Determination of Adenosine Deaminase Activity in type 1 and type 2 Diabetes Mellitus

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Abstract
Serum adenosine deaminase (ADA) activity was determined in 30 blood sample of type 1 diabetic individuals 30 blood sample for the type 2 and 15 normal children as a control for type 1 15 normal adults as control for type 2. The mean ADA activity and specific activity in type 1 was (8.85± 5.55 U/mg of protein) which is compared with control (32.11± 1.54 U/mg of protein) while in type 2 was (48.46±11.91 U/mg of protein) is compared with control (5.18± 2.27 U/mg of protein ). We conclude that the altered blood level of ADA activity may help in predicting immunological dysfunction in diabetic individuals and also has a prognostic value.

Introduction
Diabetes mellitus is a group of devastating metabolic disease caused by insufficient insulin synthesis, increased insulin destruction or in effective insulin action. All of its metabolic effect result when the body’s cells fail to acquire glucose from the blood. The metabolic imbalances that occur have serious, but not life-threatening, consequences (Figure 1). In insulin dependent diabetes mellitus (IDDM), also called type 1 diabetes, inadequate amount of insulin are secreted because the B-cells of the pancreas were destroyed. Because IDDM usually occurs before the age of 20, it has (until recently) been referred to as juvenile-onset diabetes. Non insulin dependent diabetes mellitus (NIDDM), also called type 2 or adult-onset, is caused by the insensitivity of target tissues to insulin. Although these forms of diabetes share some features, they differ significantly in other. The most obvious symptom of diabetes in hyperglycemia (high blood glucose levels), is caused by adequate cellular uptake of glucose. Because the kidneys capacity to reabsorb glucose from the urinary filtrate is limited, glucose appears in the urine (glucosuria). Glucosuria results in osmotic diuresis, a process in which an excessive loss of water and electrolytes (\(\text{Na}^+\), K, and \(\text{Cl}^-\)) is caused by the presence of solute in the filtrate. With out insulin to regulate level metabolism, its three principal target tissues (liver, adipose tissue, and muscle) fail to absorb nutrient appropriately. Instead, there tissues function as if the body were undergoing starvation.

Insulin-Dependent diabetes
In most cases of insulin-dependent diabetes the insulin produced B-cells have been destroyed by the immune system. Although the symptom of IDDM often manifest themselves abruptly, it now appears that B-cell destruction is caused by an inflammatory process over several years. The symptom are not obvious until virtually all insulin producing capacity is destroyed. As in other inflammatory and autoimmune processes, B-cell destruction is initiated when an antibody bind to cell surface antigen. Auto antibodies to insulin and the tyrosine phosphotase, IA-2 have been detected. The most acute symptom of type 1 diabetes is ketoacidosis. Elevated concentration of ketones in the blood and low blood PH a long with hyperglycemia cause excessive water losses, ketoacidoses and dehydration, it left untreated, can lead to coma and death. Certain HLA antigens are found in a large majority of type 1 diabetes.
Non-insulin-dependent diabetes

Non-insulin-dependent diabetes is a milder disease than the insulin dependent form. Its onset is slow, often occurring after the age of 40. Individual with type 2 diabetes have normal or often elevated blood levels of insulin. Type 2 patients are resistant to insulin. The most common cause of insulin resistance is the down-regulation of insulin receptor. Approximately 85% of type 2 diabetics are obese. Treatment of NIDDM usually consists of diet control and exercise. In some cases oral hypoglycemic drugs are used. When the failure of type 2 diabetic patients to control hyperglycemia is accompanied by other medical condition (e.g. infection) a serious metabolic state referred to as hyperosmolar hyperglycemic nonketosis (HHNK) can result. Because of the additional metabolic stress, insulin resistance is exacerbated, and blood glucose level would rise.[1]

Adenosine deaminase (ADA), as an enzyme that is involved in nucleic acid metabolism [2]. Its main biological activity is defected in T lymphocyte function[3]. So it was considered as good marker of cell mediated immunity [4], and it has a crucial role in lymphocyte proliferation and differentiation [5]. It has been reported that adenosine deaminase is a good marker for insulin function [6,7]. But its connection with immune system was not yet established in diabetic subject. Even though there are some reports available on ADA levels in diabetic subject there are all inconclusive and controversial [8]. We have undertaken a preliminary study to determine its blood activity and to highlight its role in type 1 and type 2 diabetes mellitus.

Material and Methods

There were 30 blood samples for adult patients form (both sex) who had a history of not less than six years of diabetes mellitus (samples were collected from AL-Yarmuk hospital). They were aged 20 to 50 years. All of them were in the category of type 2 diabetes mellitus. None of subject have a history of infection or other factor (like drugs) at the time of the study. And there were 30 blood samples of patients with type 1 diabetes mellitus and there were in range of 5 to 16 years (samples were collected from AL-Yarmuk hospital). Non of the subjects have a history of infection or other element at the time of study. A group of 15 healthy adult individuals were served as control and 15 healthy children served as control for type 1.

ADA activity was determined according to the Giusti method (9). The total activity was defined as the amount of enzyme required to release 1 mol of ammonia per minute from adenosine at standard assay condition and it was expressed as U/ml. The specific activity was expressed as U/mg of protein. The statistical analysis was performed by using T-test to compare the mean value of ADA in patients with control.

Results

Adenosine deaminase activity was significantly (p<0.01) decreased in patients with type 1 symptom, it was (8.85±5.55 U/mg of protein) as compared with control (32.11 ± 1.54 U/mg of protein). While specific activity in patients (2.01±1.25 U/mg of protein) as compared with control (9.44 ± 0.78 U/mg of protein). The results in patient with type 2 show a significant increased level in ADA activity, it was (48.46 ± 16.91 U/mg of protein) in patient as compared with control (5.18 ± 2.27 U/mg of protein). While the specific activity levels in patients were (2.01±1.26 U/mg of protein) as compared with control which were (9.44 ± 0.78 U/mg of protein). The activity and specific activity of patients and control for type 1 and type 2 were shown in table 1.

Discussion

Enzyme is useful in modern medical practice for several reasons. Enzyme assays provide important information concerning the presence and severity of disease. In addition, enzyme often provide a means of monitoring patients response to therapy. Genetic
predisposition to a certain disease may also be determined by measuring specific enzyme activities [1].

Adenosine deaminase is an enzyme necessary for the normal catabolism of purine. ADA catalyses the conversion of adenosine and deoxyadenosine to inosine and deoxyinosin. Experimental evidence indicates that adenosine, in increased amount, may result in increased cAMP activity, which is known to be associated with the inhibition of lymphocyte function so immunodeficiency may result as consequences of ADA defect [10]. In this study we observe that there were significant decreases in the ADA levels in type 1 individuals while there were elevated levels of ADA activity in type 2 individuals. In regard to type 2 our finding is similar to result reported by [6,11]. The decreased level of ADA activity in type 1 may result from the detect in the action of insulin that is required for the function of lymphocyte. It is also thought that in diabetic individuals, insulin may be a good target for killing by antibody-dependent cellular cytotoxicity response [12], that has control over T-lymphocyte function [8]. The increased level of ADA in type 2 may relate to the elevated level of insulin in blood, insulin has a modulating action on immune response [13]. So according to this study we can conclude that there were immunological disturbances associated with this disease, and the altered level of ADA may help in establishing this enzyme as a good marker for assessing CMI in diabetes individuals. However, this study has a few limitation, further studies of ADA in lymphocyte DM individuals is required.

References
4- Bayhanha, M.F.; Pego, A. Lima, M.A.etal.(1990) Serum and pleural adenosine deaminase correlation with lymphocyte population. Chest ; 87: 605-610.
Table (1): Adenosine deaminase activity in serum of diabetes patients type 1 and type 2.

<table>
<thead>
<tr>
<th>Age (both sex)</th>
<th>Number</th>
<th>ADA activity U/mg of protein</th>
<th>Specific activity U/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-13 control</td>
<td>15</td>
<td>32.11 ± 1.54</td>
<td>9.44 ± 0.78</td>
</tr>
<tr>
<td>5-13 patients</td>
<td>30</td>
<td>8.85 ± 5.55*</td>
<td>2.01 ± 1.26*</td>
</tr>
<tr>
<td>20-50 control</td>
<td>15</td>
<td>5.18 ± 2.27</td>
<td>7.66 ± 0.48</td>
</tr>
<tr>
<td>20-50 patients</td>
<td>30</td>
<td>48.46 ± 11.91*</td>
<td>46.19 ± 16.55*</td>
</tr>
</tbody>
</table>

*Significant (p<0.01)

Insulin deficiency
decreased secretion
resistance, or both

Increased hepatic
gluconeogenesis

Increased hepatic ketogenesis

Decreased glucose
Utilization

Decreased ketone
utilization

Increased hepatic
glycogenolysis

Increased lipolysis

Hyperglycemia

Hyperketonemia

Osmotic Diuresis
Excessive loss
Of H2O, K, Na and Cl

Ketoacidosis

Dehydration

Fig. (1) The metabolic consequences of insulin deficiency or resistance
Fig. (2) Activity and specific activity for patients and controls
قياس فعالية انظيم ادينوسين دي امينيز في مرضى السكري من النوع الثاني

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الخلاصة
قيست فعالية انظيم الادينوسينرى لمجموعة العدد من الأدمينين (ADA) في مرضى السكر النوع الأول (IDDM) والثاني (NIDDM) وذلك بأخذ عينات دم من ثلاثين مصاباً بالنوع الواحد وثلاثين مصاباً بالنوع الثاني. ووريت هذه العينات مع عينات الأشخاص السليمين لكل نوع. وقد أظهرت النتائج وجود ارتفاع كبير في فعالية الادينوسين في مرضى النوع الثاني مقارنة بالسيطرة، في حين وجد انخفاض معنوي في عينات النوع الأول مقارنة بالسيطرة. هدفت الدراسة إلى تحديد فعالية هذا الادينوسين لدى مرضى السكر حيث أن للＡＤＡ دور رئيس في انقسام وتمايز الخلايا. تأتي التي هي أساس المناعة الخلوية. كما أن الانسولين يعد محوراً للفعالية المناعية. لذا حددنا هذا النوع من الأمراض كهدفاً لدراسة فعالية انظيم ADA. ومن خلال النتائج يمكننا الاستنتاج أن هناك اختلافاً في مستوى فعالية ADA في كل من الأشخاص المصابين بالنوعين الأول والثاني مقارنة بالسيطرة. أن هذا الاختلاف قد يعد مؤشراً فعالاً لحصول اطراب مناعي لدى هؤلاء المرضى.