Altered Adenosine Deaminase Activity in Type 2 Diabetes Mellitus

M Shiva Prakash*, S Chennaiah**, YSR Murthy***, E Anjaiah***, S Ananda Rao****, C Suresh*****

Abstract

Background: It is widely reported that immunological imbalance is one of the key factors associated with the metabolic disturbances in type 2 diabetes mellitus. The present study is an attempt to link adenosine deaminase as a marker of altered immune function in diabetes mellitus.

AIMS: To determine the activity of adenosine deaminase and describe its immunological significance in type 2 diabetic individuals.

Settings and design: Case-control study.

Materials and methods: A group of thirty-six adult patients of either sex who had a history of not less than six years of diabetes mellitus and equal number of healthy non-diabetics were selected as subjects and controls respectively. Blood samples were collected and biochemical parameters including fasting glucose and adenosine deaminase were determined.

Statistical analysis: Analysis was carried out using student t-test.

Results: A significant (p < 0.001) increase in adenosine deaminase activity was observed with a mean (± SD) 37.2 ± 9.29 U/l in diabetic subjects when compared to controls who had normal mean (± SD) values of 18.2 ± 5.6 U/l. All other biochemical parameters except glucose levels are normal in both the groups.

Conclusions: Our study hypothesises that increased ADA activity may be due to altered immunity. Therefore, ADA may serve as an immunoenzyme marker in the aetio-pathology of type 2 diabetes mellitus.

Key words: Type 2 diabetes mellitus, Adenosine deaminase, Cell-mediated immunity.

Introduction

Immunological disturbances in type 2 diabetic individuals have an association with cell mediated responses; and inappropriate T-lymphocyte function, which is vital in this pathogenic condition, has a link with insulin defect.

Adenosine deaminase, an enzyme distributed in the human tissues, was considered as good marker of cell mediated immunity. It plays a crucial role in lymphocyte proliferation and differentiation, and shows its highest activity in T-lymphocytes.

Previously, adenosine deaminase has been reported to be a marker for insulin function. But its connection with the immune system was not yet established in diabetic subjects. Even though there are some reports available on adenosine deaminase levels in diabetic subjects, these are all inconclusive and controversial.

Since a relationship exists between adenosine deaminase and cell mediated immunity, we have undertaken a preliminary study to determine its plasma activity and highlight its importance in the immunopathogenesis of type 2 diabetes mellitus.

Materials and methods

Study Design

This preliminary case-control study was conducted during the year 2005 and the screening of subjects was done as per the guidelines recommended by the expert committee on the diagnosis and classification of diabetes mellitus. We selected a group of thirty-six adult patients of either sex who had a history of not less than six years of diabetes mellitus. They were in the range of 30 to 50 years with body-mass index (BMI) ranging between 20 and 25 (kg/m²). All the subjects were in the category of type 2 diabetes mellitus. None of the subjects were on insulin treatment, nor did they have a history of infection or other ailments at the time of the study. A group of

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thirty-six age and sex matched healthy individuals with no history of diabetes served as controls. That the selection procedure was confirmed only after performing a fasting plasma glucose test and then distinguishing the subjects into diabetic and non-diabetic (controls). The associated complications of diabetes mellitus were ruled out by performing different biochemical parameters for the function of liver, kidney, and lipid profile for hypercholesterolaemia. The cut-off value used for fasting plasma glucose concentration was 126 mg/dl.

Biochemical analysis
About 2 ml of fasting blood was collected for the determination of different biochemical parameters. The blood was drawn by venipuncture and collected into clean test tubes with ethylenediaminetetraacetic acid (EDTA). The blood samples were subjected to centrifugation at 3,000 rpm for 10 min for separation of plasma. The plasma thus obtained was analysed for biochemical parameters such as glucose, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, SGOT, SGPT, creatinine, total proteins and gamma glutamyl transferase. These were measured with the kits provided by Crest Biosystems, Goa, India.

Determination of adenosine deaminase (ADA)
The ADA levels were estimated using a commercially available kit (Tulip Diagnostics Private Limited, Goa, India). This procedure is based on the method reported by Giusti and Galanti. Adenosine deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue coloured indophenol complex, with sodium nitroprusside acting as a catalyst. The intensity of blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample. The absorbance was read against water at 635 nm using a spectrophotometer. (Unicam, Helios Gamma, England). One unit of ADA is defined as the amount of enzyme required to release 1 mol of ammonia per minute from adenosine at standard assay conditions.

Statistical analysis
The statistical analysis was performed using students’ t-test to compare mean values of ADA in patients with and without type 2 diabetes mellitus and verify the assumption of homogeneity of variances.

Results
The base line characteristics of the patient and control group are shown in Table I. The mean age of both the controls and subjects were 43.2 ± 6.2 and 44.6 ± 5.4 respectively. Body mass index was also almost similar in the both the groups with the values greater than 24 and less than 25. The blood pressure was normal in the controls and subjects. All other biochemical parameters like cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, SGOT, SGPT, creatinine, total proteins and gamma glutamyl transferase was significantly higher (p < 0.001) in the diabetic subjects with an average increase of at least 34 U/lit when compared with controls. Similarly, fasting glucose levels were normal in controls and significantly (p < 0.001) higher in the diabetic subjects (Table III). Further, adenosine deaminase levels were significantly (p < 0.001) higher in the diabetic group with an average value of 37.2 ± 5.2 when compared with the control subjects (18.2 ± 5.8).

Table I: Baseline characteristics of the participants.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td></td>
<td>Controls (n = 36)</td>
</tr>
<tr>
<td>Age</td>
<td>43.2 ± 6.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 4.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.8 ± 7.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.53 ± 3.2</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
</tr>
<tr>
<td>a. Systolic (120 mm Hg)</td>
<td>119 ± 3</td>
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<tr>
<td>b. Diastolic (80 mm Hg)</td>
<td>78 ± 4</td>
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</tbody>
</table>
Table II: Biochemical status of the participants.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Subjects</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>206.4 ± 6.8</td>
<td>210.4 ± 6.8</td>
<td>Up to 230</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>76 ± 4.2</td>
<td>68 ± 4.2</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dl)</td>
<td>110 ± 9.8</td>
<td>126.2 ± 11.2</td>
<td>&lt; 150</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>122.5 ± 7.2</td>
<td>143.2 ± 6.8</td>
<td>&gt; 150</td>
</tr>
<tr>
<td>SGOT (units/L)</td>
<td>16.7 ± 0.9</td>
<td>22.1 ± 1.6</td>
<td>8 - 40</td>
</tr>
<tr>
<td>SGPT (units/L)</td>
<td>21.2 ± 1.6</td>
<td>17.2 ± 1.4</td>
<td>3 - 35</td>
</tr>
<tr>
<td>Creatinine (mg%)</td>
<td>0.67 ± 0.06</td>
<td>0.8 ± 0.01</td>
<td>&gt; 12</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.6 ± 0.78</td>
<td>7.2 ± 0.9</td>
<td>6 - 8</td>
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Table III: Levels of gamma glutamyl transferase (γGT), fasting glucose and adenosine deaminase (ADA).

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>86.05 ± 9.2</td>
<td>146.8 ± 16.2*</td>
</tr>
<tr>
<td>γGT U/lit</td>
<td>32.6 ± 1.8</td>
<td>66.24 ± 3.2*</td>
</tr>
<tr>
<td>ADA (U/L)</td>
<td>18.2 ± 5.6</td>
<td>37.2 ± 5.0*</td>
</tr>
</tbody>
</table>

* Significantly different from controls at p < 0.001.

NB: Normal value of ADA activity is taken as 15-25 U/L.

Discussion

Immunological disturbances of cell-mediated origin are believed to initiate from T-lymphocyte dysfunction. Recent in vitro studies implicated that in type 2 diabetes mellitus, inappropriate immune responses may result from the defects in the action of insulin that is required for the function of T-lymphocytes.

Adenosine deaminase plays a crucial role in lymphocyte proliferation and differentiation and shows its highest activity in T-lymphocytes. In the present study we observed a significant elevation in the adenosine deaminase levels in diabetic subjects when compared to controls.

The high plasma adenosine deaminase activity might be due to abnormal T-lymphocyte responses or proliferation; may point towards a mechanism that involves its release into circulation. Therefore, we report that increased adenosine deaminase activity in diabetic individuals could be due to altered insulin related T-lymphocyte function. Previously, Chang and Shaio, have demonstrated that impaired cell mediated immunity was associated with abnormal lymphocyte proliferation. We report that, as adenosine deaminase is associated with T-lymphocyte activity, its altered blood levels may help in predicting immunological dysfunction in diabetic individuals and might be one of the important biomarkers in predicting diabetes mellitus.

We hypothesise that these observations may furnish better insights on the role of cell-mediated immunity in the pathophysiology of type 2 diabetes.

It is also thought that in diabetic individuals, deranged immunity may also originate from antibody dependant cellular cytotoxic responses, which are believed to target insulin that has control over T-lymphocyte function. Such non-specific cellular immune responses, described as one of the four possible mechanisms responsible for the production of lymphocytotoxins, were observed earlier in autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), where elevated adenosine deaminase levels have also been detected.

The role of adenosine deaminase in the cellular immunity was first identified in patients with severe combined immuno deficiency (SCID). The high activity of this enzyme was considered to be a reflection of immunological disturbances observed in tuberculosis, infectious mononucleosis, jaundice, leukaemia, and other conditions. Our study on adenosine deaminase activity in type 2 diabetic individuals is the first of its kind of description in the immunological context.
The use of adenosine deaminase is a cost-effective process and the efficient exploitation of this strategy may help in better establishing this enzyme as a good marker for assessing CMI in diabetic individuals. Therefore, we conclude that elevated adenosine deaminase activity may be an important indicator in the immuno-pathogenesis of type 2 diabetes mellitus.

However, this study has a few limitations. A concomitant lymphocytic/plasma adenosine deaminase and its activity on insulin or vice versa, and a correlation with oral glucose tolerance test (OGTT) are to be carried out to strengthen this concept. Further studies on ADA activity in lymphocytes is required to consider ADA as an effective prognostic and pathological marker in type 2 diabetes mellitus.

References