Evaluation of serum adenosine deaminase activity in acute lymphoid leukemia in Erbil city

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Abstract

Background and objectives: Adenosine deaminase (ADA), is an enzyme that catalyzes hydrolytic deamination of either adenosine or deoxy adenosine to produce inosine and deoxy inosine respectively. The aim of the present study is to evaluate the serum ADA activity in acute lymphoid leukemia.

Method: A prospective study was carried out from January to June 2011 by the clinical biochemistry department in College of Medicine-Hawler Medical University on (30) patients with Acute lymphoid leukemia (group 1), and (30) healthy individuals, (group 2).

Results: The mean value of serum ADA activity was significantly lower in Acute lymphoid leukemia (group 1), than those of healthy individuals, (group 2) (p<0.01), and both ions Na⁺ and K⁺ acted as an activators for Serum ADA activity.

Conclusion: Based on the findings, it can be concluded that in acute lymphoid leukemia the activity of Serum ADA was decreased due to decline in the immunity.

Keywords: Serum adenosine deaminase (ADA), acute lymphoid leukemia, immunity.

Introduction

Adenosine deaminase (E.C.3.5.4.4) (ADA) is an enzyme involved in metabolism of purine through the salvage pathway, catalyzes the irreversible hydrolytic cleavage of adenosine to inosine and (deoxy) adenosine to (deoxy) inosine and production of ammonia in both nucleotides. It is widely distributed in human tissues and shows highest activity in lymphoid tissues and it is necessary for the proliferation, maturation and function of lymphocytes, specifically for T lymphocytes. Its activity increases during antigenic and mitogenic responses of lymphocytes; therefore it is considered as an important immunoenzyme marker for assessing cell mediated immunity in diseases characterized by T lymphocyte proliferation and maturation. Adenosine deaminase is a glycoprotein consisting of a single polypeptide chain of 311 amino acids. It was sequenced by Daddona et al. The primary amino-acid sequence of ADA is highly conserved across species. Studies on the crystal structure of mouse ADA showed that the protein is composed of eight stranded. The crystal structure has also revealed that ADA is a metallo –enzyme that complexes one mole of Zn²⁺ per mole of protein. A high level of ADA in sera of cancer patients, in hepatitis, cirrhosis, and infectious mononucleosis has been reported. Investigation of ascitic fluid of tuberculosis revealed a high activity of ADA enzyme. During the last decade, different disorders has been investigated and they revealed elevation in ADA activity in leprosy patients and in secondary liver tumors. In a study conducted by Hasan G.H. etal, who indicated that synovial fluid ADA activity increased in patients with rheumatoid arthritis and reactive arthritis, Sary et al, study showed that serum total ADA and ADA-2 activity is closely associated with rheumatoid arthritis and these non-invasive investigations can be used as biochemical markers for inflammation status.

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These provide additional information about the regarding disease activity along with the traditional indices such as ESR and C-reactive protein (CRP). In a recent study, the highest value of ADA activity was observed in synovial fluid of patients with tubercular arthritis followed by rheumatoid septic, osteo and post traumatic arthritis. Finally deficiency of ADA activity was observed in severe combined immune deficiency disorders. Acute leukemia is a malignancy of the hematopoitic progenitor cell. The malignant cell loses its ability to maturation and differentiation. These cells proliferate in an uncontrolled fashion and replace normal bone marrow elements. Most cases arise with no clear cause. However, radiation and some toxicant (benzene) are leukemogenic. In addition, a number of chemotherapeutic agents (especially procarbazine, melphalan, other alkylating agents, and etoposide) may cause leukemia. Most of the clinical findings in acute leukemia are due to replacement of normal bone marrow elements by the malignant cells. Acute lymphoblastic leukemia (ALL) comprises 80% of the acute leukemias of childhood. It is also seen in adults causing approximately 20% of adult acute leukemias. Acute myelogenous leukemia (AML) is chiefly an adult disease with a median age at presentation of 60 years and an increasing incidence with advanced age. This study aimed to investigate serum ADA activity in acute leukemia in order to evaluate its role in the diagnosis of acute lymphoid leukemia.

Method

A-SUBJECTS: This study was conducted over a period of six months, from January to July 2011. 30 patients with acute lymphoid leukemia (group 1), were included in the study (15 males & 15 females) with the mean ages (24.26±7.8), and (30) healthy volunteers, (15 males & 15 females), (group 2), mean ages (23.15±6.5), and. All the cases in both groups (1&2), were non smokers and non alcohol drinkers.

B-SERUM SAMPLING: Four to six mls of venous blood was withdrawn from each individual using disposable syringes. The samples were immediately centrifuged for [10] min at 3000 rpm, and the serum was obtained and analyzed directly.

C-METHOD

Estimation of Serum ADA activity:- Serum ADA activity was determined for the two groups according to the method which was described by (Giusti, and Galanti, 1968).17

Principle of the Methodology:- Adenosine deaminase is easily assayed by measuring the amount of ammonia formed during its incubation for 60 minutes at (37°C). Ammonia reacts with sodium hypochlorite at (pH6.5), in the presence of phenol-nitroprusside as a catalyst, producing blue indophenols. The ammonia concentration is directly proportional to the intensity (measured at 630nm) of the color of indophenols produced.

Reagents:

2. Phosphate buffer solution (PH 6.5).
3. Stock solution of ammonium sulphate: dissolving (0.4955) g of ammonium sulphate in 250 ml of D.W.
4. Standard solution of ammonium sulphate: prepared freshly by diluting 0.5 ml of stock solution to 100ml with D.W.
5. Phenol nitroprusside solution: (10) g of phenol mixed with (50) mg of sodium nitroprusside then dissolved in (1) liter with D.W.
6. Hypochlorite solution: (125) ml of (1 N) NaOH mixed with (16.9) ml NaOCl solution then mixture diluted to 1 liter with D.W.
7. Adenosine solution: preparation of (0.25) Mm of adenosine as a substrate for ADA enzymatic reaction. Substrate solution was dissolved in phosphate buffer solution (PH 6.5) (reagent No. 2).
Method:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Test</th>
<th>Control</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer solution (pH6.5).</td>
<td>------</td>
<td>--------</td>
<td>--------</td>
<td>1ml</td>
</tr>
<tr>
<td>Substrate</td>
<td>1ml</td>
<td>1ml</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>Standard</td>
<td>------</td>
<td>--------</td>
<td>1ml</td>
<td>------</td>
</tr>
<tr>
<td>Serum sample</td>
<td>50uL</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>DW</td>
<td>------</td>
<td>50uL</td>
<td>50uL</td>
<td>50uL</td>
</tr>
</tbody>
</table>

Mix well, then incubate for 60 minutes in water bath at 37°C.

Phenol-nitroprusside. 3ml 3ml 3ml 3ml

Hypochlorite 3ml 3ml 3ml 3ml

Mixed well, and then incubate for 30 minutes in water bath at 37°C. Read the absorbance at 630nm for all duplicated tubes against DW.

Calculation:
Adenosine deaminase activity was calculated using the following equation:
Activity of ADA in IU/L = (Atest-Acontrol/ Astandard-Ablank) ×50* (50*): is the concentration of working standard solution of ammonium sulphate.

D-Effect of Na⁺ & K⁺ ions on ADA activity: The same protocols of the enzyme activity determination as shown in section (C) were implemented by the addition of (0.1 ml, of 0.5mM), of each of the ions (Na⁺ and K⁺), from the salts NaCl &KCl respectively.

E-STATISTICAL ANALYSIS:
The statistical evaluation of the results [mean, standard deviation(S, D) and standard error of mean (S.E.M.)] were calculated using the scientific calculator [prop, 4h, 105].

The different variables were compared to each other, simple correlations were tested with the unpaired student test [T-Test]. Only P values of <0.05 were regarded as statistically significant. 18

Results

Table (1) illustrate the mean S.ADA activity in the acute lymphoid leukemia and normal groups. The results obtained indicated that the mean S.ADA activity was (5.38±1.72 IU/L) (Mean±S.D), in acute lymphoid leukemia group. This value was significantly higher than that obtained in normal group (10.26±2.48IU/L) (p<0.01).

Table 1: The Mean±S.D of S. ADA activity in normal and acute lymphoid leukemia groups:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sex</th>
<th>No.</th>
<th>S.ADA(IU/L) (Mean±S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoid leukemia (Group 1)</td>
<td>Males</td>
<td>15</td>
<td>5.71±2.4</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>15</td>
<td>4.95±2.6</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>30</td>
<td>5.38±1.72</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>15</td>
<td>10.82±3.5</td>
</tr>
<tr>
<td>Normal (Group 2)</td>
<td>Females</td>
<td>15</td>
<td>9.44±4.53</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>30</td>
<td>10.24±2.48</td>
</tr>
</tbody>
</table>

S. ADA of acute lymphoid leukemia. V Normal Z=16.5 P<0.01
S. ADA of Normal (Females V Males) Z=0.135 P>0.01 NS
S. ADA of acute lymphoid leukemia (Males V Females) Z=0.483 P>0.01 NS
V (versus). S (serum).
Tables (2 and 3), shows the effect of (0.1ml of 0.5mM, Na⁺ and K⁺) ions, as ions effect on S.ADA activity, the results showed that both ions increased the activity of S.ADA at different percentages in both groups.

**Table 2:** The effect of (0.5mM) Na⁺ ions on S.ADA activity:

<table>
<thead>
<tr>
<th>Groups</th>
<th>S.ADA activity with (0.1ml of 0.5mMNa⁺)</th>
<th>S.ADA activity without (0.1ml of 0.5mM Na⁺)</th>
<th>%stimulation activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoid leukemia</td>
<td>8.2±2.18</td>
<td>5.38±1.72</td>
<td>35.01%</td>
</tr>
<tr>
<td>(Group 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>15.24±3.48</td>
<td>10.24±2.48</td>
<td>33%</td>
</tr>
<tr>
<td>(Group 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** The effect of (0.5mM) K⁺ ions on S.ADA activity:

<table>
<thead>
<tr>
<th>Groups</th>
<th>S.ADA activity with (0.1 ml of 0.5mMk⁺)</th>
<th>S.ADA activity without (0.1 ml of 0.5mMk⁺)</th>
<th>%stimulation activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphoid leukemia</td>
<td>8.9±2.18</td>
<td>5.38±1.72</td>
<td>39.51%</td>
</tr>
<tr>
<td>(Group 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>16.34±3.58</td>
<td>10.24±2.48</td>
<td>37.2%</td>
</tr>
<tr>
<td>(Group 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The mean activity of serum ADA in patients with acute lymphoid leukemia was significantly lower than that of healthy individuals (p< 0.001). Our results are in agreement with those obtained by John Zimmer, A. Samy Khalifa and James J. Lightbody, BS Michell, et al, and Robertson Parkman et al. However, it was in disagreement with WG Yasmineh, who indicated that adenosine deaminase is highly important for lymphocyte proliferation in children with ADA deficiency. Adenosine deaminase is an enzyme present in most mammalian tissues with highest activity in organs containing many lymphoid cells. Current interest in this enzyme has been stimulated by the finding that patients with inherited deficiency of ADA have a combined immunodeficiency. Two ions (Na⁺ and K⁺) were used in this study, the results showed that they increased the activity of ADA, this by (AL-Taii. 2006).

1. Binding to enzyme active site of 5'NT.
2. Facilitate the binding of substrate to enzyme.
3. Altering the equilibrium constant of the enzymatic reaction.
4. Be a part of enzyme components and thus bind to side groups. Studying the effects of metal ions could provide a normal approach to studying Cation-Cation interactions. In addition, it may permit the inclusion of substrate imbricate between the Cations supramolecular structure. Furthermore, it may display successive binding of different substrate yielding “cascade” type complexes. Recently, both in vivo and in vitro specific proteins was found to bind metal ions, as it has been suggested that metal ions could influence the structural stability and, potentially the transition to the infectious scrapic form.
Conclusions

From the results of this study, one can con-\-
clude:
1-The mean serum activity of ADA, in\-
acute lymphoid leukemia significantly is\-
lower than that of healthy individu-\-
als. Hence, the measurement of serum\-
activity of ADA may be used as\-
biomarkers in the diagnosis of acute\-
lymphoid leukemia.
2- ADA activity is related to the immunity\-
status, more studies are required to\-
confirm this theory.

References

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