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Original Article

Augmentation of IL-6 production contributes to development of gestational diabetes mellitus: An Indian study

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ABSTRACT

Aim: Inflammatory mediators like interleukin-6 (IL-6) and acute phase protein like C-reactive protein (CRP) are supposed to contribute to development of GDM, however clinical data supporting this hypothesis is limited. This study was designed to analyze the association of IL-6 and CRP with development of GDM in Indian females.

Methods: This case control study included pregnant women diagnosed as GDM (n = 53) and those having normal glucose tolerance (n = 50). Serum levels of IL-6 and CRP were analysed and correlated with various clinical parameters.

Results: Serum IL-6 levels were significantly high ($p < 0.05$) in GDM females as compared to control females. IL-6 levels correlated with pre-pregnancy body mass index (BMI), fasting blood sugar (FBS) and postprandial sugar (PPBS). Unlike IL-6, CRP levels did not show significant differences between GDM and control females. However, positive correlation of CRP levels with BMI, FBS and PPBS was observed.

Conclusion: High IL-6 levels in gestational diabetes may indicate a possible role for inflammation in pathophysiology of GDM.

1. Introduction

Gestational Diabetes Mellitus (GDM) is a common clinical condition characterized by glucose intolerance of varying severity in the second or third trimester of pregnancy [1]. It is increasing all over the world [2], therefore it is important to investigate the underlying mechanisms. Studies have also shown that South Asian females particularly those from India are at higher risk for developing GDM (11-fold higher) as compared to the European females [3]. In India, it is estimated that nearly 4 million women are affected by GDM at any given time point [4] and the prevalence of GDM is increasing steadily from 2% in 1982, 7.62% in 1991 to 16.5% in 2003. It is anticipated that the prevalence will reach 79.4 million by the year 2025 [3,5]. GDM is associated with maternal complications such as preeclampsia, polyhydramnios, infection and fetal complications like sudden intra-uterine demise, macrosomia, hypoglycemia, hyperbilirubinemia, birth trauma and respiratory distress. GDM also increases the risk of developing type 2 diabetes later in life [6,7]. Considering the complications and high prevalence rate associated with GDM, it is imperative to determine the factors contributing to pathophysiology of the disease.

Metabolic changes are a normal feature in pregnancy and are primarily attributed to placental derived hormones [8]. However, elevated secretion of proinflammatory cytokines in pregnancy may disrupt insulin signalling and induce development of GDM [9]. The production of proinflammatory factors may be further amplified in obesity [10]. Several studies in literature have demonstrated a definite link between increased inflammation and development of GDM [11]. However, despite the presence of number of studies on inflammatory markers in GDM, the literature is largely insufficient to draw conclusions on association of inflammation with GDM. In addition, studies have largely overlooked the role of factors like BMI and glucose levels in inducing inflammation and GDM. We, therefore undertook this study, to analyze the association of inflammatory mediators like IL-6 and CRP with development of GDM in Indian females. We also investigated the correlation of these factors

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with variables like BMI and glucose levels in pregnant women with GDM and pregnant women with normal glucose tolerance.

2. Materials and methods

The study included 53 patients with GDM and 50 pregnant women with Normal Glucose Tolerance (NGT), between 24 and 31 weeks of gestation, attending the Gynaecology and Obstetrics clinic at Max Super Speciality Hospital, Saket, New Delhi from July 2015–May 2017. All subjects were Indians. The diagnosis of GDM was done according to American Diabetes Association screening criteria [1]. Blood samples for immunoassay were collected before the initiation of diet or insulin therapy. Serum samples were stored at −70 °C until analysed. All women were non-smokers and had not taken any drugs known to affect carbohydrate metabolism in the previous 3 months. Patients with abnormal glucose readings before pregnancy, as well as with pregnancy-induced hypertension, pre-eclampsia and other pregnancy complications (except GDM) were excluded from the study. Written informed consent was taken from each subject and protocol was approved by institutional ethics committee. The estimation of pregnancy duration was based on routine ultrasonographic examination preformed between 12 and 26 weeks of gestation. Control group included non-GDM pregnant females attending the Gynaecology and Obstetrics clinic at Max Super Speciality Hospital, Saket, New Delhi.

2.1. Anthropometric and biochemical measurements

The information on maternal age and pre-pregnancy weight were collected from patient records. Maternal height and weight were measured by standard methods, and body mass index (BMI) was calculated by using the formula; weight in kilograms divided by height in meters squared. Plasma glucose was measured by the glucose oxidase method, glycosylated haemoglobin (HbA1c) was assayed by BIORAD Immunoassay method.

2.2. Estimation of IL-6 and CRP

Briefly, serum was separated from **v**enous blood and stored at −70 °C until analysed. Each serum sample was run in duplicates for immunoassays. Estimation of IL-6 and CRP was done using commercially available immunoassay kits for IL-6 (Diaclone) and CRP (Xema Ultra-CRP analysis). The minimum detectable dose of IL-6 using Diaclone IL-6 ELIZA kit was 2 pg/ml and the sensitivity of the assay using Xema Ultra-CRP analysis kit was 0.05 mg/l. Linear standard curves were prepared by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-6 or CRP standard concentration on the horizontal axis. Standard curve for IL-6 and CRP was fitted using 4 parameter logistic (4 PL) regression analysis. Actual concentration of IL-6 and CRP in patient samples was calculated by extrapolating OD values using the 4 PL curve.

2.3. Ethics approval

This study was approved by the Scientific and Institutional Ethics Committees of Max Super Speciality Hospital, Saket, New Delhi (Ref No.: TS/MSSH/DDF/ENDO/IEC/15–19). Written informed consent was taken from all the subjects and only those women who elected to participate were enrolled into the study.

2.4. Statistical analysis

The SPSS software version 16.0 was used to analyse the data.-Normality test for each variable tested was performed by Shapiro–Wilk test. When the data did not follow normality assumptions, one-way ANOVA was performed on log-transformed data. Mean of FBS, Age, Height, pre-pregnancy weight, BMI, Blood Pressure (Systolic and Diastolic), post-prandial blood sugar (PPBS), total leucocyte count (TLC), Platelet and Thyroid Stimulating Hormone (TSH) was compared between the two groups (GDM & non-GDM) by independent student t-test or mann whitney U test. Relationships between IL-6 and CRP with other variables (Age, pre-pregnancy weight, BMI, FBS, PPBS, HbA1c and TSH) were tested by Spearman's rank correlations. All statistical comparisons were considered significant if the p values were less than 0.05 ($p < 0.05$). Graph pad software was used to make graphs. Continuous variables like age, height, weight, BMI, FBS, PPBS, Haemoglobin (Hb), TSH, platelet, TLC and HbA1c were expressed as Mean ± SD.

3. Results

3.1. Characteristics of study population

Demographic and biochemical characteristics of study subjects are listed in Table 1. Mean age of study participants was 30.74 ± 3.91 years. Maternal age and pre-pregnancy BMI were significantly higher in GDM group as compared to control group (Table 1). Mean fasting blood glucose, random blood sugar levels and glycosylated haemoglobin were significantly higher in GDM group as compared to non GDM group. Significantly higher percentage of women with GDM had family history of diabetes as compared to control group. Gestational age and parity were not significantly different between GDM and non-GDM groups. Females in GDM group were also analysed based on their age. Proportion of women who developed GDM was significantly higher in age group of >30 as compared to those in <30 ($p = 0.01$). Approximately, 73% females who were diagnosed as GDM were in the age group of >30 years. 20.75% (11/53) of them were on insulin during pregnancy, with 13.21% (7/53) on insulin plus oral hypoglycaemic agents (OHAs), 18.87% (10/53) on OHAs and rest (47.17%, 25/53) were managed on diet only.

3.2. Association of maternal serum Interleukin-6 (IL-6) and C-reactive protein (CRP) concentration with disease parameters

Significantly increased levels of mean log IL-6 were observed in GDM group (0.72 ± 0.67 pg/ml) as compared to non-GDM group (0.39 ± 0.70 pg/mL); ($p = 0.001$). Fig. 1 represents comparison of mean ± SEM values of log transformed IL-6 levels (pg/ml) in GDM and non-GDM groups. Correlation analysis of IL-6 (Table 2) with various parameters indicated a positive association of IL-6 with pre-pregnancy BMI ($r = 0.289$, $p = 0.003$), fasting blood sugar ($r = 0.249$, $p = 0.011$) and PPBS ($r = 0.237$, $p = 0.016$). Fig. 2 represents the correlation analysis of IL-6 with BMI, FBS and PPBS. Mean CRP levels were comparable in GDM group (6.615 ± 5.195 mg/dL) *vs* control group (5.176 ± 4.216 mg/dL) $p = 0.104$).

3.3. IL-6 levels increase with BMI in GDM and non-GDM females

Further comparison of IL-6 and CRP was done by stratifying the GDM and non GDM females on basis of BMI. GDM and non-GDM group females were divided in three groups based on BMI <25, 25–29.9, >30 kg/m^2. GDM females in all three BMI groups exhibited high IL-6 levels as compared to control females in corresponding BMI group (Fig. 3). Mean values of log IL-6 were significantly different between GDM and non-GDM group in BMI<25 kg/m^2 ($p = 0.01$) and BMI>25–29.9 kg/m^2 ($p = 0.03$) groups. Significant proportion of females in control group had

Table 1
Demographic and biochemical characteristics of study population.

S. No	Variable	GDM (n = 53)	Non-GDM (n = 50)	*p*-value
Demographic Parameters				
1.	Age (yrs)	31.77 ± 4.017	29.64 ± 3.498	0.005
2.	Height (cm)	1.59 ± 0.062	1.58 ± 0.055	0.303
3.	Pre-pregnancy Weight (Kg)	64.12 ± 11.76	58.26 ± 7.46	**0.003**
4.	BMI (Kg/m^2)	25.30 ± 4.08	23.28 ± 2.95	0.005
5.	BP (mm of Hg)			
	Systolic	112.28 ± 10.47	109.46 ± 16.41	0.301
	Diastolic	72.03 ± 8.99	71.67 ± 7.09	0.434
6	Smoking	0%	0%	–
7	Parity			
	1	69.81% (37/53)	64% (32/50)	NS
	2	26.41% (14/53)	30% (15/50)	NS
	3	3.77% (2/53)	6% (3/50)	NS
8	Gestational age	25.75 ± 7.04	23.46 ± 7.56	0.114
9	Family history of diabetes	62.26% (33/53)	12% (6/50)	<0.001
Biochemical Parameters				
6.	Hb (g/dL)	11.41 ± 1.174	11.34 ± 1.097	0.731
7.	FBS (mg/dL)	101.45 ± 13.72	81.88 ± 5.95	**0.000**
8.	PPBS (mg/dL)	151.13 ± 23.15	111.62 ± 15.86	**0.000**
9	HbA1c (%)	5.48 ± 0.74	4.94 ± 0.42	**0.001**

Abbreviations: BMI-Body Mass Index; BP– Blood Pressure; Hb- Haemoglobin; FBS– Fasting Blood Sugar; PPBS– Post-prandial Blood Sugar.

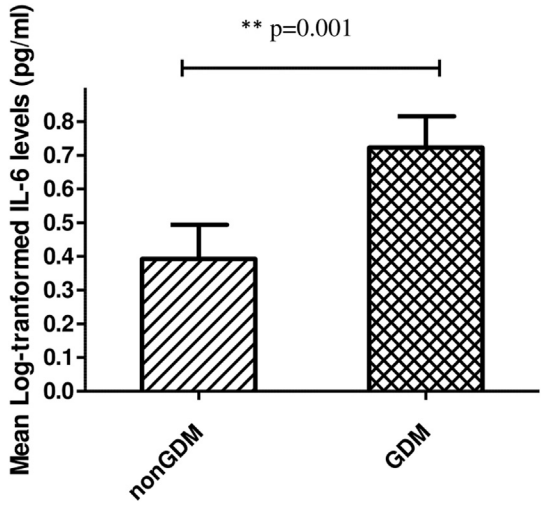

Fig. 1. Interleukin-6 (IL-6) concentrations in gestational diabetes mellitus (GDM) patients compared with control women at the time of GDM screening. Graph represents log transformed mean ± SEM of IL-6 levels in GDM (n = 53) and control (n = 50) group, **p = 0.001 between GDM and control group).

BMI of <25 kg/m^2 (38/50; 76% in control group *vs* 29/53; 60% in GDM group, p = 0.01). Proportion of females in control group having BMI in range of 25–29.9 kg/m^2 was not different from that in GDM group (11/50; 35% in control group vs 16/53; 22% in GDM group, p = 0.17). Fifteen percent females in GDM group (8/53; 15%) had BMI>30 kg/m^2 as compared to only 2% (1/50; 2%) in control group. Since, there was only one female in control group having BMI>30, therefore statistical comparisons could not be done for this group.

4. Discussion

Our study indicates that production of inflammatory mediator such as IL-6 is altered in women with GDM as compared to pregnant females with normal glucose tolerance. Inflammation has now been recognized as one of the key mechanisms that can disrupt insulin signalling and cause diabetes [12,13]. Overt levels of inflammation may serve to amplify pre-existing low levels of inflammation in pregnancy and cause development of GDM. Augmentation of IL-6 production during pregnancy has been linked with GDM in several studies [14–17]. Our results are consistent with other studies which have compared the circulating serum IL-6 levels in women diagnosed with GDM. IL-6 is an inflammatory molecule secreted by monocytes and macrophages in the adipose tissue. The level of IL-6 is also known to increase in obesity causing multiple effects on insulin sensitivity in liver and β-cells of the pancreas, thereby causing insulin resistance [18,19]. During pregnancy, IL-6 produced by placenta may lead to chronic inflammatory process in the adipose tissue contributing to pregnancy-induced insulin resistance [20]. Studies suggest that high body mass index (BMI) and body fat mass are associated with elevated levels of serum IL-6 in non-pregnant females [21,22]. Therefore, we also analysed the correlation of BMI with serum IL-6 levels in the present study. Serum IL-6 levels in our study population were strongly correlated with pre-pregnancy BMI. A small study by Morisset et al. (2011) has also reported positive correlation between circulating IL-6 and BMI [14]. IL-6 levels were also shown to correlate positively with fasting (FBS) as well as post-prandial (PPBS) blood sugar levels.

C-reactive protein (CRP) is one of the acute-phase proteins, synthesized by liver and secreted into the circulation. CRP is used for diagnosis and treatment of inflammatory disorders and is found to be associated with Type 2 diabetes and GDM [23,24]. It is

Table 2
Correlation of IL-6 & CRP with various parameters.

Parameters	IL-6	CRP	Age	Pre-pregnancy BMI	FBS	PPBS	HbA1c	TSH
IL-6	1.000	0.227	0.063	0.289**	0.249*	0.237*	0.157	0.156
CRP	–	1.000	0.137	0.142	0.062	0.171	−0.055	0.148

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

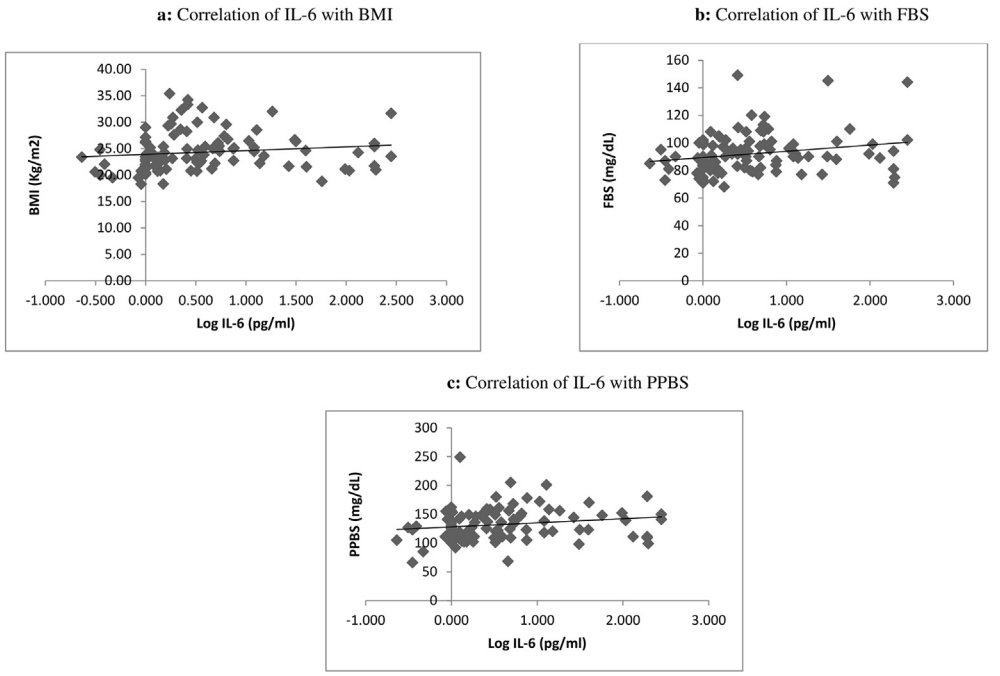

Fig. 2. Correlation of IL-6 with a) BMI, r = 0.289 p = 0.003 b) Fasting blood sugar, r = 0.249 p = 0.011 c) Post Prandial Blood Sugar, r = 0.237 p = 0.016.

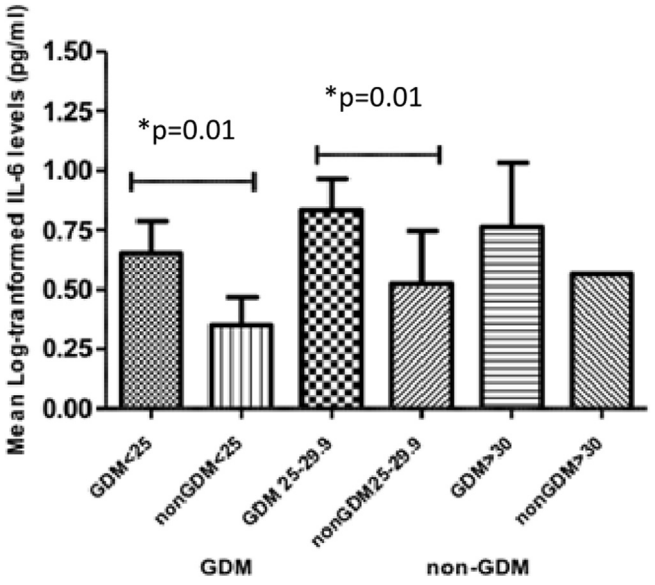

Fig. 3. Variation in IL-6 levels in GDM and non-GDM groups according to BMI levels. The differences in IL-6 levels between GDM and non-GDM groups in <25 kg/m^2 group (*p = 0.01) and 25–29.9 kg/m^2 groups are significant (*p = 0.01).

reported that IL-6 induces CRP production in liver by januskinases [25]. Though, there was difference in the levels of CRP between the GDM and non-GDM groups (4.178 ± 0.045 mg/dL and 3.750 ± 0.043 mg/dL, respectively); the difference was not statistically significant (p = 0.299). A prospective study by Retnakaran et al. (2003) also did not find any significant association between CRP and gestational diabetes. They instead reported a significant correlation between pre-pregnancy obesity and CRP [26]. We also found a positive correlation of CRP with BMI, FBS and PPBS in the present study.

Obesity could lead to adverse pregnancy outcomes including metabolic dysfunction manifested as preeclampsia or GDM [18]. In our study, IL-6 levels were elevated in GDM females having higher BMI as compared to normal pregnant females. This suggests that there is a link between enhanced IL-6 levels and obesity; increase in BMI levels may lead to enhanced IL-6 levels and disturbed insulin signalling leading to development of GDM. We are however unable to comment on whether IL-6 is a cause or effect of increased BMI from our study. A study on healthy individuals by Vittal et al. (2010) has reported a positive correlation of BMI with FBS, which reiterates the effect of adipose tissue in impairing blood glucose metabolism [19]. Our data also suggests a positive correlation of pre-pregnancy BMI with FBS and HbA1c indicating that pre-pregnancy BMI might be an important determinant for development of GDM. Observations from several large sample studies have also reported similar findings [19,22]. Although measurement of HbA1c in pregnancy is controversial and is not a routine practice, but it has been shown to predict adverse pregnancy outcomes [23,24].

GDM is more likely to develop during the second and third trimester of pregnancy because insulin resistance and diabetogenic effect of pregnancy hormones are maximum during this period. As per the standard guidelines of ADA, it is recommended that all pregnant females should be screened for GDM between 24 and 28 weeks of gestation [1]. Therefore, in this study, we screened pregnant females in their second and third trimester of gestation period for GDM to determine the role of inflammatory mediators in pathogenesis of GDM. Based on the data reported in this work, further studies may be initiated in larger sample size to determine the precise association of inflammatory parameters such as IL-6 and CRP with development of GDM. Further, whether elevated level of IL-6 in GDM is a primary reason for development of GDM or an effect induced indirectly by some other factors in GDM also needs to be investigated. In conclusion, our study implicates a possible role for IL-6 in inducing insulin resistance and a crosstalk between obesity and inflammatory mediators in GDM.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2018.12.023.

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