Elevated levels of serum adenosine deaminase in type 2 diabetes mellitus patients

Venkata Bharatkumar Pinnelli¹*, Jayashankar C. A.², Shrabani Mohanty³, Asha G.⁴, Minu Mary Mathai⁴, Raghavendra D.S.⁵

¹Associate Professor, ²Professor, ³Assistant Professor, ⁴Professor and Head, Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, EPIP Area, Nallurhalli, Whitefield, Bangalore, India
²Associate Professor, Department of Medicine, Vydehi Institute of Medical Sciences and Research Centre, EPIP Area, Nallurhalli, Whitefield, Bangalore, India

Received: 12 November 2015
Revised: 23 November 2015
Accepted: 17 December 2015

*Correspondence:
Dr. Venkata Bharatkumar Pinnelli,
E-mail: pvbharatkumar@yahoo.co.in

ABSTRACT

Background: Diabetes Mellitus (DM) is a metabolic disorder characterized by an absolute or relative deficiency of insulin and insulin resistance or both. Adenosine deaminase (ADA) is an enzyme, that catalyses the irreversible hydrolytic deamination of adenosine to uric acid. Since ADA activity is associated with T-lymphocyte activity and insulin resistance, in the present study, we measured serum ADA activity in type 2 Diabetes mellitus (T2DM) patients to evaluate the relationship between serum ADA activities with glycemic status.

Methods: A total of 100 T2DM patients and controls were recruited for the study. Estimation of fasting plasma glucose (FPG), postprandial plasma glucose (PPG), HbA1c and fasting lipid profile was done. Serum ADA level was estimated by Colorimetric method. Statistical analysis of data was performed using the SPSS version 15.

Results: ADA level was significantly higher (p<0.001) in patients with T2DM (45.5±4.6 U/L) than controls. A significant positive correlation was observed between serum ADA and HbA1c (r=0.585), FPG (r=0.495), PPG (0.387) and serum triglycerides (r=0.375) among subjects with T2DM but not among non-diabetic controls.

Conclusions: In the present study, serum ADA activity in T2DM patients has been increased. High ADA activity reduces the glucose uptake into cells; therefore, insulin resistance is related to ADA activity.

Keywords: Adenosine deaminase, Type 2 Diabetes Mellitus, Hyperglycaemia, Insulin resistance

INTRODUCTION

Diabetes mellitus is one of the major noncommunicable diseases on the rise worldwide, causing 4.8 million deaths and morbidity in 371 million people every year.¹ India has the second largest number of people with diabetes in the world (62.4 million) with 3.8% in rural and 11.8% in urban adults, and this number is expected to reach 100 million by the year 2030.² In the recent past numerous studies have attempted to evaluate the role of ADA activity in diabetic patients but the data is inconclusive and controversial. ADA, an enzyme, which is present in red blood cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine. Inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid.³,⁴ Highest ADA activity is observed in the lymphoid and fatty tissues, liver, skeletal muscle, and heart, although the activity is widely distributed in most organs.⁵ An increase in ADA activity in T2DM patients has been reported by several researchers.⁶,⁷ Elevated ADA levels are also found in obesity, metabolic syndrome, liver cirrhosis and hepatitis, TB, brucellosis, typhoid fever, hypoxic states and cell mediated immune responses.⁶,⁷ However, it is difficult to conclude whether changes in ADA activity are
the cause or result of actual insulin resistance.\textsuperscript{12-15} In the present study, we measured serum ADA activity in T2DM patients to evaluate the relationship between serum ADA activity with glycemic status and various metabolic parameters in T2DM patients.

**METHODS**

The study was approved by the institutional ethics committee; a written informed consent was obtained from all participants for participation in this study. A total of 100 patients (aged 30-70 years) with T2DM were recruited from the Institute’s General Medicine department. The diagnosis of T2DM was confirmed by biochemical investigations as per WHO criteria. Patients were excluded when diagnosed with type 1 DM, acute complications such as severe infection, major surgeries, trauma, gastrointestinal disorders, severe cardiovascular/respiratory diseases, pregnant and breast feeding women. Patients taking supplements such as antioxidants, vitamins, minerals were also excluded. Age and sex matched 100 controls were recruited after clinical and biochemical evaluation. The baseline demographic data was obtained. 5 mL of venous blood sample was collected after 12 hours of fasting for estimation of fasting plasma glucose, HbA1c and lipid profile and 2 ml venous blood sample 2 hours after breakfast for postprandial plasma glucose. Hb and A1C concentration were measured separately by Hb reagent using colorimetric method and A1C by turbidimetric immune-inhibition method.\textsuperscript{16} The final result reported as % HbA1c using IFCC reference method. Lipid profile was performed by timed end point method. All the above mentioned parameters were measured using the autoanalyzer Beckman Coulter DXC 600. Serum ADA was estimated by Colorimetric method described by Guiuseppe Guisti.\textsuperscript{17}

**Statistical analysis**

Statistical analysis of data was performed using the SPSS (Version 15.0). For comparison of parameters between the two groups, students t test was used. Statistical significance was considered at a ‘p’ value of < 0.05. For correlation, Pearson’s correlation coefficient (r) was used.

**RESULTS**

The study included 100 subjects with T2DM and 100 healthy controls. The mean age was 54.36±11.25 years in cases and 51.81±10.25 years in controls, and the majority (70%) was male. There was a statistically significant increase in mean value of fasting blood glucose 218.62 mg/dl with p<0.001 and postprandial blood glucose 285.04 mg/dl with p<0.001 in cases as compared to controls.

There was a statistically significant increase in mean value of HbA1c 9.95% with p<0.001 in cases compared to controls and also a statistically significant increase (p<0.001) in mean value of ADA 45.5 U/L with p<0.001 in cases compared to controls. A significant positive correlation was observed between serum ADA with HbA1c, FBS and PPBS in cases compared to controls with P value <0.001 and r value 0.585, 0.495, 0.387 and 0.375 respectively.

**Table 1: General characteristics and the study design with routine biochemical parameters of type 2 diabetes mellitus patients.**

<table>
<thead>
<tr>
<th>Study Parameters</th>
<th>Cases(n=100)</th>
<th>Controls (n=100)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>70/30</td>
<td>70/30</td>
<td>-</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>54.36±11.25</td>
<td>51.81±10.25</td>
<td>0.13</td>
</tr>
<tr>
<td>Duration of Diabetes (years)</td>
<td>11.02±8.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fasting plasma Glucose (FPG) (mg/dL)</td>
<td>218.62±121.7</td>
<td>81.96±12.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post Prandial plasma Glucose (PPG) (mg/dL)</td>
<td>285.04±121.7</td>
<td>113.56±22.39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.95±3.12</td>
<td>4.83±0.70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Total Cholesterol (mg/dL)</td>
<td>204.3±57.5</td>
<td>182.5±33.9</td>
<td>0.015</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dL)</td>
<td>221.4±69.2</td>
<td>142.8±46.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mg/dL)</td>
<td>31.2±3.1</td>
<td>35.5±4.6</td>
<td>0.15</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mg/dL)</td>
<td>131.15±31.35</td>
<td>125.45±29.25</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum Adenosine deaminase (ADA) (U/L)</td>
<td>45.5±4.6</td>
<td>19.5±4.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are presented in Mean ± SD

There was a statistically significant increase in mean value of serum total cholesterol p=0.015, and triglycerides p<0.001 in cases as compared to controls, and there was also positive correlation between triglycerides and serum ADA.
Table 2: Pearson correlation between serum ADA and HbA1c, FPG, PPG and TG.

<table>
<thead>
<tr>
<th>Pearson correlation</th>
<th>Cases r value</th>
<th>P value</th>
<th>Controls r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ADA v/s HbA1c</td>
<td>0.585</td>
<td>&lt;0.001</td>
<td>0.056</td>
<td>0.376</td>
</tr>
<tr>
<td>Serum ADA v/s FPG</td>
<td>0.495</td>
<td>&lt;0.001</td>
<td>0.164</td>
<td>0.256</td>
</tr>
<tr>
<td>Serum ADA v/s PPG</td>
<td>0.387</td>
<td>&lt;0.001</td>
<td>0.181</td>
<td>0.208</td>
</tr>
<tr>
<td>Serum ADA v/s TG</td>
<td>0.375</td>
<td>&lt;0.001</td>
<td>0.176</td>
<td>0.216</td>
</tr>
</tbody>
</table>

DISCUSSION

The world-wide burden of the diabetes is increasing day by day and has reached in epidemic proportions, being a chronic metabolic disorder, its long term complications could have devastating consequences. ADA is an enzyme that converts adenosine into inosine through an irreversible deamination reaction. It is hypothesized that adenosine has got insulin like activity on glucose and lipid metabolism particularly in adipose tissue and skeletal muscles. ADA is found as a producer of reactive oxygen species (ROS), stimulator of lipid peroxidation and marker of both T-cell activation and glycemic status in diabetes mellitus (DM). An increase in ADA activity in T2DM patients has been reported, while the mechanism that increases serum and tissue ADA activity is not well known, with higher ADA activity in insulin-sensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced. Glucagon-like peptide-1 (GLP-1), an incretin, promotes insulin secretion in a glucose concentration-dependent manner in pancreatic beta cells, inhibits glucagon secretion in alpha cells, decreases the gastric discharge rate, and mediates appetite suppression. GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). DPP-4 is an enzyme that acts as an important immune regulator by interacting with CD3 and acting as a co-stimulator for CD4+ T cells. It also regulates glucose homeostasis by hydrolysing integrins. DPP-4 binds ADA with high affinity and as adenosine causes apoptosis and inhibits differentiation of T lymphocytes by activating P1 adenosine receptors, interaction of ADA with DPP-4 can lead to T cell proliferation and increased cytokine production which can interfere with insulin signalling, there were several reports that DPP4 might increase the incidences of some infectious diseases (e.g. nasopharyngitis and urinary tract infection), so further experimental and clinical studies are needed to determine the effects of DPP-4 on immune cell function. Our study found an elevated serum ADA activity in cases of T2DM when compared to age and sex-matched controls. These results of elevated ADA in T2DM also correlated with studies done by Kurtul et al., Lee et al and Shiva Prakash et al. Similar study done by Hoshino et al found the cases of T2DM with chronic hyperglycaemia favours auto oxidation and also increases free radical activity. Several other researchers also found elevated ADA levels and ADA activity correlated with glycemic control in T2DM patients.

However, there are few limitations in our study which includes the non-estimation of serum transaminase and serum insulin levels which are known to be related to ADA. Moreover, a correlation study between serum ADA level and oral glucose tolerance test will further enhance the serum level of ADA in T2DM subjects. Prediabetic subjects were also not considered in this study as screening of serum ADA may be an alarming factor in the pathogenesis of T2DM subjects. Despite these limitations, our study shows higher serum ADA in T2DM patients and a strong positive correlation of ADA with FPG, PPG and HbA1C which suggests an association between ADA and glycemic status. A larger cross-sectional study needs to be done to strengthen the fact. Thus, if ADA activity is suppressed, insulin sensitivity may be improved, and cellular proliferation, inflammation, and T-cell activity, which are associated with the pathophysiology of insulin resistance, can be affected.

CONCLUSION

Results showed a clear and significantly positive correlation between serum ADA levels and glycemic parameters. Though it is evident that there is an elevation of serum ADA values in individuals with T2DM, the exact mechanism behind the elevation and the implication of altered expression need to be further elucidated.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES


