NON-ALL	34	49.19	12.74	2.18
Absolute lymphocyte count (ALC)	CONTROL	33	2541.85	430.07	74.87
	NON-ALL	34	6287.53	1823.89	312.79

ADA (Z =3.72 P =0.000), ALC (Z=11.48, P=0.000)

The above table shows that both plasma level of ADA and higher in Non-ALL and lower in control.

Table 2. Plasma concentration of ADA and absolute lymphocyte count in control and ALL

Marker	Types of Groups	N	Mean	SD	SD Mean
ADA	CONTROL	33	39.72	7.30	1.27
	ALL	35	48.85	14.32	2.42
Absolute lymphocyte count (ALC)	CONTROL	33	2541.85	430.07	74.87
	ALL	35	1935.46	1346.71	227.64

ADA (Z=3.28, P=0.002), ALC (Z=2.47, P=0.016)

The above table shows that plasma level of ADA is higher in ALL and lowers in control. And absolute lymphocyte count (ALC) is higher in control group and lower in ALL groups.

Table 3. Total leukocyte counts in children suffering from ALL and those of control

	Types of Groups	N	Mean	SD	SD Mean
TLC	ALL	35	3308.57	2370.20	400.64
	CONTROL	33	7836.36	1265.36	220.27

Z = 11.11, P = 0.00 (TLC)

The above table shows that mean total leukocyte count is lower in ALL and higher control but it does not tell about the absolute lymphocyte count

Table 4. Total leukocyte counts Non-ALL (with leukocytosis and lymphocytosis) and Control

	Types of Groups	N	Mean	SD	SD Mean
TLC	NON-ALL	34	12705.88	2185.48	374.81
	CONTROL	33	7836.36	1265.36	220.27

Z = 9.74, P = 0.00(TLC)

The above table shows that mean blood total leukocyte count is higher in Non-ALL group and lower in control groups.

Table 5. Plasma absolute lymphocyte count and ADA Level in ALL, Non-ALL, and healthy Control

	Types of groups	N	Mean	SD	SD Mean
Absolute Lymphocyte Count (ALL)	ALL	35	1935.46	1346.71	227.64
	NON-ALL	34	6287.53	1823.89	312.79
	CONTROL	33	2541.85	430.07	74.87
	TOTAL	102	3582.33	2348.01	232.49
ADA	ALL	35	48.85	14.32	2.42
	NON-ALL	34	49.19	12.74	2.18
	CONTROL	33	39.72	7.30	1.27
	Total	102	46.01	12.57	1.24

High plasma ADA level (mean= 49.19 IU/L, SD mean = 2.18 IU/L) was found in Non-ALL in compare to control group (mean= 39.72 IU/L, SD =1.27 IU/L). This ADA level in Non-ALL was significantly higher by student's two tailed t-test (P=0.000, Z=11.29) than control subject. The high ADA level in Non-ALL patient has been reported by other workers.[7,8] Ungerer JPJ, et al has been demonstrated high ADA activity in Non-ALL patient as Hepatitis A, infectious mononucleosis pneumonia, Rheumatoid Arthritis conducted at Department of chemical pathology, University of Pretoria-0001, South Africa.

There was a significant increase in plasma ADA level in our acute lymphoblastic leukemia patients as compared to Normal control subject. The plasma ADA level was 48.85±2.42 IU/L in ALL patients, which was significantly higher by student's two-tailed t-test (P=0.002) than plasma ADA level in control subject (mean ADA=39.72 IU/L SD mean =1.27 IU/L). Other worker has also demonstrated this increased activity of plasma ADA in ALL patients as well.[4-9] Coleman MS. et al.[9] in their study of ADA activity in peripheral blood and bone marrow of pediatric ALL patients also observed that plasma ADA level was elevated in ALL patients as well as non-ALL case as compared to control group. There was also significant decrease in Absolute lymphocyte count in ALL patient (mean ALC=1935.46, SD mean=227.64) as compared to normal subjects (mean =2541.85, SD=74.87). Blood ALC were 1935.46 in ALL, which was significantly lower by student's two-tailed t-test (P<0.016) than ALC in control subject. Hatzistilianou M et al,[12] in their study of prognostic significance of ADA in children with malignancies also observed that ADA activity of plasma at onset of disease were 60.2±6.2 IU/L in compare to control group.

Increased plasma ADA level was found in Non-ALL and ALL patients. Here no significant increase in Non-ALL (P=0.0918, Z=1.04) was found in compare to ALL groups. Increased ADA level in Non-ALL patients as infectious mononucleosis.[7] conducted at Department of chemical pathology, University of Pretoria- 0001, South Africa. The absolute lymphocyte count in peripheral blood of ALL patients was 1935.46±227.64 which was significantly lower by student's two tailed t-test (P=0.000, Z=11.29) than absolute lymphocyte count in Non- ALL patient (6287.53±312.79).

Peripheral blood total leukocyte count was lower in ALL patients (mean TLC=3308.57/cumm, SD mean =400.64/Cu mm) in compare to Non-ALL subjects (mean TLC=12705.88/cumm, SD=374.81 Cu mm). Blood TLC in ALL patients was significantly lower by student's two-tailed t-test (P=0.000, Z=17.10) than TLC in Non-ALL patients.

Peripheral blood total leukocyte count was found lower in ALL children (mean= 3308.57± 400.64/ cumm) in compare to control group (mean =7836.36± 220.27/ cumm). The low TLC level in ALL patients has been demonstrated by Boggs et al. The TLC level in ALL was significantly lower by student's two tailed t-test (P=0.000, Z=11.11) than TLC in control subject.

The peripheral blood total leukocyte count was found to be higher in Non-ALL patient (mean TLC=12705.88/ Cumm, SD=374.81/cumm) in compare to control subject (mean =7836.36/cumm, std. error =220.17/cumm). This increased TLC in Non-ALL in compare to control group.[12] The peripheral TLC of ALL patients were 1200-9800/ Cumm (mean=7836.36/cumm ±220.27/cumm) which was significantly lower by student's two tailed t-test (P=0.000, Z=9.74) than peripheral TLC count in Non-ALL patients.

CONCLUSIONS

Plasma concentration of ADA is increased in Acute Lymphoblastic leukemia (ALL) and Non Acute Lymphoblastic Leukemia in comparison to control group. Increased plasma ADA level is the non-specific biomarker for ALL as it also increases in other diseases and benign condition where there is leukocytosis with lymphocytosis. Therefore use of ADA as bio-marker of ALL patients may provide to certain extent clinically useful information in prognosis. The laboratory method for measuring ADA is inexpensive, relatively simple to perform. It may thus be useful in laboratories with limited resources, especially in underdeveloped and developing countries.

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