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Original Article

Early pregnancy biochemical markers of placentation for screening of gestational diabetes mellitus (GDM)

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ABSTRACT

For the effective management and screening of patients with diabetes, lipid profile has been a useful mean. Here, we hypothesized that biochemical analyses of blood serum in pregnant women with GDM will develop an insight on the pathogenesis of the disease and possibly uncover new biomarkers. In order to test our hypothesis, antenatal pregnant women (n = 300) were selected for blood samples including 176 with positive clinical/family history and 124 with negative clinical/family history of GDM during the early second trimester (14–18 weeks of gestation). All the subjects were followed up to the early third trimester (24–28 weeks of gestation) for second sampling until the onset of GDM. Lipid profile data shows that mean values of triglycerides, total cholesterol, low density lipids and very low density lipids were significantly higher ($p < 0.05$) and mean HDL was significantly lower in early second trimester in those patients who subsequently developed GDM during late third trimester when compared with those who didn't develop GDM. Inflammatory biomarker such as High-sensitivity C-reactive protein (hs-CRP) levels were also found to be significantly higher by 69% increase in patients who developed GDM later in third trimester in comparison with those who didn't develop. About 32% patients who finally developed GDM belonged to positive clinical/family history group. The results of our study indicate that abnormal serum cholesterol; triglycerides, HDL, LDL, VLDL and hs-CRP play a vital in pathophysiology of gestational diabetes. Early diagnosis of GDM based on these biochemical markers will help decrease adverse neonatal and maternal outcomes.

1. Introduction

GDM deteriorates insulin sensitivity or glucose intolerance in pregnancy [1]. If GDM goes untreated, it can lead to diabetes type-2 in both mother and fetus. The risk factors of GDM involve impaired fasting glycemia, pre-diabetes, family history of GDM, obesity and backdrop of having child more than 4 kg body weight [2,3]. The above mentioned risk factors which causes alterations in metabolism are inflamed with the induction of GDM, including changes in fasting and postprandial glycemia and lipids [4]. Diabetes is estimated to complicate approximately 2–5% of all pregnancies of which 90% are identified during pregnancy i.e. GDM and the rest are overt or pre-gestational [5].

GDM is known as a glucose metabolism disorder; hence, level of glucose in blood has become the "key player" for detecting and leading cure in pregnancy [6]. Females diagnosed with GDM have a major long-term threat of being diseased that eventually cause death because of heart failure [7–10]. Lipid profile testing is recognized as an effective method for diagnosing diabetic patients, since pregnancy adds the effect of diabetes to atherogenic lipid profile development. Lipid aberrations linked with insulin resistance affect all lipid fractions [11], causing elevated levels of triglycerides and low density lipoproteins (LDL) cholesterol in association with low levels of high density lipoproteins (HDL) cholesterol.

Several studies show that metabolism of lipid during gestation might play a significant role in the etiology and development of GDM [12]. For a strong pregnancy growth, proper lipid metabolism is very necessary. A lipid profile that immitates the pathologic outcomes of the metabolic disorder is a usual result during the

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second half of gestation [13–15]. But the mechanisms featuring such changes of the lipid metabolism are not fully understood [16]. Lipid profile assessment of blood samples in pregnant women with gestational diabetes can help understand the pathogenesis along with the discovery of potent biomarkers of GDM.

Maternal serum markers, measureable early in pregnancy and in routine clinical practice, have been identified as possible predictors of later pregnancy glucose intolerance [17,18]. High-sensitivity C-reactive protein (hsCRP) is an acute-phase reactant that, at subclinical elevations, is a marker for endothelial damage, cardiovascular disease, and obesity in nonpregnant patients [19]. In pregnancy, hs-CRP is associated with maternal serum glucose when measured at the time of standard third-trimester GDM screening [18,20,21].

Inflammation by expanded serum levels of hs-CRP, is a critical autonomous hazard for cardiovascular disease [22]. Although, GDM speaks to be a novel forerunner of type 2 diabetes, the fact that inflammation is likewise connected with the GDM development is obscure. In this manner, we tested hypothesis that increased CRP levels, estimated early in pregnancy, are related with the consequent development of GDM. Here, we studied the predictive role of lipid profile in the development of GDM and the association of inflammatory markers with the risk of developing GDM in pregnant women.

2. Materials and methods

2.1. Study participants and study site

Out of total 300 pregnant women, 176 with positive and 124 with negative familial background of GDM were selected from different hospitals of Lahore, Pakistan, at their first antenatal visit during early second trimester. Informed consent, clearly mentioning the purpose of the study, was signed by the patient or their attendants. All of the women were screened for GDM both in early and late stages of their pregnancy.

During the early care visits of gestation, participants were questioned about parity, age, history of previous maternal diabetes, family history of type 2 diabetes mellitus, and habit of smoking. Body mass index (BMI) was determined by recording patient's weight in kilograms and height in meters. Systolic and diastolic blood pressure was recorded.

2.2. Inclusion criteria

Inclusion criteria was based on positive or negative family history of GDM and T2DM, maternal age between 18 and 40, glycated hemoglobin (HBA1c) of less than 6.5%, with no hypertension, renal and cardiac diseases and current medical treatments which could affect patients' hormonal concentration, lipid profile, liver and renal function tests.

2.3. Exclusion criteria

Subjects with prior history of GDM, T2DM multiple pregnancies, ectopic pregnancy, hypertensive disorders, history of smoking/alcohol abuse, assisted reproductive technology treatment, fetal congenital irregularities and any other confounding pathologies (hyper- or hypothyroidism), polycystic ovarian syndrome, glycated haemoglobin greater than 6.5%, renal or hepatic failure, uncontrolled endocrine or any other metabolic disorder that may influence glucose regulation were excluded. Fasting glycemia was determined and the women with fasting plasma glucose (FPG) > 110 mg/dl were considered to possess undiagnosed pregestational diabetes mellitus.

2.4. Blood sampling

Ethical approval for the study was obtained from Institutional Ethical Review Board of Punjab University. Cases were followed-up until the development of GDM that was diagnosed if any of the glucose level was equal to or greater than 5.1 mmol/l (fasting), 10.0 mmol/l (1 h post-load), or 8.5 mmol/l (2 h post-load). Blood was collected by venipuncture from all of the subjects, at 14–18 week of pregnancy. After the determination of fasting glycemia and routine pathology testing, 5 cc blood was drawn from all the subjects and serum was separated by centrifugation.

2.5. Sample analysis

Samples were brought to room temperature before analyses. TC, TG, LDL, and HDL were determined using commercially available kits using clinical chemistry analyzer (Crescent diagnostics). Hs-CRP protein was analyzed as inflammatory marker using commercially available ELISA kits (DiaMetra, Italy).

2.6. Data analysis

Statistical analysis was done by one-way ANOVA and SPSS PROC GLM in SAS software to compare the results in comparable groups. P values (<0.05) were considered as statistically significant.

3. Results

All the subjects were followed until the development of GDM. The subjects who developed GDM were termed as "GDM" group and those who did not develop GDM were treated as "control" group. Blood samples for both groups were collected at predefined intervals; early second trimester, early third trimester (for control group) and at the onset of GDM development (for GDM group). All the subjects were further analyzed on the basis of positive and negative familial history of GDM.

3.1. Overall biochemical comparison among GDM group and control group

Overall biochemical comparisons among pre and post GDM group ($n = 58$) and pre and post control group ($n = 100$) were carried out by one-way ANOVA technique. Group means were separated through SNK multiple range test.

3.2. Serum total cholesterol and triglycerides levels

The results showed statistically significant ($P \leq 0.05$) elevation in cholesterol concentration in GDM group compared to control group at early second trimester and early third trimester. Similarly, mean serum TG concentration for the GDM group was statistically significantly higher ($p < 0.0001$) than controls at early second trimester and early third trimester (Table 1).

3.3. Serum lipoprotein levels

Statistically significant ($P \leq 0.05$) decrease in HDL cholesterol concentration was observed in GDM group compared to control group. However, mean LDL cholesterol level for the GDM group showed significant increase in early third trimester in comparison with controls not only in early second trimester but also in early third trimester. The results further showed that mean VLDL cholesterol level was significantly higher in GDM group when compared with controls at early second trimester and early third trimester.

Table 1
An overall comparison of lipid profile in GDM group (n = 58) and control group (n = 100) at early 2nd trimester (14–18 weeks) and early 3rd trimester (24–28 weeks) of gestation.

Parameters	Early Second Trimester		Early Third Trimester		
	T2C	T2GDM	T3C	T3GDM	*P*-val
Cholesterol (mg/dL)	287.71d ± 1.67	308.91c±1.27	340.43b ± 1.58	367.86a±2.39	<0.0001
Triglycerides (mg/dL)	$346.42^{d} \pm 3.52$	369.52^{c}±3.34	$423.94^{b} \pm 3.38$	450.45^{a}±4.21	<0.0001
HDL (mg/dL)	59.80^{a}±0.78	$45.71^{b} \pm 0.74$	$41.63^{b} \pm 0.87$	33.42^{c}±1.93	<0.0001
LDL (mg/dL)	$131.16^{d} \pm 1.02$	165.62^{c}±2.02	$201.60^{b} \pm 2.75$	227.13^{a}±3.43	<0.0001
VLDL (mg/dL)	$31.88^{d} \pm 0.30$	39.95^{c}±0.95	$47.38^{b} \pm 0.48$	54.39^{a}±1.11	<0.0001
Hs-CRP (mg/L)	4.67 ± 0.33^{d}	6.23 ± 0.45^{c}	7.27 ± 0.53^{b}	9.86 ± 0.37^{a}	<0.0001

a,b,c,d on indicate significant differences at $p < 0.05$.
Order of significance is as: a > b > c > d.
T2C = 2nd trimester control, T3C = 3rd trimester control.
T2GDM = 2nd trimester GDM, T3GDM = 3rd trimester GDM.

All subjects showed elevated levels of serum lipoproteins at early third trimester but their elevations were more pronounced in GDM group.

3.4. Levels of hs- C reactive protein

The level of C-reactive protein was significantly higher in GDM group at early second and third trimester in comparison with control group.

3.5. History wise biochemical comparisons among risk group and control group

All the subjects were further analyzed on the basis of positive and negative family history of GDM or T2DM. Among 300 pregnant women, the percentage of GDM occurrence in females with positive family history was found to be 32% while it was 14% for those with negative family history. A significant difference in serum cholesterol, triglycerides, HDL, LDL, VLDL and hs-CRP was observed in group with negative family history of GDM when compared with group having positive family history as shown in Table 2. The same group showed significant elevation of all above mentioned parameters except HDL in early third trimester of pregnancy in comparison with control group (T3C) while there was a significant decrease in early third trimester of gestation in HDL levels as compared to control group (T3C) in Table 1. The percent increase in TC, TG, LDL, VLDL and hs-CRP was higher while HDL levels were lower among those patients who had positive family history in comparison to those with negative family history. The results above demonstrate that patients with positive family history of GDM or T2DM are more prone to develop GDM as compared to those with negative family history.

4. Discussion

In our study, we investigated the biochemical parameters as potential indicators of GDM. Elevated estrogen levels in pregnant women may have triggered liver to synthesize more triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) which alternatively led to an increase in total plasma cholesterol and triglyceride levels. Maternal GDM also leads to cardiometabolic effects and utero-hyperinsulinaemia in offspring [23]. In this study, mean values of triglycerides, total cholesterol, LDL, and VLDL, were found to be significantly higher ($p < 0.05$) and mean HDL was significantly lower in pregnant patients during second trimester as compared to those who didn't develop GDM at all. These alterations were more pronounced in subjects with positive family history of GDM as compared to those with negative history of said disease. VLDL may combine to form LDL which is the major transporting form of TG in the blood and for GDM patients, levels of both LDL and TG go hand in hand. Total cholesterol concentrations were significantly higher in GDM patients as compared to controls in our study mainly because of the fact that GDM considerably modifies cholesterol metabolism prompting dyslipidaemia [24].

Fat accumulation increases in the second trimester of pregnancy prompting plasma triglyceride concentration [25]. LDL is produced from VLDL which is the vital transport type of triglyceride in the blood thus in GDM, when triglyceride concentration raises, LDL increases accordingly. In this research, VLDL and LDL cholesterol were essentially higher in GDM patients with positive family history in mid second and third trimester than the controls with negative family history. This could be because of the high triglycerides levels reported in this study. VLDL is produced from triglycerides formed in the liver all over again or by re-esterification of free unsaturated fats. In this manner, VLDL level rises when triglyceride level increases [24,26].

Table 2
An overall comparison of lipid profile and inflammatory marker in negative family history group (n = 124) and positive family history group (n = 176) during early 2nd trimester (14–18 weeks) and early 3rd trimester (24–28 weeks) of gestation.

Parameters	Early Second Trimester		Early Third Trimester	
	Negative History	Positive History	Negative History	Positive History
Cholesterol (mg/dL)	206.55 ± 4.88^{c}	256.07 ± 2.67^{b}	295.77 ± 2.92^{a}	305.27 ± 3.87^{a}
Triglycerides (mg/dL)	235.96 ± 8.81^{c}	286.78 ± 4.54^{b}	313.38 ± 7.52^{a}	322.79 ± 7.89^{a}
HDL (mg/dL)	62.13 ± 1.18^{a}	53.26 ± 0.84^{b}	46.79 ± 0.82^{c}	39.28 ± 0.92^{d}
LDL (mg/dL)	99.58 ± 2.40^{d}	118.47 ± 2.43^{c}	149.93 ± 3.97^{b}	163.22 ± 3.65^{a}
VLDL (mg/dL)	38.91 ± 0.65^{d}	41.43 ± 0.74^{c}	$48..75 \pm 0.94^{b}$	53.76 ± 1.03^{a}
Hs-CRP (mg/L)	4.13 ± 0.21^{d}	5.96 ± 0.68^{c}	8.27 ± 0.99^{b}	11.48 ± 0.27^{a}

a,b,c,d indicate significant differences at $p < 0.05$.
Order of significance is as: a > b > c > d.

Increased levels of TG, TC and LDL for GDM group might be due to the increased storage of progesterone [25] and fat [27] in the early second trimester of pregnancy to reset the lipostat in the hypothalamus prompting increment in the lipids concentration. .

Levels of inflammatory marker hs-CRP were found to be increased by 69% in GDM women in late third trimester as compared to non-GDM control subject. Most of the GDM patients, in our study, were having familial history of the disease. Increased serum CRP is a responsive indicator of systematic inflammation that has developed as an autonomous hazard for cardiovascular sickness [28,29]. Developing facts likewise incriminate inflammation in the pathogenesis of type 2 diabetes. Moreover, females with the polycystic ovary disorder, characterized by insulin resistance and extended danger of type 2 diabetes and cardiovascular mortality, show essentially elevated CRP levels further support our hypothesis of positive association of inflammation with hyperglycemia [30].

Studies show that in females who develop GDM, there are signs of increased inflammation in the late first trimester, and intervene to some degree by expanded BMI [29].

Here, we report that TG, LDL, VLDL, abnormal serum cholesterol and hs-CRP play significant role in pathophysiology of GDM. All the factors that might be responsible for the above-mentioned changes were excluded. In addition, during the analysis, those GDM subjects were also excluded who were found to have other clinical complications than GDM. This indicates that the above-mentioned changes in pregnant women were attributed by GDM exclusively. This study opens a new horizon to investigate clinical application of risk algorithms for GDM development. Early diagnosis of GDM will reduce neonatal and maternal metabolic complications. GDM during early pregnancy can be sorted out with maternal demography and clinical attributes to find novel biochemical markers.

5. Conclusions

The levels of TG, TC, LDL and VLDL in early 2nd and 3rd trimester were found higher in subjects who developed GDM as compared to that of controls. So these high levels in early second trimester can be considered as a strong predictors of GDM in later stages of pregnancy. Pregnant females can be screened in early stages of the gestation for these biomarkers to reduce the possibility of GDM development. Similarly, low levels of HDL in early pregnancy can also be treated as an indicator for GDM in later stages of gestation. Our results also revealed hs-CRP as a strong pathogenic factor for the development of GDM. If levels of these biomarkers can be controlled via therapeutic measures at early stages of pregnancy, the onset of said disease can be delayed or prevented in later stages of pregnancy. As clinical history of GDM is associated with susceptibility to develop T2DM and/or other metabolic dysfunctions, screening of such pregnancies for dyslipidemia and inflammatory markers in early stages can greatly reduce the chances of GDM development, improve therapeutic efficacy and reduce complications.

Conflicts of interest statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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