

Study of Adenosine Deaminase Activity and Inflammatory Status in Gestational Diabetes Mellitus

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Abstract

OBJECTIVE: To determine the serum levels of adenosine deaminase (ADA) and inflammatory marker in normal pregnancy, nonpregnant women and gestational diabetes.

METHODS: 40 pregnant women, 40 normal non-pregnant women, 40 gestational diabetes were recruited for the study. Serum adenosine deaminase enzyme (ADA) activity was measured by the Giusti and Galanti method and IL -6 by ELISA in all study subjects.

RESULTS: The mean serum ADA level in the gestational diabetes was higher than that in the normal pregnant women. Correlation was found between insulin resistance with serum ADA and IL-6.

CONCLUSION: 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 Gestational diabetes.

Keywords: Adenosine deaminase, Insulin resistance, Cytokines

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a glucose intolerance of varying severity with onset or first recognition during pregnancy that complicates approximately 2–4% of pregnancies [1]. It is a significant cause of fetal macrosomia, perinatal mortality, and long-term maternal risk of developing type 2 diabetes [2]. GDM is characterized by hyperglycemia, insulin resistance, and hyperlipidemia, biochemical abnormalities that are common to type 2 diabetes. Gestational diabetes mellitus has been considered a prediabetic state [3], and the pathophysiology of the two is clearly related.

The prevalence of GDM is increasing worldwide especially in developing countries. In India, the prevalence of GDM is high and varies according to geographical areas and diagnostic methods employed [4]. According to a random national survey conducted in 2004 the prevalence was 16.55% [5]. In 2008, a hospital based survey showed a combined prevalence of GDM and IGT to be 21.6% [6]

Adenosine deaminase (ADA) is a purine metabolic enzyme that catalyzes the deamination of adenosine to inosine, thereby involved in the regulation of intracellular and extra cellular concentrations of adenosine, and probably modulates energy metabolism [7-9]. The physiological function of ADA is crucial in regulating the steady state concentrations of adenosine in a variety of systems, especially immunology, neurological and cardiovascular systems [9]. Adenosine is proved to be an anti-inflammatory agent (10). It is also found to directly modulates insulin activity via several processes such as glucose transport, lipid synthesis, pyruvate dehydrogenase activity, on and cyclic nucleotide phosphodiesterase activity [11]. Therefore, adenosine and ADA play an important role for modulating the bioactivity of insulin [12].

Hoshino et al. (13) reported a significant elevation in serum ADA activity in diabetic patients. In addition to it, recently Kurtul et al. [14] reported significant increase in ADA activity in serum of patients with type 2 diabetes compared to normal healthy controls. There is also evidence for higher levels of endogenous extra cellular adenosine in obese animals [15].

During pregnancy, one of the most significant maternal metabolic adaptations is a decrease in insulin sensitivity. Studies with the hyperinsulinemic-euglycemic clamp technique indicate that insulin mediated glucose disposal decrease as much as 40-60% from early to late pregnancy [16, 17]. Although pregnancy induced insulin resistance and GDM are generally reversible after pregnancy, 30-50% of women with a history of GDM have been predicted to develop type 2 diabetes in their later life, particularly if they are obese [18,19,20]

Growing evidence in the literature suggests that insulin resistance is the result of an inflammatory milieu. Plasma levels of several markers of inflammation, C - reactive protein, lipopolysaccharide (LPS), interleukin (IL)-6, TNF- α and leptin are elevated in

individuals with obesity and type 2 diabetes [21-24]. TNF- α , has been recognized as the most prominent factor contributing to insulin resistance in obesity and diabetes [25-29].

TNF- α , IL-6 and IL-8 are released from the placenta at term [30] and have been linked to various states of insulin resistance [31]. In addition to performing a physiological role in the fetoplacental unit during pregnancy, cytokines may also play a pathophysiological role if expressed in abnormal amounts or sites. Though the relation between insulin sensitivity and cytokines, ADA and insulin activity has been studied, reports studying their inter relation-ship are not available.

With this background, we want to explore status of ADA in GDM cases of our region and to study the inter-relation between ADA activity in insulin resistance and inflammatory cytokines.

MATERIALS AND METHODS

This study was conducted in Department of Obstetrics and Gynaecology and Department of Biochemistry, ESIC medical college, Chennai. The project was approved by institutional ethics committee and written informed consent was obtained from all patients and healthy individuals.

Inclusion criteria

- Group 1- 40 Antenatal woman of age group between 20-35 years of gestational age 20-35 weeks diagnosed as GDM
- Group 2- 40 Age matched healthy pregnant woman who were normoglycemic and not suffering from any diseases complicating pregnancy.
- Group 3- 40 age matched healthy non pregnant woman

Exclusion criteria were type 1 and 2 diabetes, Rheumatoid arthritis, Sarcoidosis, Tuberculosis, SLE, Hydatidiform mole, Preeclampsia etc) will be excluded from our study.

Fifty grams of oral glucose challenge test (OGCT) was performed at 26–28 weeks of gestation and the 1-h venous plasma glucose concentration higher than 140 mg/dl was considered as a positive screening result. Individual with a positive OGCT subsequently underwent a 100 g 3-h oral glucose tolerance test (OGTT), which was considered as the actual diagnostic test for GDM [32].The fasting sample was centrifuged and collected serum was stored at -20°C for further analysis.

Estimation of Serum Glucose by glucose Oxidase & Peroxidase (GOD-POD) [33]. Estimation of Fasting insulin by Fasting insulin –ELISA method [34]. Estimation of Adenosine deaminase (ADA) ADA -Giusti's method [35]. Cytokine (IL-6) IL-6 -

ELISA method [36]. Insulin Resistance HOMA-IR = fasting glucose (mmol/l) × fasting insulin (µg/ml) / 22.5 [37].

STATISTICAL ANALYSIS

Statistical analysis was performed by commercial software (SPSS for Windows, V 17). The results were expressed as mean ± SD, and data were analyzed using one-way ANOVA and the Tukey's multiple comparison tests. Pearson's correlation coefficient was used to determine the correlation between the parameters. *P value* <0.05 were considered statistically significant.

RESULTS

In table 1 we can see significant weight increase in gestational diabetic women compared to normal pregnant women. BMI was also increased in gestational diabetic women compared to normal pregnant women. Table 2 shows significant increase in blood glucose levels in gestational diabetic mothers compared to normal pregnancy. High insulin levels and significant insulin resistance was seen in gestational diabetic women compared to normal pregnant women and non pregnant women. Table 3 shows significant correlation of adenosine deaminase with insulin resistance and IL-6.

Table 1: Demographic characteristic of normal women, normal pregnant women and gestational diabetes mellitus (GDM)

	Non pregnant women	Pregnant women	Gestational DM
Number of cases	40	40	40
Age (year)	27.2±5.6	26.1±4.1	29.1±5.5
Gestational age(week)		30.3 ±7.0	29.2 ±6.5
Ht (cm)	161.8± 4.3	163.3 ±8.6	164.5 ±6.7
Wgt (kg)	58.6±6.4	64.9±11.5*	69.6±12.2*
BMI	23.3±3	25.4±3.7*	26.6±4.2*
SBP(mm/hg)	110.7±5.9	117.6±6.4*	118±7.5*
DBP(mm/kg)	70.9±8.4	72.8±6.9	73.2±6.1

*p<.05 statistically significant

Table 2: Laboratory characteristic of normal women, normal pregnant women and gestational diabetes mellitus (GDM)

Analyte	Non pregnant women	Pregnant women	Gestational diabetes mellitus
FASTING BLOOD GLUCOSE (MG/DL)	80.2±8.7	81.5±8.7	142.8±4.6 a* b*
INSULIN μ IU/ML	8.7±1.9	10.9±4.6	14.4±4.9 a* b*
INSULIN RESISTANCE (IR)	1.7±.4	2.1±.7	5.1±1.2 a*b*

a*p<.01 compared to nonpregnant women

b*p<.01 compared to pregnant women

Table 3: Correlation in GDM (Gestational DM)

VARIABLE	R VALUE	P VALUE
ADA vs. IR	0.559	0.016*
IL-6 vs. IR	0.523	0.026*

*p<.05 statistically significant

Figure1 shows the comparison of ADA in the three groups. ADA is found to be significantly increased in both normal pregnancy and gestational diabetes. Figure 2 shows the comparison of IL-6 in the three groups. IL-6 is found to be significantly increased in both normal pregnancy and gestational diabetes. But IL-6 is comparatively increased more significantly in gestational diabetes compared to normal pregnant women

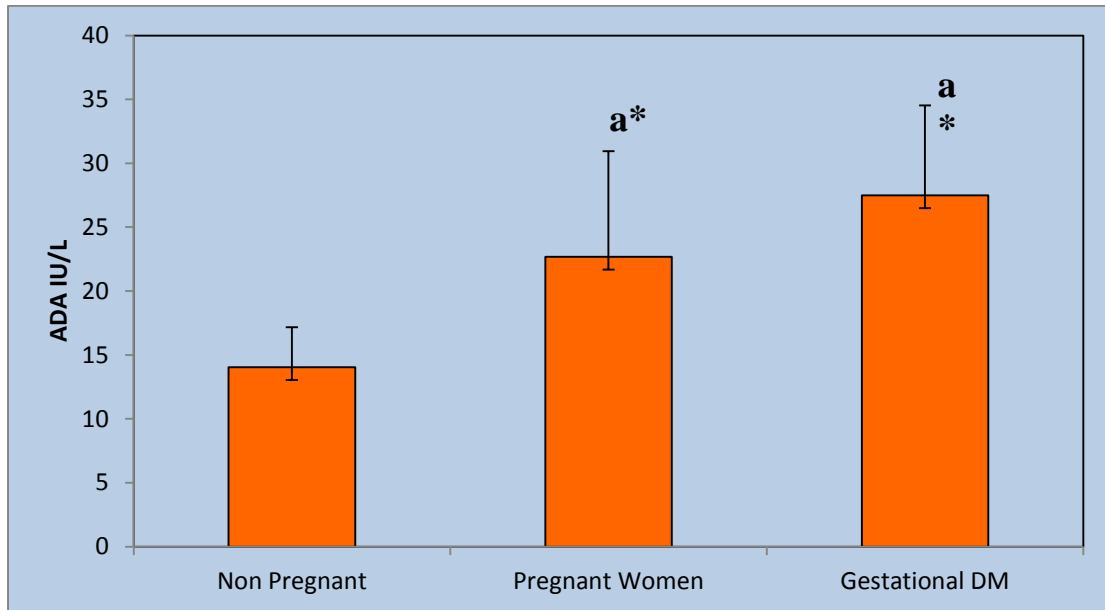


Figure 1: Comparison of ADA in the Groups
(a* p<.01 compared to nonpregnant)

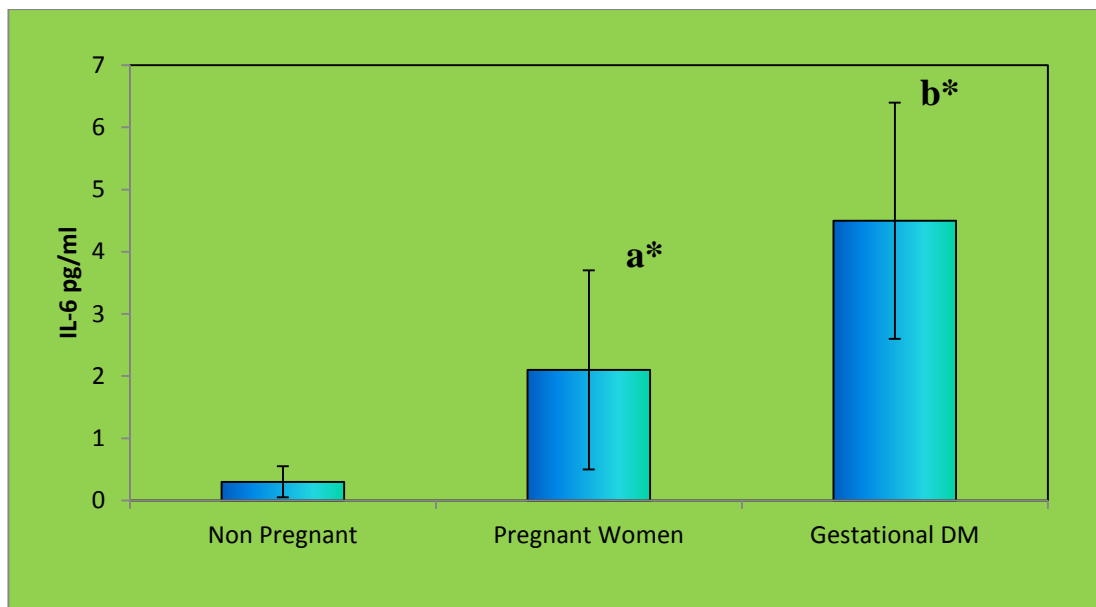


Figure 2: Comparison of IL-6 in the groups
(a* p<.01 compared to nonpregnant b* p<.001 compared to pregnant)

DISCUSSION

A significant increase in ADA was found in GDM and normal pregnant women than normal non-pregnant women, while there was no significant difference between GDM and normal pregnant group in ADA activity. Our results are consistent with a study of Henkiewicz and Michalski, which had found that the serum ADA in normal pregnant women was significantly higher than that of non-pregnant women.

Adenosine deaminase plays a crucial role in lymphocyte proliferation and differentiation [38] and shows its highest activity in T- lymphocytes [39]. 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 [40], 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 [39], 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.

A close correlation has been found between the severity of inflammation and a local increase in both expression and activity of ADA. Cytokine-induced insulin resistance appears to be a primary mechanism underlying the association between inflammation and glucose intolerance. Besides, cytokines exert their effect on insulin resistance by interfering with the insulin receptor–signaling cascade. Adenosine deaminase activity is mainly located in haematopoietic cells, such as T helper 1 (Th1) cells, monocytes and macrophages [41]. T helper (Th) cells play central regulatory roles in the immune response during pregnancy. In addition, Th1 and Th2 cells produce various cytokines that regulate membrane adenosine deaminase on human lymphocytes [42]. Th1 cytokines stimulate and Th2 cytokines inhibit adenosine deaminase expression on lymphocytes.

Besides, cytokines exert their effect on insulin resistance by interfering with the insulin receptor–signalling cascade. The insulin receptor is a protein tyrosine kinase that upon binding to insulin results in the phosphorylation of intracellular substrates. Upon phosphorylation, these substrates interact and activate other molecules that modulate intracellular metabolism. Cytokines can directly interfere with the intracellular signaling cascade of the insulin receptor [43]. In GDM pregnancy IL-6 increase is caused by the oxidative stress and the inflammation associated with the hyperglycemia.

CONCLUSION

Therefore, our observation showed an increase in ADA activity in pregnant and GDM group compared with normal non-pregnant group. This may be due to the cytokine imbalance in our case groups which may affect ADA expression and activity

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