Original Research Article

Altered Interleukin 6 level in Gestational Diabetes across Eastern India

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Abstract

Background: Gestational diabetes mellitus (GDM) is the most common pregnancy-associated metabolic disorder that is steadily increasing worldwide. Early diagnosis of pregnant women susceptible to GDM is the first step for deploying effective preventive treatment to reduce maternal, fetal, and neonatal complications. **Materials & Methods:** The cross-sectional study consists of 52 pregnant females routinely examined for GDM with a 75 g 2 hour oral glucose tolerance test (OGTT) at the gynecological out-patient clinic were taken as cases whereas 48 women with normal glucose tolerance (NGT) were taken as control subjects in ICARE Institute of Medical Sciences and Research, Haldia. The cases as well as controls were matched for age, gestational age as well as BMI for this study. Overnight fasting venous blood samples were obtained from all participants by arm venous puncture to assess serum IL-6 levels and other biochemical parameters in the second trimester (24–28th weeks of gestation) during GDM screening. The samples were primarily stored at room temperature for 30 min to allow the blood to clot, followed by centrifugation at 2500 rpm for 15-20 min to separate serum. Serum specimens were aliquoted and stored at −80 °C until IL-6 levels were analyzed. Glucose levels were measured with the hexokinase method using a commercially available kit whereas Insulin levels were determined using a chemiluminescent assay (Beckman Coulter, CA). Serum IL-6 levels were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Raybiotech, USA). **Results:** There was no significant difference in age and BMI in either of the two groups between GDM cases and control subjects (Table 1). OGTT levels were elevated in GDM cases as compared to controls which were found statistically significant. Serum IL-6 levels were increased in GDM cases as compared to controls and were statistically significant (7.85 ± 4.71 vs 4.27 ± 1.85 pg/ml; $p < 0.0001$). It was also observed that serum Insulin levels were higher in GDM cases as compared to controls which was statistically significant (17.14 ± 7.53 versus 9.61 ± 3.97 µ IU/ml; $P < 0.0001$). However, no correlation was observed between serum insulin with serum IL 6 level ($r = 0.141$; $P = 0.398$) among GDM subjects. **Conclusion:** Despite correlation has not been established between IL-6 and insulin in GDM cases there is a sharp rise in their levels indicating early screening of these markers could be of diagnostic importance in the prevention of the pathogenesis of Gestational diabetes. Moreover, a large longitudinal study needs to be done to conclude the fact.

Keywords: Gestational diabetes, interleukin 6 (IL-6), oral glucose tolerance test (OGTT), normal glucose tolerance (NGT)

Introduction

Gestational diabetes mellitus (GDM) known to be a metabolic disorder during pregnancy leads to acute and chronic complications due to glucose intolerance in both mother and newborn currently obscure nearly 18% of pregnancies worldwide with an estimate of 25% in Southeast Asia [1, 2]. Moreover, it is a heterogeneous disorder due to exchanges between environmental and genetic factors. Maternal hyperglycaemia may plausibly be linked with higher rates of both birth weight and cord blood, C-peptide levels above the 90th percentile, primary caesarean section, clinical neonatal hypoglycaemia, shoulder dystocia and birth injury, premature delivery, neonatal hyperbilirubinaemia, preeclampsia and neonatal intensive care admission [3]. The prevalence of GDM is increasing due to associated factors such as Obesity and advanced maternal age worldwide. Moreover, it enhances the potential risk of type 2 diabetes onset in the mother and her offspring [4]. The pathogenesis of GDM has not been fully understood, However, since the disease shares risk factors with type 2 diabetes mellitus (T2DM), a relationship between these two disease states is

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plausible [1]. Interleukin 6 (IL -6), a cytokine which is produced by immune, adipose, and endothelial cells have significant effects on glucose metabolism as well as affects pancreatic islet beta cells further leads to enhanced insulin secretion [5]. Moreover, pro-inflammatory markers such as IL-6 have also been implicated in the pathogenesis of type 2 diabetes [6]. There were various studies done on the association of IL-6 with GDM. Few studies have shown a statistically significant association between elevated IL-6 levels and GDM while others did not report such relationships [4, 7-10]. Therefore, we aimed to investigate the relationship between IL-6 levels and GDM and the possible benefits of the metabolic profile.

Materials & Methods

The cross-sectional study consists of 52 pregnant females routinely examined for GDM with a 75 g 2 hour oral glucose tolerance test (OGTT) at the gynecological out-patient clinic were taken as cases whereas 48 women with normal glucose tolerance (NGT) were taken as control subjects in ICARE Institute of Medical Sciences and Research, Haldia. The cases as well as controls were matched for age, gestational age as well as BMI for this study. The diagnosis of GDM was made if one or more plasma glucose levels were elevated during a 75 g, 2 h oral glucose tolerance test (OGTT) according to the criteria of the American Diabetes Association [11]. The following threshold plasma glucose levels were used: fasting ≥100 mg/dl (5.5 mmol/l), 1 h ≥ 180 mg/dl (10.0 mmol/l) and 2 h ≥ 140 mg/dl (7.8 mmol/l). Patients with multiple pregnancy, pre-existing glucose intolerance, pregnancy-induced hypertension, bronchial asthma, preeclampsia, acute or chronic inflammation, as well as active smokers were not included. Routine biochemical parameters were done for both cases as well as controls. Informed consent was taken by cases and control groups. The study was approved by the Institutional Ethics committee.

Overnight fasting venous blood samples were obtained from all participants by arm venous puncture to assess serum IL-6 levels and other biochemical parameters in the second trimester (24–28th weeks of gestation) during GDM screening. The samples were primarily stored at room temperature for 30 min to allow the blood to clot, followed by centrifugation at 2500 rpm for 15 – 20 min to separate serum. Serum specimens were aliquoted and stored at −80 °C until IL-6 levels were analyzed. Glucose levels were measured with the hexokinase method using a commercially available kit whereas Insulin levels were determined using a chemiluminescent assay (Beckman Coulter, CA).

Serum IL-6 levels were assayed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Raybiotech, USA).

Statistical analysis of different biochemical parameters was performed by Students' *t*-test. All variables were expressed as mean ± SD (standard deviation). Means obtained from two normally distributed sample groups were compared by Student's unpaired two-tailed "*t*"-test and for nonparametric Mann-Whitney *U* "*t*" test. To find out the correlation between two variables, Pearson's product moment correlation coefficient was used. A value of $P < 0.05$ was considered as statistically significant. All statistical analyses were performed by using Graph Pad prism software (version 5, 2007, San Diego, California, USA).

Results

The demographic and biochemical profile of the GDM subjects and healthy controls is presented in Table 1. There was no significant difference in age and BMI in either of the two groups between GDM cases and control subjects (Table 1). OGTT levels were elevated in GDM cases as compared to controls which were found statistically significant (Table 1).

Serum IL-6 levels were increased in GDM cases as compared to controls and were statistically significant (7.85 ± 4.71 vs 4.27 ± 1.85 pg/ml; $p < 0.0001$) (Figure 1). It was also observed that serum Insulin levels were higher in GDM cases as compared to controls which was statistically significant (17.14 ± 7.53 versus 9.61 ± 3.97 µ IU/ml; $P < 0.0001$) (Figure 2). However, no correlation was observed between serum insulin with serum IL 6 level ($r = 0.141$; $P = 0.398$) among GDM subjects.

Table 1: Demographic and biochemical profile of the subjects

	Control (n = 48)	Cases (n = 52)
Age	26.82 ± 5.91	28.91 ± 4.72
BMI	25.72 ± 2.84	26.62 ± 1.82
Gestational age at blood sampling (in weeks)	25.78 ± 1.46	25.62 ± 1.54
FBG (mg/dl)	84.23 ± 7.62	106.7 ± 11.36 *
OGTT 2 h (mg/dl)	115.2 ± 9.24	148.64 ± 19.44*
Total Cholesterol (mg/dl)	192.84 ± 22.18	212.26 ± 38.2

FBG, fasting blood glucose; oral glucose tolerance test (OGTT); Age, BMI, and serum levels of biochemical parameters were expressed as the means ± SD. Statistically significant, * $p < 0.001$ vs Control.

Discussion

GDM is recognised by an amplification of the low-grade inflammation which exists in normal pregnancy. The role of inflammatory markers has been emerging and plays a crucial factor in the pathogenesis of T2DM and GDM. On the other hand, Insulin resistance has been linked with abnormal secretion of proinflammatory cytokines such as TNF-a and interleukin IL-6 and decreased production of anti-inflammatory mediators such as IL-4 and IL-10. It is also a fact that T2DM at present is regarded as a chronic inflammatory disease. As there is simultaneous association between T2DM and GDM as well as inflammation it may be presumed that inflammation may be associated in the patho-physiology of GDM.[12] Moreover, IL-6, a pro- inflammatory cytokine encoded by IL-6 gene in humans, secreted by T cells and macrophages to stimulate immunity as well as plays a vital role in the fight against infection[16,17]. IL-6 is produced as a proinflammatory cytokine in muscle cells within the tunica media layers of several blood vessels. IL-6 acts as a first stimulator of acute phase proteins and also acts as an anti-inflammatory cytokine mediated through the activation of IL-10 by inhibitory effects on TNF-α [18].Furthermore, IL-6 stimulates the production of the IL-1 receptor antagonist, which acts an anti-inflammatory mediator[19].Our study reveals that the serum IL-6 levels were found to be higher in GDM subjects which were statistically significant with the control group (Figure 1). Further serum insulin was found to be higher in GDM cases than controls (Fig. 2). This suggests that the proinflammatory protein has a potential role in acute as well as chronic inflammation. Moreover, the activation of inflammatory cascade triggered by cytokines and characterised by the influx of neutrophils and myriad of IL 6 trans-signalling play an important role in the pathogenesis of GDM [20]. A study by Kuzmicki et al. found higher IL-6 levels measured between weeks 24 and 31 in women with GDM compared to control subjects [21]. Another study demonstrated significantly higher IL-6 levels at 27 weeks of pregnancy in GDM patients compared with control subjects from the same group [22]. Moreover, serum insulin and glucose levels were also significantly higher in GDM cases as

compared to controls[21,22]. Our study are also shows the same and were in agreement with the above studies. Another study by Lappas et al. did not show any difference in release of IL-6 from placenta, adipose tissue or skeletal muscle from normal pregnant women and GDM subjects [23]. It might be plausible that IL-6 may have a protective effect. Thus a rise in IL-6 during pregnancy may be associated with gestational insulin resistance, particularly due to placental production [24]. Few studies done earlier have found a positive correlation between the concentration of IL-6, insulin sensitivity and plasma glucose levels, and gestational and postpartum body fat percentages [25-27]. In case-control studies, plasma IL-6 levels are a significant predictor of GDM [28]. The mechanism of IL-6 associated with gestational diabetes may be due the inflammation of macrophages in the pancreas and adipocytes which causes an increase in the production of IL-6. Associated immunocytes linked to IL-6 also contributes to infiltration [29]. Thus the destruction of pancreatic β-cells results in decreased synthesis of insulin and further leads to apoptosis resulting in high levels of blood glucose [30-31].

Serum IL-6 levels were significantly high (p<0.05) in GDM females as compared to non-GDM females. The IL-6 levels correlated with pre-pregnancy body mass index (BMI), fasting blood sugar (FBS) levels and postprandial sugar (PPBS) levels. Unlike IL-6, CRP levels did not show significant differences between the GDM and non-GDM females. However, we did observe a positive correlation of CRP levels with BMI, FBS and PPBS. GDM group also showed association with age, BMI and family history of diabetes. The occurrence of GDM was observed more in the females > 30 years of age in our population subset. High IL-6 levels in gestational diabetes may indicate a possible role for inflammation in pathophysiology of GDM [32].

There were few limitations in our study which needs to be mentioned. The sample size of the study was less. Secondly, few of the patients were taking some other drugs such as antihistamines, topical corticosteroids which might interfere with serum IL-6 or Insulin levels. Despite these limitations it has been observed that serum IL-6 levels and serum Insulin levels were higher in GDM patients.

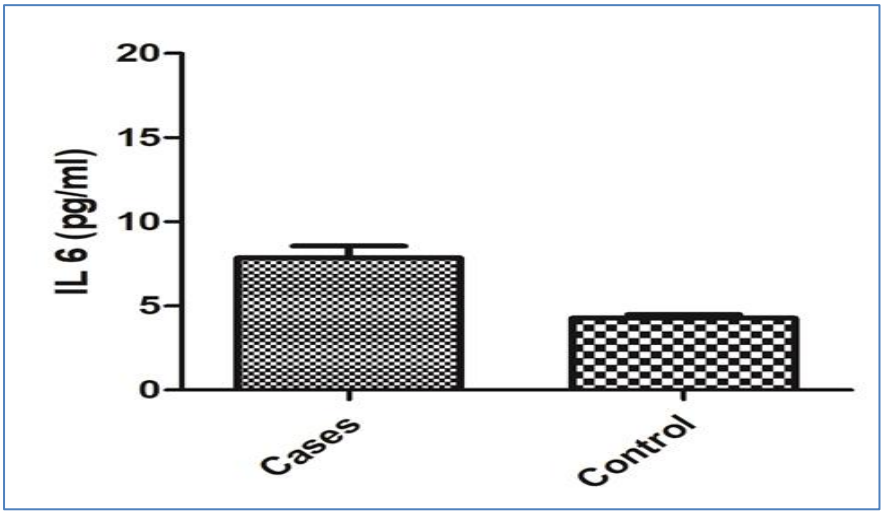

Fig 1: Serum levels of IL-6 in control and GDM subjects.

Serum levels of IL-6 were determined as described in methods for control and GDM subjects. Values expressed as the means ± SD. Statistically significant, * p < 0.0001, vs GDM.

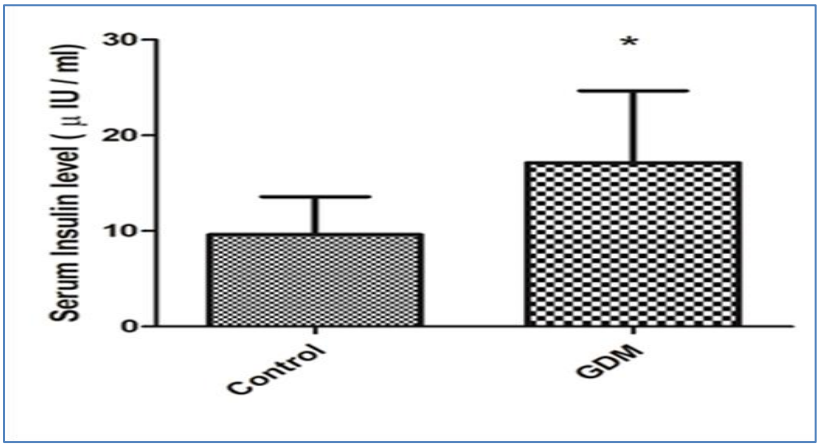

Fig 2: Serum levels of Insulin in control and GDM subjects

Serum levels of Insulin were determined as described in methods for control and GDM subjects. Values expressed as the means ± SD. Statistically significant, * p < 0.0001, vs GDM.

Conclusion

Despite correlation has not been established between IL-6 and insulin in GDM cases there is a sharp rise in their levels indicating early screening of these markers could be of diagnostic importance in the prevention of the pathogenesis of Gestational diabetes. Moreover, a large longitudinal study needs to be done to conclude the fact. Based on the results of the present study, assessing the serum IL-6 levels can be investigated a newly established diagnostic biomarker for GDM. Therefore, through early diagnosis of susceptible women, effective measures can be implemented to reduce its complications.

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