Is adenosine deaminase level a predictive biochemical marker of type II diabetes mellitus in obese Indian subjects

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Abstract
Objective: To evaluate serum Adenosine Deaminase (ADA) levels in obese and non obese Type II Diabetes Mellitus (T2DM) and compare it with healthy controls (obese and non obese).

Materials and Methods: Total 100 subjects were included in the study. Fasting blood sugar (FBS) and ADA activity along with other routine biochemical parameters were determined in their serum. Based on their BMI and FBS level study subjects were divided into four groups: Control, Obese, Non obese T2DM and Obese T2DM.

Results: ADA activity was significantly increased (P < 0.0001) in obese (non diabetic) and Non obese T2DM subjects compared to controls.

Conclusion: In this study we observed an increased ADA activity in obesity and T2DM. A close relation may be present between insulin resistance produced in obese subjects and diabetics. Therefore ADA could therefore be used in routine lab investigations, especially in obese patients as a predictive marker for assessing the development of insulin resistance and hence diabetes.

Keywords: Diabesity, ADA, Obesity, Insulin resistance, Fasting blood sugar.

Introduction
Diabetes Mellitus is a disease of multiple aetiologies including genetic and environmental factors. Unhealthy food habits, increased level of stress, sedentary lifestyle and consequent Obesity, especially central obesity are major contributing factors for increasing incidences of Type II Diabetes Mellitus (T2DM). In most developed countries it is one of the top five causes of death. The World Health Organisation (WHO) projections indicate that by 2030 around 300 million might suffer from the disease, out of which, 228 million might be from India and China. The relationship between Diabetes with obesity is termed “Diabesity” to characterise the close association of these two disorders.

Obesity is a disorder caused by excess calorie intake, coupled with lack of physical activity. It occurs due to an increase in both size and number of adipose tissues. Biochemically, obesity is linked with a low grade systemic inflammation, where the adipose tissue secretes several chemical mediators like Tumour Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) and leptin. Further, TNF-α has been shown to have generate insulin resistance by affecting insulin receptors phosphorylation and the insulin receptor substrate. Impaired insulin secretions and insulin resistance are associated with T2DM. So obesity and Diabetes Mellitus show strong correlation. There is also strong evidence that Indians have a greater degree of insulin resistance and a stronger genetic predisposition to Diabetes.

Adenosine is a nucleotide produced in the body, and has potent anti-inflammatory effects, one of them being its ability to inhibit TNF-α by A3 receptor activation. Adenosine Deaminase (ADA) facilitates irreversible deamination of adenosine to inosine. ADA is present in all mammalian tissues. Glucose uptake into cells is increased by adenosine. Higher ADA activity also decrease adenosine levels in insulin sensitive tissues which ultimately results in decreased uptake of glucose into cells. Thus estimating ADA levels might provide an insight regarding insulin sensitivity and inflammation.

This will prove to be a powerful tool for early diagnosis of Type II Diabetes Mellitus in the population at high risk of developing the disease. Since Diabetes is a disease which can be prevented by making a few lifestyle changes, early diagnosis can prove to be very beneficial. Moreover Diabetes is growing at alarming rates, almost epidemic proportions in urban areas, and the projections for the population afflicted by Diabetes in the near future are also very disheartening. Hence, early diagnosis and prevention is our only hope to prevent this disease from becoming an epidemic.

Materials and Methods
Study Design
The study was conducted in the Department of Biochemistry, Gandhi Medical College, Bhopal (M.P.). The patients with T2DM and control subjects visiting outpatient department of Medicine, Gandhi Medical College, Bhopal were recruited for the study. The institutional ethical committee approval was obtained prior to the conduct of study and written informed consent was taken from the participants, after explaining the motives and procedure of the study.
Body mass index (BMI) of the subjects was calculated (weight in kilograms divided by the square of heights in meters).\textsuperscript{15,16}

A total of 100 subjects of age group 18-65 years (male or female) were included in the study and they were divided into four groups:

| Group | Characteristics |
|-------|-----------------|
| 1     | Healthy control subjects. (n = 25, BMI < 25kg/m$^2$) |
| 2     | Obese subjects NOT suffering from Type II Diabetes Mellitus. (n = 25, BMI \geq 30 kg/m$^2$) |
| 3     | Non-Obese subjects suffering from Type II Diabetes Mellitus. (n = 25, BMI < 25kg/m$^2$) |
| 4     | Obese subjects suffering from Type II Diabetes Mellitus. (n = 25, BMI \geq 30 kg/m$^2$) |

**Inclusion Criteria**
The inclusion criteria for diabetic subjects was male/ female diagnosed with Type 2 Diabetes mellitus, aged 18-65 years, with FBG \geq 126 mg/dL or on oral anti-diabetic drugs for more than one month.

**Exclusion Criteria**
Type 1 diabetes mellitus, Patients on insulin treatment, hypertension, neurological disorders, ischemic heart disease, renal failure, cancer, chronic liver disease, tuberculosis, immunological disorders, rheumatoid arthritis and pregnancy.

**Study Duration**
The study was carried out between August and September 2016.

**Sample Collection**
After taking the informed consent of the subjects, they were instructed to fast overnight. The morning after, their venous samples were collected by a trained technician, using fluoride vials for the estimation of Fasting blood glucose and plain vials for the other biochemical parameters.

**Processing and Lab Analysis**
The serum samples were stored at -20°C until analysis. All routine biochemical parameters like FBS, LFT, KFT, Lipid profile were analyzed on Biosystem A25 fully automated biochemistry analyser. The serum ADA level was measured on semi auto analyser (MERCK) using Biosystem kit (kinetic reaction kit).

**Statistical Analysis**
The data obtained was analyzed using SPSS statistical program version 16.0 (SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was applied for differences in concentrations between the groups. P value < 0.05 is considered statistically significant.

**Results**
Table 1 shows characteristics of study population. The subjects were divided into four groups as shown in Table 1. Control (n = 25), Obese Non diabetic (n = 25), Non Obese T2DM (n = 25) and Obese T2DM (n = 25). Age was 38.5 ± 14.3 (mean ± SD) in control, 26.0 ± 10.3 (mean ± SD) in Obese, 49.2 ± 14.3 (mean ± SD) in Non Obese T2DM and 48.8 ± 9.1 (mean ± SD) in Obese T2DM and BMI was 22.2 ± 4.8, 31.1 ± 1.6, 24.4 ± 2.4 and 31.4 ± 1.6 (mean ± SD) respectively in above groups (Table 1).

The fasting blood sugar levels were found to be significantly increased obese and non obese T2DM subjects as compared

| Table 1: Baseline characteristics of the study population |
|--------------------------------------------------------|
| Characteristic                | Normal (Control) (n=25) | Obese (Non Diabetic) (n=25) | Non obese T2DM (n=25) | Obese T2DM (n=25) |
| Age                         | 38.5 ± 14.3            | 26.0 ± 10.3                 | 49.2 ± 14.3           | 48.8 ± 9.1        |
| BMI                         | 22.2 ± 4.8             | 31.1 ± 1.6                  | 24.4 ± 2.4            | 31.4 ± 1.6        |
| FBS, mg/dl                  | 87.3 ± 7.9             | 98.3 ± 9.1                  | 141.6 ± 80.5          | 144.8 ± 50.5      |
| Bilirubin, mg/dl            | 0.6 ± 0.2              | 0.6 ± 0.2                   | 0.5 ± 0.1             | 0.7 ± 0.1         |
| SGOT, U/L                   | 21.5 ± 5.3             | 26.9 ± 8.4                  | 24.4 ± 5.8            | 24.4 ± 5.9        |
| SGPT, U/L                   | 21.9 ± 8.8             | 34.0 ± 18.1                 | 23.0 ± 8.4            | 22.4 ± 7.1        |
| Urea, mg/dl                 | 24.3 ± 6.3             | 23.7 ± 6.6                  | 28.3 ± 10.8           | 32.8 ± 13.0       |
| Creatinine, mg/dl           | 0.7 ± 0.1              | 0.7 ± 0.1                   | 0.8 ± 0.2             | 0.7 ± 0.2         |
| Total cholesterol, mg/dl    | 170.9 ± 30.1           | 183.8 ± 27.2                | 167.9 ± 37.6          | 174.8 ± 37.4      |
| Triglyceride, mg/dl         | 126.2 ± 47.4           | 164.8 ± 60.5                | 165.7 ± 61.0          | 156.7 ± 42.1      |
| HDL-C, mg/dl                | 43.5 ± 7.4             | 40.1 ± 9.2                  | 39.7 ± 11.3           | 37.6 ± 11.5       |
| LDL-C, mg/dl                | 95.4 ± 18.5            | 112.2 ± 26.4                | 94.8 ± 25.3           | 97.9 ± 24.9       |

Number in parentheses shows number of samples analysed. Values expressed as mean ± SD.
to control group (P < 0.0001, Table 3). Mean fasting blood sugar levels in control were 87.3 mg/dl, 98.3 mg/dl in obese, 141.6 mg/dl in non obese T2DM and 144.8 mg/dl in obese T2DM subjects. FBS levels were significantly increased in non obese T2DM and obese T2DM as compared to obese subjects (P<0.05), (Table 1).

Mean serum ADA levels in were 18.1 U/L in control subjects, 28.4 U/L in obese, 34.6 U/L in non obese T2DM and 36.2 U/L in obese T2DM subjects respectively (Table 2). As compared to control group the serum ADA levels were found to be significantly increased (P< 0.001) in obese, non obese T2DM and obese T2DM subjects (Table 2, Fig 1). Significantly increased (P<0.05) serum ADA levels were found in non obese T2DM and obese T2DM as compared to control as well as obese subjects (Table 2, Fig. 1). But there is no significant difference in ADA levels between obese and non obese T2DM. The serum bilirubin levels in obese, non obese T2DM and obese T2DM subjects as compared to control group (P > 0.05, Table 3).

| Characteristic | F* | P value |
|----------------|----|---------|
| FBS, mg/dl     | 9.45 | < 0.0001* |
| Bilirubin, mg/dl | 1.89 | 0.1365 |
| SGOT, U/L      | 2.83 | 0.0421* |
| SGPT, U/L      | 6.39 | 0.0005* |
| Urea, mg/dl    | 4.75 | 0.0039* |
| Creatinine, mg/dl | 2.54 | 0.0606 |
| Total cholesterol, mg/dl | 1.06 | 0.3675 |
| Triglyceride, mg/dl | 3.01 | 0.0339* |
| HDL-C, mg/dl   | 1.45 | 0.2328 |
| LDL-C, mg/dl   | 2.92 | 0.0380* |

F* = One way analysis of variance (ANOVA)
P < 0.05 is significant.

**Study Groups**

![Graph showing the comparison of Serum adenosine deaminase (ADA) activity levels (mean±standard deviation) between Normal, Obese non diabetic, Non obese T2DM and Obese T2DM](image)

Table 2: Serum ADA levels in the study population (mean ± standard deviation)

|                  | Normal (Control) | Obese (Non Diabetic) | Non obese T2DM | Obese T2DM | F*  | P value |
|------------------|------------------|----------------------|----------------|------------|-----|---------|
| Serum ADA, U/L   | 18.1 ± 1.1       | 28.4 ± 3.1           | 34.6 ± 3.0     | 36.2 ± 2.9 | 231.7 | <0.0001* |

Number in parentheses shows number of samples analysed.

F* = One way analysis of variance (ANOVA).
P < 0.05 is significant.
Mean serum creatinine levels in control were 0.7 mg/dl, 0.7 mg/dl in obese, 0.8 mg/dl in non-obese T2DM and 0.7 mg/dl in obese T2DM subjects respectively (Table 1).

There were no significant differences found in serum total cholesterol levels in obese, non-obese T2DM and obese T2DM subjects as compared to control group (P > 0.05, Table 3).

Mean serum cholesterol levels in control were 170.9 mg/dl, 183.8 mg/dl in obese, 167.9 mg/dl in non-obese T2DM and 174.8 mg/dl in obese T2DM subjects respectively. (Table 1)

Mean serum triglyceride levels in control were 126.2 mg/dl, 164.8 mg/dl in obese, 165.7 mg/dl in non-obese T2DM and 156.7 mg/dl in obese T2DM subjects respectively. (Table 1). And the difference is statistically significant. (P < 0.05, Table 3).

The serum HDL cholesterol levels were found to be non significant in obese, non-obese T2DM and obese T2DM subjects as compared to control group (P > 0.05, Table 3).

Mean serum HDL cholesterol levels in control were 43.5 mg/dl, 40.1 mg/dl in obese, 39.7 mg/dl in non-obese T2DM and 37.6 mg/dl in obese T2DM subjects respectively (Table 1).

Mean serum LDL cholesterol levels in control were 95.4 mg/dl, 112.2 mg/dl in obese, 94.8 mg/dl in non-obese T2DM and 97.9 mg/dl in obese T2DM subjects respectively (Table 1). And the difference is statistically significant (P < 0.05, Table 3).

Discussion

T2DM, is a multifactorial disease, expressed by deranged fat, protein and carbohydrate metabolism secondary to insulin resistance. Early identification of insulin resistance plays a major role in diminishing the associated complications. Biochemically, obesity is linked with a low grade systemic inflammation, where the adipose tissue secretes several chemical mediators, one of them being TNF-α.5-7

TNF-α generates insulin resistance by influencing phosphorylation of insulin receptors and their substrate.7-9

The pathogenesis of insulin resistance is characterised by adipose tissue inflammation,17 so obesity and T2DM are strongly correlated. Evidences suggest that Indians are genetically more prone to diabetes10,11 and have greater degree of insulin resistance.

With highest concentrations in fatty and lymphoid tissues, ADA distribution varies between different tissues.18

Adenosine is converted to inosine by ADA. Adenosine increases glucose uptake in cells12 and hence ADA contributes to insulin resistance.14

Various different methods are used for insulin resistance determination but they cannot be used for all patients of T2DM, the reason being various complexities associated with those methods. This study carries with it the objective of assessing the serum level of ADA in the pathogenesis of insulin resistance in T2DM (obese and non-obese) patients, as well as to see whether ADA can be used to as a predictive biochemical marker of T2DM in obese subjects.

Elevated ADA levels in serum of T2DM patients has been reported by previous studies.19-23

In the present study the ADA levels are coherent with previous work done by other researchers and the findings displayed that ADA activity is significantly increased in T2DM patients (obese and non-obese) as compared to that of non-obese control subjects.

In our awareness there are very few studies evaluating ADA activity in obesity.20,24,25 They have found significantly increased ADA activity was in serum of obese and overweight subjects in comparison to that of non-obese control subjects.

In our study, a significant difference between the ADA levels of the obese subjects and as compared to control was found.

In our awareness, only one24 study has estimated serum ADA activity in obese non-diabetic, obese diabetic and diabetic subjects and their results were significantly higher as compared to controls.

The results of the present study are consistent with the aforementioned study. Although difference in serum ADA levels of non-obese T2DM and obese T2DM subjects is not significant in our study, we found significant increase in serum ADA activity in T2DM (obese and non-obese) patients as compared to non-diabetic.

Inflammation plays a minor role in non-obese subjects due to lesser amount of adipose tissues, ADA levels are considered to play a vital role in them in comparison with obese patients.

As adipose tissue inflammation is the trademark of insulin resistance in obese T2DM patients and ADA has been apparently linked with inflammation, the serum level of ADA in non-obese T2DM are not well defined.

We tried to estimate the role of serum ADA in non-obese T2DM subjects and to find out any correlation with normal and obese subjects.

Adenosine is released as a reaction to the inflammatory condition of obesity and results in subsequent generation of insulin resistance by TNF-α.7-9 Insulin resistance is the main physiological anomaly associated with T2DM. However, increased serum ADA acts on this adenosine and converts it to inosine, thereby elevated concentration of ADA results in decreased glucose uptake into cells. The ADA gene is located on the long arm of chromosome 20 (20 q12-q13.11)26 and this locus is linked to T2DM. There is also proof for connectivity with percentage of body fat and markers spanning ADA locus in humans.27 There may be presence of close relation between insulin resistance produced in obesity and diabetes which develops subsequently in people with an Indian background who are more prone to diabetes.28

Our study also supports this mechanism as we found increased ADA in both obese and non-obese T2DM and obese non-diabetic subjects as compared to control.

Conclusion

It has been established that diabetes mellitus patients show elevated ADA. Thereupon we report that increase in ADA activity is more than what was anticipated in obesity. In conclusion, our study shows elevated serum ADA activity
in obesity and T2DM. A close relation may be present between insulin resistance produced in obese subjects and diabetics, which develops subsequently in Indians who are more prone to develop diabetes. ADA could therefore be used successfully in daily routine lab investigations, especially in obese patients as an predictive marker for assessing the development of insulin resistance and hence diabetes.

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Conflict of Interest:
None.

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