SERUM ADENOSINE DEAMINASE ACTIVITY AMONG NEWLY DIAGNOSED PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS CORRELATION WITH INSULIN RESISTANCE

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ABSTRACT

Aims: Insulin resistance is consistence feature in type 2 diabetes mellitus (DM). Several studies demonstrated that serum adenosine deaminase activity (ADA) is elevated in type 2 DM patients. The present study was conducted to estimate serum ADA activity in newly diagnosed type 2 DM patients, compared with control groups and to find out correlation of insulin resistance with serum ADA activity.

Method: Fifty-one type 2DM patients were selected as cases and fifty healthy individuals served as controls. Their body mass index (BMI), fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostatic model for assessment of insulin resistance (HOMAIR), % beta cell activity, % sensitivity of insulin and serum ADA activity were measured using appropriate methods.

Results: ADA activity was significantly increased among cases in comparison to controls (p value <0.001). Serum ADA activity was positively correlated with BMI (r=0.386; p=0.005); FPI (r=0.318; p=0.023); HOMAIR (r=0.363; p=0.009) & negatively correlated with % sensitivity (r=-0.381; p=0.006) among case group.

Conclusion: ADA activity might be considered as a predictive marker of insulin resistance in obese or overweight type 2 DM.

Key word: ADA activity, HOMA-IR, obesity, type 2 DM.
INTRODUCTION:

According to International Diabetes Federation 2012, in the world more than 371 million people suffer from diabetes mellitus (DM). 71.3 million Adults with diabetes in the world live in the South-East Asia Region, 61.3 million of whom are in India. The most common form of this epidemic is type 2 DM [1].

Type 2 DM consistently demonstrates three cardinal abnormalities: resistance to the action of insulin in peripheral tissues, particularly muscle and adipose tissue; decreased insulin secretion, and increased glucose production by the liver [2]. Insulin resistance is central to the pathophysiology of type 2 DM. It is defined as a decreased biological response to normal concentrations of circulating insulin [3]. Insulin resistance is manifested by decreased insulin-stimulated glucose uptake and its metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output resulting hyperglycemia [2]. However, the development of frank diabetes mellitus require insulin secretion defect also. In the absence of β-cell dysregulation, individuals can compensate indefinitely for insulin resistance with appropriate hyperinsulinemia [4]. GLUT4 is the main glucose transporter activated by insulin in skeletal muscle cells and adipocytes in maintaining blood sugar. Insulin sensitivity is influenced by a number of factors including age, weight, ethnicity, body fat (especially abdominal) [2], physical activity [5], dietary habits [6] (excessive carbohydrate diet), free fatty acid [2, 7], vitamin D deficiency [8], inflammation [9, 10] genetic factors and medications such as glucocorticoid. In addition to the above mentioned there are many more factors; such as adenosine molecule.

Adenosine, a degradation product of adenine nucleotides, has been proven to play an important role in modulation of insulin action on glucose metabolism in different tissues. Some of the adenosine that is produced intracellularly is released into the extracellular space, where it interacts with adenosine receptors to regulate various physiological processes in an autocrine manner. One such action is to modulate insulin-stimulated glucose transport (via GLUT4) in striated muscle and adipocytes [11]. Allan Green et al in 1987 demonstrated that adenosine and other A1 receptor agonists (N6 phenyl isopropyl adenosine, PIA), increases insulin sensitivity and inhibit lipolysis in adipocytes [12]. Intracellular adenosine concentration is maintained by adenosine deaminase enzyme.

Adenosine deaminase (ADA 3.5.4.4) is a purine catabolic enzyme that catalyzes the deamination of adenosine to inosine and maintain cellular adenosine concentration. ADA was considered as good marker of cell mediated immunity. It plays a crucial role in lymphocyte proliferation and differentiation. Previously, ADA activity has been reported to be increased in type 2 DM [13-16]. But it is not yet established whether ADA activity rises at early phase of onset of type 2 DM or raised ADA activity is affected by therapy. It is also precisely not known whether there is any correlation between insulin resistance and serum ADA activity. This study was conducted to determine serum ADA activity in newly diagnosed patients with type 2 DM who have not started any treatment and its correlation with insulin resistance.

Materials & methods:

This preliminary descriptive cross-sectional study was conducted during the year 2012-13. Cases were selected from the patients during their first visit at outpatient clinic for diabetes mellitus (R. G. Kar Medical College). Cases were selected whose FBG >126 mg/dl and HbA1C >6.5%. Type 2 DM was diagnosed according to American Diabetes Association criteria [17]. Institutional ethics committee
permission was taken prior to start of the work. Informed consent was taken from all participants before inclusion in the study in their convenient language as applicable.

A group of fifty one adult patients of either sex, who had not started any medication for diabetes mellitus, selected as cases. All the subjects were in the category of type 2 DM. Neurological complications were excluded by taking history. Nephrological complications were excluded by measuring serum urea, creatinine. Cardiovascular complications were excluded by taking history, measuring blood pressure, performing electrocardiogram (ECG). Retinopathy was excluded by ophthalmological examination. Tuberculosis, leprosy, HIV infections, viral hepatitis, and liver cirrhosis that may increase ADA level at the time of the study [18] were excluded by performing liver function test, HIV, HbsAg assay, chest x ray and taking previous history. The above mentioned diseases were excluded from both case & control groups.

A group of fifty age and sex matched healthy individuals with no history of diabetes mellitus served as controls. The selection procedure was confirmed only after performing both fasting plasma glucose test and glycated hemoglobin assay, then distinguishing them into two groups: case and controls. The cut-off value used for fasting plasma glucose concentration was 126 mg/dl (fasting is defined as no caloric intake for at least 8 hours) [17] and cutoff value for HbA1C was 6.5% [17]. As participants attended the hospital OPD from a large rural base, they had approximately similar ethnicity, socioeconomic status and dietary habits. After recording of weight and height of the individual BMI was then calculated by dividing their weight (kg.) with their height’s square in meters [19]. World health organization (WHO) proposed BMI cut-points 23.0 to 24.9 kg/m2 for being overweight and ≥25.0 kg/m2 for obesity in the adult Asian population [20].

Fasting blood was collected by venipuncture for the determination of different biochemical parameters. The blood samples were subjected to centrifugation for separation of plasma or serum. The plasma or serum was analysed for different biochemical parameters. These were measured with standard kits provided.

Fasting plasma glucose was determined by glucose oxidase –peroxidase method [21] using kits provided by Crest Biosystems, Goa, India (Coefficient of variation for glucose- level 1 control - 4.78%, level 2 control - 3.66%). Glycated hemoglobin by ion exchange resin method provided by Crest Biosystems. Fasting plasma insulin was assayed [22, 23] by ELISA kit AccuBind from Monobind Inc. USA (Coefficient of variation for insulin- level 1 control-10.78%, level 2 control- 7.56%). This method has been reported to show a high degree of correlation with reference radioimmunoassay method. No cross reactivity with C-peptide was detected. The plasma was separated & stored at -20°C till assay was done. Assays were done next day of the date of collection in fully automated ELISA reader and washer from TECAN, Austria. Homeostatic model assessment (HOMA2 model) was used for assessing [24] β-cell function and insulin resistance (IR) and % sensitivity from basal (fasting) glucose and insulin concentration using HOMA2 model. David R. Matthews, summarized the physiological basis of HOMA, a structural model of steady-state insulin and glucose domains, constructed from physiological dose responses of glucose uptake and insulin production. HOMA has been validated against a variety of physiological methods. HOMA2: the updated HOMA model, the correctly solved computer model [25, 26], has nonlinear solutions, and these should be used when HOMA is compared with other model [27]. The insulin secretion curve has been modified to allow for an increase in insulin secretion in response to a plasma glucose concentration of >10 mmol/l. This version incorporates an estimate of proinsulin secretion into the model and thus allows the use of either total (radioimmunoassay [RIA]) or specific insulin assays. %Sensitivity is a function of glucose metabolism driven by the action of insulin. β-cell function were modeled by changing the β-cell response to plasma glucose concentrations.
Serum ADA level was estimated in serum using a commercially available kit (Tulip Diagnostics Private Limited, Goa, India) (Coefficient of variation for serum ADA- of pooled serum 11.51%). This procedure is based on the method reported by Giusti and Galanti [18]. 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. 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 (ECIL- UV5704SS). One unit of ADA activity releases three nanomoles of ammonia in the reaction in 1 hour at 37°C. ADA activity was measured on the day of sample collection.

Suitable statistical methods and techniques were applied with the help of software based computer programme (SPSS version17) to analyse the results.

RESULTS

Total study population of comprised of 61 cases and 57 controls of 21-58 years age group of either sex (after exclusion by history and clinical examination). Final exclusion was done after implementation of exclusion criteria and by outlier detection (Horn & Coworker) [28]. After implementation of the exclusion criteria, 10 cases and 07 controls were excluded from the study, resulting final population of 51 cases and 50 controls. The distribution of males & females in both groups were found insignificant by chi-square test (χ²=0.06;df-1; p= 0.7). After ensuring comparability between cases & controls; unpaired t test was performed to find out significant difference between mean value of BMI, FPG, FPI, HbA1C, HOMA-IR, %β cell function, % sensitivity, ADA activity among cases and controls & p value <0.05 was considered significant. Correlation analysis of serum ADA activity and different parameter of insulin resistance was done by pearsons’ correlation study.

In our study FPG (mg/dl) were significantly increased in cases (159.84 ± 34.7) in comparison to controls (85.80±10.17) (t=14.48; p = <0.001) (table no 1). FPG values were >126mg/dl in all cases. HbA1C of case (7.35±0.91) were significantly increased (t= 12.72; p <0.001) in comparison with controls (5.53± 0.45)(table no 1). HbA1C values were >6.5% in all cases. BMI of case (25.66±2.64) was significantly increased in comparison of controls (21.54±1.67) (t= 9.33; p = <0.001). FPI μIU/ml of cases (19.96 ± 5.28) was significantly increased compared to control (8.56 ±1.96) (t=16.42; p = <0.001) (table no 1). HOMA-IR was calculated as an indicator of insulin resistance [24]. HOMA-IR were significantly increased in cases (2.88±0.74) comparison with controls (1.09±0.24) (t=16.14; p = <0.001) (table no 1). In this study % β cell activity of case (69.64±26.40) were significantly decreased in comparison of controls (117.92±37.79) (= - 7.45; p = <0.001) (table no1). % sensitivity of cases (36.87± 8.47) was also significantly decreased in comparison of controls (95.83±19.71)(= -19.59; p = <0.001).

In this study group, mean serum ADA level in cases and controls were 38.41±10.53 U/L; 16.30±2.97 U/L respectively which was increased significantly in cases (p<0.001) (table no1). Serum ADA activity was increased significantly among cases & positively correlated with BMI (r= 0.386; p=0.005), FPI (r=0.318; p=0.023) (figure 1) and HOMAIR value (r= 0.363; p = 0.009) (figure 2). ADA activity was negatively correlated with % sensitivity (r = - 0.381; p = 0.006) (figure 3), but FPG & % β cell activity were not significantly correlated with ADA activity.
DISCUSSION:

In our study FPG values and HbA1C values were >126mg/dl and >6.5% respectively in all cases. Insulin resistance is a consistent finding in patients with type 2 DM. However, in the absence of β-cell function defect, individuals can compensate indefinitely for insulin resistance with appropriate hyperinsulinemia [4].

Fasting plasma insulin (FPI) of cases were significantly increased compared to control (t=14.48; p < 0.001) (table no 1). HOMA-IR were significantly increased in cases comparison of controls (t=18.81; p < 0.001) (table no1). In this study % β cell activity (t= -7.85; p = <0.001) (table no 1) and % sensitivity significantly decreased among cases (t= -19.68; p = <0.001) (table no 1). The above observation confirms that the cases in this study were suffering from type 2 DM. Newly diagnosed fifty one type 2 DM patients (both male and female) who had not started any medication were considered as cases.

Serum ADA activity was significantly increased among cases in respect to control (t= 14.30; p<0.001) (table 1). Similar finding was observed by Naciye Kurtul in 2004. In 2006 M Shiva Prakash et al. also demonstrated that there is a significant (p < 0.001) increase in ADA activity with a mean (± SD) 37.2 ± 9.29 U/l in diabetic subjects when compared to controls (18.2 ± 5.6 U/l). It is positively correlated with HOMAIR value (r =0.363; p = 0.009) (figure 2) & negatively correlated with % sensitivity (r = - 0.381; p = 0.006) (figure 3).

Elevated ADA activity results in decrease cellular adenosine concentration. Adenosine has role on carbohydrate and fat metabolism [11, 12]. Some of the adenosine that is produced intracellularly is released into the extracellular space, where it interacts with adenosine receptors and modulates insulin-stimulated glucose transport (via GLUT4) in striated muscle and adipocytes [11].

Susan J. Vannucci, et al proposed that adenosine appears to stabilize the fully activated form of glucose transporter (GLUT4) and addition of ADA inhibits glucose transport activity [29]. So increased ADA activity decreases glucose entry within cell, ultimately leads to increased insulin resistance. Thus rise of serum ADA activity in this study might be one important factor in development of insulin resistance. The adenosine is also strong antilipolytic & helps to maintain plasma free fatty acid level (FFA) [30]. Adenosine deaminase produces a strong lipolytic response in fat cells accompanied by increase in cyclic AMP levels [31, 32] & raised plasma FFA increases burden of insulin resistance [2, 33]. Thus adenosine plays an important role in glucose homeostasis by both this mechanism and its concentration is dependent upon cellular ADA activity. Adenosine also modulates the function of polymorphonuclear leukocytes (PMN) and monocytes & inhibits the production of TNF-alpha, IL-6 and IL-8 by lypopolysachharide (LPS)-activated human monocyte [34]. Oscar J. Cordero et al found that ADA activity is regulated by cytokines [35].

It has been increasingly accepted that chronic subacute inflammation plays an important role in the development of insulin resistance and type 2 DM in animals and humans [36]. Macrophage invasion of adipose tissue is frequently detected in obese. Adipocyte acts as a large endocrine organ secreting large number of hormone & cytokines (tumor necrosis factor TNF-α, IL-6 and MCP-1etc) [2, 37]. Particularly supporting this is that suppression of systemic inflammation in type 2 DM improves glycemic control [36, 38]; this also points to a new potential therapeutic target for the treatment of type 2DM. In addition, insulin resistance due to a high-fat diet causes macrophage accumulation in adipose tissue [10]. Tumor necrosis factor (TNF)-α has been proposed as a link between obesity and insulin resistance [39]. Obesity is also associated with an increase in adipose tissue infiltration of macrophages, which contributes to the inflammatory process through the additional secretion of cytokines [40]. The mechanisms by which adipose tissue recruits and maintains macrophages could involve expression of monocyte chemoattractant...
proteins and intercellular adhesion molecule-1, as was described recently [6, 41]. Adenosine deaminase (ADA) is important in acute and protracted inflammatory response. Recent work has demonstrated elevated ADA activity during inflammatory responses in macrophage rich tissues, such as liver and spleen. Specifically, a disproportionate increase in the enzyme activity attributable to the isoenzyme ADA2 has been found in pleural effusions of tuberculosis patients and serum of patients of HIV patients [42].

The study population here is newly diagnosed & before start of any therapy. So any modification due to therapy is excluded. In this study most cases were overweight or obese. BMI of case (25.66±2.64) significantly increased in comparison of controls (21.54±1.67) (t= 9.33; p = <0.001) & positively correlated with serum ADA activity. With increase mass of adipose tissue in obese & overweight person, there is increased release of hormone & cytokines. They might act in two ways: on one hand cytokine increases expression of ADA in macrophages which are accumulated in adipose tissue [36], on other hand they inhibit insulin signaling pathway as described above.

It is well known that insulin sensitivity is influenced by multiple factors, adenosine is one of them. So elevated ADA activity leads to fall of adenosine concentration might have an important role in development of insulin resistance. The gold standard for investigating insulin resistance is hyperinsulinemic euglycemic clamp study [24]. This is rarely done in clinical practice. The modified insulin suppression test is another method of measuring insulin resistance. But this is mainly used in research field. Homeostatic model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) are recently introduced method of assessing insulin resistance. Both this value correlates significantly with clamp technique. But both this value also requires measurement of fasting plasma insulin by ELISA (Enzyme linked imunosorbtant assay) technique which requires sophisticated Elisa reader and skilled manpower. Serum ADA activity is measured by simple colorimetric method. The serum ADA activity might be considered as surrogate marker of insulin resistance in obese or overweight type 2 DM but more number of study is required for it. Further studies on drugs which inhibit ADA activity, in animal model, are required to consider ADA as an effective prognostic marker in type 2 DM and may show immense prospect in treatment of type 2 DM.

Acknowledgements:
We take this opportunity to express our special sense of gratitude to The Principal of R. G. Kar Medical College & Hospital, Ethical Committee members & The West Bengal University of Health Sciences for kindly allowing us to carry out this work.

Bibliography

Table No.1 shows mean & SD of different biochemical parameters among cases and controls.

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Case (mean±SD)</th>
<th>Control (mean±SD)</th>
<th>‘t’ value</th>
<th>‘p’ value (df-99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>25.66±2.64</td>
<td>21.54±1.67</td>
<td>9.33</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FPG</td>
<td>159.84±34.70</td>
<td>85.80±10.17</td>
<td>14.487</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HbA1C</td>
<td>7.35±0.91</td>
<td>5.53±0.45</td>
<td>12.72</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>FPI</td>
<td>19.43±4.26</td>
<td>8.55±1.95</td>
<td>16.421</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.80±0.59</td>
<td>1.09±0.24</td>
<td>18.815</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>%β Cell Activity</td>
<td>68.28±24.45</td>
<td>117.92±37.79</td>
<td>-7.852</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>% Sensitivity</td>
<td>37.30±7.84</td>
<td>95.83±19.71</td>
<td>-19.683</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ADA activity</td>
<td>38.41±10.52</td>
<td>16.29±2.97</td>
<td>14.302</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

p value <0.05 considered as significant.

BMI, FPG, FPI, HbA1C, HOMA-IR and serum ADA activity were significantly increased; % β cell activity, % sensitivity were significantly decreased among case group.

Figure No. 1 Scatter diagram shows correlation between FPI and ADA activity among cases

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According to pearsons correlation study FPI was positively correlated with ADA(r = 0.318; p = 0.023), and it was significant
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According to pearson's correlation study, HOMA-IR was positively correlated with ADA (r = 0.363; p = 0.009), and it was significant.

According to pearson's correlation study, % sensitivity was negatively correlated with ADA (r = -0.381; p = 0.006), and it was significant.