Estimation of Adenosine Deaminase in Adult Iraqi Patients with β-Thalassemia

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Abstract

Beta-thalassemia is a well-known hereditary hemoglobinopathy. Patients with major and intermedia thalassemia require periodic blood transfusions. The diagnosis of this disease involves the investigation of several biochemical and hematological markers. Adenosine deaminase (ADA) has received much attention due to its role in purine catabolism, and in this work the activity of ADA was estimated among two patient groups of thalassemia (major and intermedia) and control group. A significant increase was found in studied groups as compared with control group. The levels of Iron, ferritin, transferrin, ALT and AST were also estimated in the current study. It was found that the levels of these parameters were correlated with the activity of ADA.

Keywords: Beta-thalassemia; Adenosine deaminase; ALT; AST

Introduction

Thalassemia is a congenital hemolytic disorder caused by a partial or complete deficiency of α or β-globin chain synthesis. Homozygous carriers of β-globin gene defects suffer from severe anemia and other serious complications from early childhood.

The disease is treated by chronic blood transfusion. However, this can cause severe iron overload resulting in progressive organ failure. Despite the difficulties associated with treatment, standards of care for thalassemic patients have improved in recent years, resulting in almost doubling of the average life expectancy (1).

The clinical signs of β-thalassemia include cardiac failure and arrhythmia, changes in liver, gall bladder and spleen, the skull and other bones may be deformed, in addition, pallor skin from anemia and jaundice (2). Iron overload is the most important complication of β-thalassemia and is a major focus of management (3).

Adenosine Deaminase (ADA) is an enzyme which is involved in purine catabolism and is responsible for the conversion of adenosine deoxyadenosine to inosine, deoxynosine respectively and ammonia. Further metabolism of these deaminated nucleosides leads to hypoxanthine and finally to uric acid (4). ADA presents in all cell types, however, the amount of enzyme differs widely among tissues. The highest ADA levels in humans are found in lymphoid tissues (5). It has an important role in development of human immune system (6).

Adenosine deaminase is observed to bind to the cell surface of T-lymphocytes through the activation marker CD26, Which is also known as dipeptidyl peptidase IV or ADA binding protein (7). In addition to adenosine breakdown, ADA stimulates release of excitatory amino acids and is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins (8).

The gene for ADA is located on chromosome 20q13.12 (9). Some mutations in this gene lead to ADA deficiency, which is one of causes of severe combined immunodeficiency (SCID) (10).
Adenosine deaminase deficiency leads to pulmonary fibrosis, suggesting that chronic exposure to high levels of adenosine can worsen inflammation responses instead of suppressing them (11). Deficient levels of ADA have also been associated with pulmonary inflammation, thymic cell death, and defective T-cell receptor signaling (12). Also mutations causing this enzyme to be elevated are one cause of hemolytic anemia (13). High levels of ADA associated with AIDS has also been reported (14,15). In this study we estimated the levels of ADA and examined the relationship between this enzyme and other related biomarker in two groups of β-thalassemia patients and compared the results with control group.

Materials and Methods

The samples were collected from Ibn- Balady Hospital during the Period from November 2015 to March 2016. They have been classified as the following:

- Group one: β-Thalassemia Major (TM) as pathological they were diagnosed by specialist physicians depending on the laboratory analysis of blood film, Hb-electrophoresis and biochemical iron study, Included 73 patients their ages range 18-30 years with a mean age 22.99 years.
- Group two: β-Thalassemia Intermedia (TI) as pathological they also were diagnosed by specialist physicians depending on the same laboratory analysis that used in diagnosing of β-Thalassemia Major but they are less than Thalassemia Major in their needing to blood transfer. This group included 20 patients their ages range 18-30 years with a mean age 23.6 years.
- Group three: Control group included, their ages range 18-27 years with a mean age 22.93 years no previous disease which may interfere with the parameters analyzed in this study.

The activity of ADA in patients and control determined by the method described by Giusti (16), the method depends on following reaction:

\[
\text{Adenosine + H}_2\text{O} \xrightarrow{\text{ADA}} \text{Inosine + NH}_3
\]

So, the ammonia is released from the hydrolysis of adenosine by ADA. The ammonia converted to blue indophenols in the presence of sodium hypochlorite with phenol in alkaline medium. The absorbance was read at 628 nm at 37 °C. The catalytic factor in the reaction is sodium nitroprusside and the reaction stops by adding phenol nitroprusside solution. All the solutions were prepared immediately (in the day of use) to prevent any interpenetration in the results because of ammonia losing.

The levels of ALT, AST and Iron have been determined spectrophotometrically by using Randux kits. Ferritin levels had been examined according to one step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). This method had been done by minividas device as a one instrument of BioMeriux Company. All of the assay steps were performed automatically by the instrument. Transferrin was determined by radial immunodiffusion method (RAD), in which transferrin protein diffuses in agarose gel containing transferrin antibody and will form an immuno-complex, which appears as a visible ring around the well. The ring diameter is directly proportional to the concentration of transferrin after diffusion time (72 hours).

Results and Discussion

Adenosine deaminase is an enzyme involved in purine salvage. The determination of ADA may show the reflection of purine accumulation or affected gene that is responsible of ADA production (17).

Table (1) and figure (1) show the result of ADA activity level in study groups. These results show high significant increase in the
two patients groups (TM and TI) when compared with control.

Hereditary hemolytic anemia with increased red cell adenosine deaminase has been reported by Valentine et al. (18) who found the activity of ADA significantly increased. They have attributed this to defect in feedback regulation concerned with induction and suppression of ADA synthesis in the nucleated red cell precursor. Glader et al (19) have been reported also elevated red cell ADA activity in patients of Diamond-Blackfan anemia (DBA) and other hematologic diseases. They have suggested that this disorder is caused by perturbation of normal erythropoiesis might be as a result of the defect of erythroid stem cell differentiation. Our results may be agreed with the previous explanations.

Table 1: Adenosine deaminase activity in serum of TM, TI and control

<table>
<thead>
<tr>
<th>Variables</th>
<th>Statistics</th>
<th>Major</th>
<th>Intermediate</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>Mean± SD</td>
<td>70.107±24.814</td>
<td>59.688±22.759</td>
<td>22.344±10.95</td>
</tr>
<tr>
<td>ANOVA</td>
<td>&lt;0.001</td>
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On other hand, the results of iron and ferritin levels for the two patients groups showed highly significant increase as compared with control group, while transferrin levels are significantly decreased for the two patients groups as compared with control, as shown in table (2).

Iron is one of the most abundant element, which has much important roles, in human body (20). Periodic blood transfusion as a treatment of thalassemia often leads to accumulation of excess iron may be an acquired condition. It is well known that blood transfusion provides the body with approximately 250 mg of iron, while the body cannot excrete more than 1 mg/day of iron typically added to the body’s stores (20). The cirrhosis of liver is associated with increase in serum ferritin levels. However, as in primary iron overload, the majority of morbidity and mortality ultimately results from progressive heart and liver failure (21).

Serum ferritin protein is an acute phase reactant, rising with any inflammation process from infection through chronic disease. To determine whether a high serum ferritin protein is due to iron overload or inflammation, it is also necessary to determine serum iron and transferrin (22). Iron is one of the most abundant element, which has much important roles, in human body (23).
Transferrin has a much longer half life in plasma than iron and shows short term of fluctuation (24). Consequently, it can be said that the high levels of ferritin accompanied with high level of serum iron and the low level of transferrin in two patients groups compared with control may be an evidence for iron overload in these patients.

Table 2: Iron Study in serum of TM, TI and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Statistics</th>
<th>Major</th>
<th>Intermedia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Mean± SD (µg/dl)</td>
<td>218.94±132.33</td>
<td>203.54±112.49</td>
<td>90.88±33.6</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>&lt;0.001</td>
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<tr>
<td>Ferritin</td>
<td>Mean± SD (ng/dL)</td>
<td>3943.99±2793.1</td>
<td>1800.6±1985.73</td>
<td>117.19±57.64</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>&lt;0.001</td>
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<tr>
<td>Transferrin</td>
<td>Mean± SD (mg/dl)</td>
<td>208.28±53.92</td>
<td>191.55±46.9</td>
<td>330.65±52.5</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>&lt;0.001</td>
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The results of ferritin are show positive correlation with ADA levels ($p < 0.01$), so as mentioned previously the, the elevation of ferritin and iron cause iron overload. Depending on these result, the activity of ADA may be elevated due the damage of the organs that caused by iron overload. The levels of ALT and AST had been determined as shown in table (3). The results show high significant increase in the two patients groups compared with control. Liver fibrosis and cirrhosis are well known complications of thalassemia so they lead to elevate this enzyme because of iron overload (25). Abnormal elevated of ALT activities may arise as a result of iron overload which is a common blood transfusional symptom in thalassemia as reported by Li et al (26).

Table 3: AST and ALT activity in Serum of TM, TI and control groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Statistics</th>
<th>Major</th>
<th>Intermedia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>Mean± SD (U/l)</td>
<td>38.90±28.1455</td>
<td>32.03±18.7620</td>
<td>13.20±3.4323</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>&lt;0.001</td>
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<tr>
<td>ALT</td>
<td>Mean± SD (U/l)</td>
<td>50.37±45.5082</td>
<td>29.11±19.1569</td>
<td>18.43±4.3600</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>&lt;0.001</td>
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Also positive correlation had been founded between liver enzyme and ferritin in patients groups. So it can be said: the elevation of ADA levels in thalassemia patients it caused by iron overload and the positive correlation between ADA and ferritin enhance this explanation. On other hand, the positive correlation between ADA and liver enzymes(ALT and AST) is reveal the elevation of ADA happened because of the damage of organs (especially the liver) because of iron overload.

References


18 Valentine WN, Paglia DE, Tartaglia AP, Gilsanz F. "Hereditary hemolytic anemia with increased red cell adenosine deaminase (45-to 70-fold) and decreased adenosine triphosphate." Science 195.4280 (1977): 783-785.


