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RESEARCH ARTICLE

Maternal circulating levels of Adipocytokines and insulin resistance as predictors of gestational diabetes mellitus: preliminary findings of a longitudinal descriptive study

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Abstract

Purpose The present study aimed to assess the role of adipocytokines as predictive markers of gestational diabetes mellitus (GDM). We undertook this study to explore the association of adiponectin, resistin, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) and insulin resistance in the development of GDM.

Methods This longitudinal descriptive study was done in the Medical College Hospital, involving the departments of Biochemistry and Gynecology. The study subjects were pregnant women with normal body mass index. They were recruited at 12–14 weeks of gestation, The healthy pregnant women were selected and were followed at 24–28 weeks for screening of GDM. The participants were categorized as healthy and GDM based on oral glucose tolerance test results. Blood samples were collected on both the occasions at 12–14 weeks and 24–28 weeks. The serum samples were analyzed for levels of insulin, adiponectin, resistin, IL-6 and TNF-alpha. Insulin resistance (HOMA-IR) was calculated from serum insulin and plasma glucose values.

Results The levels of HOMA-IR, resistin, IL-6 and TNF-alpha were significantly higher and level of adiponectin was significantly lower in GDM subjects in comparison to healthy pregnant women, when compared both at 12 weeks and 24 weeks. The levels of resistin, IL-6 and TNF-alpha were significantly higher and level of adiponectin was lower at 24 weeks in comparison to 12 weeks, in the healthy pregnant women as well as those with GDM .

Conclusions Significant difference in the levels of adipocytokines between the healthy and GDM pregnant women suggest potential clinical application of these molecules as biomarkers of GDM. The increase or decrease in the levels of adipocytokines during the course of pregnancy in GDM subjects suggests their role in GDM and potential use as predictive markers.

Keywords Adipocytokines · Gestational diabetes mellitus · Insulin resistance · Interleukin-6 · Tumor necrosis factor

Introduction

Gestational diabetes mellitus (GDM) is one of the most common medical conditions associated with pregnancy, and is known to increase the risk of various adverse pregnancy outcomes [1, 2]. GDM also increases the risk of future type 2 diabetes in the mother and her offspring, thereby fuelling the increasing burden of diabetes seen in many countries, including India [3, 4]. GDM has been reported to affect between 1.4% to 12.3% of pregnancies [5], and its prevalence is increasing and parallels the rising incidence of type 2 diabetes mellitus worldwide [6]. Risk factors for developing GDM in pregnancy include obesity, glycosuria, family history, ethnicity and hypertension [7].

Insulin resistance is shown to increase during pregnancy, and has been shown to be the catalyst of GDM in late gestation [8–10]. The molecular and metabolic events that lead to insulin resistance have been proposed to be mediated by pro inflammatory molecules such as cytokines and the regulators of adiposity and metabolism such as adipokines. Adiposity is an important modifiable risk factor for the development of GDM, although mechanisms linking excess adiposity to elevated risk of GDM are not completely understood [11]. Adipose tissue is not only the main energy storage depot but, also an active endocrine organ secreting adipokines [11]. The crosstalk between adipocytes and other insulin target tissues, which is

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mediated by adipokines such as leptin, adiponectin, resistin, retinol-binding protein, tumor necrosis factor-alpha and interleukins, has been proposed to be a major mechanism in the development of insulin resistance during pregnancy. Dysregulation of the network of adipokines, the molecular pathways and the insulin target tissues is the critical factor in the deterioration of insulin sensitivity [11–14].

In recent years, there has been increased interest in the role of inflammation as a mediator of metabolic disarrangements due to maternal obesity. Various studies done on pregnant women with GDM have observed altered levels of inflammatory molecules such as cytokines during the progression of pregnancy [15]. The cytokines TNF-α and IL-6 are inflammatory mediators produced by monocytes and macrophages in the adipose tissue. These molecules are increased in obesity and have multiple effects on insulin sensitivity in muscles, liver, or beta cells of the pancreas, ultimately leading to insulin resistance. In pregnancy, TNF-α and IL-6 production occurs in placenta, while it is considered that a chronic inflammatory process in the adipose tissue may contribute to pregnancy-induced insulin resistance [16, 17].

The pathogenesis of GDM is not completely and clearly understood. The role of adipokines and cytokines in the development of insulin resistance in pregnancy needs to be explored. There is paucity of studies which have correlated the levels of adipokines and cytokines with insulin resistance, especially in the Indian population. In the present study, we have made an attempt to assess the levels of adiponectin, resistin, interleuin-6 (IL-6) and tumor necrosis factor (TNF-alpha) in the serum of healthy pregnant women and pregnant women with gestational diabetes mellitus in the first and third trimester of pregnancy.

Materials and methods

Source of data The present study was done at the Medical College Hospital, involving the Departments of Obstetrics and Gynecology, and Biochemistry. Study subjects were pregnant women. The study was started after obtaining clearance from Institutional Ethics Committee, and voluntary informed consent was obtained from all participants.

Study Design: This was a longitudinal descriptive study.

Sampling Purposive sampling method was adopted. The findings in this study are part of an ongoing study in which 160 each of normal pregnant women and pregnant women with GDM were planned to be recruited during the study period of 2 years from October 2018 to September 2020. In the present paper, findings of the study with 38 each of healthy and GDM pregnant women are presented.

Methods

The initial screening for DM was done during the first trimester. Subjects were recruited at 12 weeks of gestation, and initial screening was done before the completion of 14 weeks. At 24–28 weeks, pregnant women were screened for GDM using 50 g glucose challenge test (GCT), further confirmed by 100 g oral glucose tolerance test (OGTT) as per International Association of the Diabetes and Pregnancy Study Groups *(I*ADPSG) criteria [18, 19].

Inclusion criteria Pregnant women of the age 20 to 40 years with BMI of 18.5 to 24 (normal weight) were included. Only singleton, viable pregnancy was considered.

Exclusion criteria Pregnant women with a history of diabetes mellitus**,** Chronic medical conditions (uncontrolled hypertension, chronic liver disease, heart diseases, chronic kidney disease, chronic infections, polycystic ovarian syndrome, thyroid disease), history of substance abuse or smoking in the current pregnancy, GDM in previous pregnancy, and hypertension if diagnosed during the visits.

Collection of blood samples

Fasting blood samples were collected from normal pregnant and GDM group, taking aseptic precautions. The samples were centrifuged at 3000 rpm for 15 min. Separated serum samples were stored at −80 degree Celsius till analysis.

Assays

1) Serum insulin was estimated by Electrochemiluminescence Immunoassay (ECLIA) [20], with the reagents kits from Roche Diagnostics, and the Cobas 6000 automated chemistry analyzer. The manufacturer's instructions were followed for performing the assay.
2) Insulin resistance calculated by the Homeostasis Method Assessment (HOMA) model [21], as follows:

$$\text{HOMA-IR} = \frac{\text{Serum Insulin (IU/L) X Plasma Glucose (mmol/L)}}{22.5}$$

3) Levels of adiponectin, resistin, IL-6 and TNF-alpha in the serum were assayed by a sandwich enzyme linked immunosorbent assay (ELISA), with the reagent kits from Biovendor, USA and the Biorad PR4100 microplate reader was used in measuring the absorbance readings [22].

Statistical Analysis

Statistical analysis was performed using SPSS software. Normally distributed data were presented as mean ± SD, and non-normally distributed data were presented as median. Comparisons between the groups were done using Student's t test and Mann-Whitney U-test. Value of $P < 0.05$ was considered statistically significant.

Results

Total number of participants whose data is presented were, 38 each in healthy and GDM groups. The demographic details and baseline laboratory data of participants are given in Table 1. At 12 weeks of gestation, there was no significant difference between the pregnant women who continued to have normal pregnancy and the pregnant women who eventually developed GDM with regard to age, BMI, systolic blood pressure, diastolic blood pressure, fasting plasma glucose and blood glycated hemoglobin (HbA1c). The results are presented in Table 2. The pregnant women were categorized as "GDM" or "Normal" based on OGTT at 24 weeks. Then, the levels of biochemical parameters were compared between the two groups at 12 weeks and 24 weeks. In comparison to normal pregnant women, GDM group showed significant difference in the values of HOMA-IR, adiponection, resistin, IL-6 and TNF-alpha both at 12 weeks and 24 weeks. The GDM group showed significantly higher HOMA-IR, resistin, IL-6 and TNF-alpha values, and significantly lower adiponectin level, when compared to the normal pregnants (Table 2).

In the normal pregnant group, the values of resistin, IL-6 and TNF-alpha were significantly higher and adiponectin was lower at 24 weeks when compared to their respective values at 12 weeks. There was no significant difference in the insulin resistance (HOMA-IR) between 12 and 24 weeks, among the normal pregnant women. In the GDM group, the values of HOMA-IR, resistin, IL-6 and TNF-alpha were significantly higher, and adiponectin was lower at 24 weeks in comparison to 12 weeks (Table 2).

Discussion

The present study observed alterations in the levels of adipokines and cytokines during pregnancy. The findings indicate the role of adipocytokines in the pathogenesis and progression of insulin resistance in pregnancy. Insulin resistance is shown to be increased during pregnancy as demonstrated by increased value of HOMA-IR from 12 weeks to 24 weeks of gestation in the GDM subjects, and significant difference was observed in the HOMA-IR values between GDM group and healthy pregnant women both at 12 and 24 weeks of gestation. Most of the studies have reported higher insulin resistance in GDM subjects when compared to healthy pregnant women in the third trimester of pregnancy, without finding any change in the first trimester [23]. We have not observed any difference in IR among the healthy pregnant women from first to third trimester.

We observed significant increase in the levels of resistin and decrease in the levels of adiponectin during the pregnancy in both healthy and GDM subjects. Adiponectin levels in GDM were significantly lower than those of healthy pregnant both at 12 and 24 weeks. Even in normal pregnancy, the levels decreased significantly from 12 weeks to 24 weeks. Adiponectin, the protective adipokine produced exclusively in adipose tissue has been shown to play important role in regulating insulin resistance and glucose homeostasis. There are contradicting reports on serum adiponectin levels in normal pregnancy. While few studies have reported decreased adiponectin level from first trimester to third trimester, others have not found any significant change [24]. As per the

Table 1 Demographic details of study participants at 12 weeks of gestation

	Healthy Pregnant Women (at 12 weeks; and continued to have normal pregnancy when followed up at 24 weeks); Group 1 a; $N = 38$	GDM (Healthy Pregnant women at 12 weeks who eventually developed GDM at 24 weeks); Group 2 a; N = 38
Age (Years)	26.2 ± 5.5	25.5 ± 5.1
BMI (Kg/m^2)	22.5 ± 4.2	23.3 ± 3
Systolic Blood Pressure (mm Hg)	121 ± 5	119 ± 6
Diastolic Blood Pressure (mm Hg)	77 ± 7.2	80 ± 6.5
Family history of diabetes mellitus	Nil	Nil
History of PCOS	Nil	Nil
Fasting Plasma Glucose (mmol/L)	4.7 ± 0.6	4.8 ± 0.5
HbA1c (%)	4.8 ± 1.9	4.9 ± 1.2

Table 2 Values of HOMA-IR, adiponectin, resistin, IL-6 and TNF-alpha in pregnant women (Values are mean ± SD; The percentage difference in the values is shown in the parantheses)

	Healthy Pregnants (at 12 weeks); Group 1 a; *N* = 38	Healthy Pregnants (at 24 weeks); Group 1 b; N = 38	GDM (at 12 weeks); Group 2 a; N = 38	GDM (at 24 weeks); Group 2b; N = 38
HOMA-IR	1.3 ± 0.3	1.4 ± 0.2	1.6 ± 1.1**(+ 23.1%)	2.5 ± 0.4 ***(+56.2%); **** (+78.6%)
Adiponectin, Serum(micrograms/ml)	10. 2 ± 3. 2	8.7 ± 2.4* (− 14.7%)	9.7 ± 1.96** (− 7.8%)	5.54 ± 1.7***(− 42.9%); **** (− 36.3%)
Resistin, Serum (ng/ml)	6.8 ± 2.3	7.9 ± 1.3*(+16.2%)	7.3 ± 0.98**(+ 7.35%)	9.77 ± 2.2***(+33.8%); **** (+23.7%)
IL-6, Serum (pg/ml)	3.6 ± 3.1	5.5 ± 2.1*(+ 52.8%)	4. 2 ± 2.3**(+ 16.7%)	8.56 ± 2.1***(+103.8%); **** (+55.6%)
TNF-alpha, Serum (pg/ml)	48 .1 ± 9.3	56.2 ± 8.9*(+ 16.8%)	67.3 ± 9.8**(+39.9%)	115.6 ± 22.1***(+71.8%); **** (+105.7%)

*Significant difference ($P < 0.001$) when Group 1 b is compared to Group 1 a

**Significant difference (P < 0.001) when Group 2a is compared to Group 1 a

***Significant difference (P < 0.001) when Group 2 b is compared to Group 2 a

****Significant difference (P < 0.001) when Group 2b is compared to Group 1 b

observations of few studies with healthy pregnant women, serum adiponectin reaches lowest concentrations in the third trimester when maternal insulin resistance is the greatest [25, 26]. Though there are many studies showing decreased adiponectin level in GDM when compared to healthy pregnant [12–14, 25–27], our findings additionally demonstrate that significant difference in serum adiponectin level among GDM and healthy pregnant women could be observed not only at 24 weeks of gestation but, also in early stages, at 12 weeks of gestation itself.

Resistin is the adipokine which promotes insulin resistance and its levels are shown to be increased in GDM [12, 28, 29]. In the present study, we observed significantly increased serum resistin levels in GDM subjects when compared to healthy pregnant women, both at 12 and 24 weeks of gestation. There was significant difference in the serum resistin levels at 12 and 24 weeks in healthy pregnant women, suggesting changes in resistin level with gestational age even in normal pregnancy. Serum resistin level showed significant positive correlation with insulin resistance at 24 weeks among the GDM subjects. A meta analysis of association of serum resistin level with GDM risk revealed that the association between elevated serum resistin level and GDM was only found in the third-trimester and that the serum level of resistin increases with gestational age [28]. However, Bawah et al. observed significant increase of serum resistin in GDM women when compared to healthy pregnant women at first trimester [29], which is consistent with the findings of our study.

We observed significantly elevated levels of the pro inflammatory molecules IL-6 and TNF-alpha in GDM subjects in comparison to healthy pregnant women, both at 12 and 24 weeks, and there was significant increase of IL-6 and TNF-alpha from 12 weeks to 24 weeks in both the study groups (healthy and GDM). The findings indicate the role of inflammation in development insulin resistance during pregnancy. Previous studies have observed increased levels of IL-6 and TNF-alpha in GDM [30]. Inflammation has been proposed to be the major molecular event in the development of insulin resistance and diabetes mellitus [31]. Salient finding of our study is the significantly higher levels of IL-6 and TNF-alpha even at 12 weeks of gestation among the pregnant women who eventually developed GDM.

Gestational Diabetes is one of the most frequent complications of pregnancy, with significant risk to mother as well as fetus. Development of insulin resistance and inflammation are the key phenomena in GDM. Altered levels of adipocytokines which are the key molecular players in the metabolic changes in insulin resistance and inflammation, indicate the mechanisms involved in pathogenesis of GDM. Assessing the adipocytokines in the early stages of pregnancy at 12 weeks itself, could help in predicting the insulin resistance later in pregnancy and also to prescribe necessary dietary and other life style changes to prevent the complications of pregnancy.

Conclusions

The biomarkers assessed in this study showed significant changes from the first to third trimester in pregnant women with GDM. Significant difference in the levels of biomarkers between normal pregnant and GDM subjects both in the first and third trimesters indicates increased insulin resistance contributing to GDM, and potential application of adiponectin, resistin, IL-6 and TNF-alpha in early detection and as predictive biomarkers of GDM. Completion of our study with the estimated sample size, and future studies with detailed analysis of sensitivity, specificity and predictive value of adipocytokines as biomarkers of GDM are required.

Authors' contributions SSAR was involved in concept formation, research design, analysis of samples, manuscript preparation and finalizing the paper. P D'Cunha was involved in research design, participant recruitment, informed consent process, collecting clinical data and finalizing the paper. C Rebeiro was involved in informed consent process, laboratory data collection, sample collection and analysis.

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Availability of data and material The study data are available for any inspection/ audit.

Compliance with ethical standards

Conflict of interest Nil

Ethics approval Approval of Institutional Ethics Committee was obtained (FMMCIEC/CCM/214/2018, dated 18.04.2018).

Consent to participate Voluntary, informed consent was obtained from the study participants. Ethical principles as per Declaration of Helsinki and the guidelines of ICMR, 2017 (national guidelines on conduct of biomedical and health research involving human participants) were followed.

Consent for publication In the informed consent from participants, provision was kept for consenting for publication of the study data.

Code availability Not applicable.

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