Maternal Leptin, Adiponectin, Resistin, Visfatin and Tumor Necrosis Factor-Alpha in Normal and Gestational Diabetes

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Received: 6 August 2013 / Accepted: 3 October 2013 / Published online: 29 October 2013
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Abstract Gestational diabetes mellitus (GDM) is a common medical complication associated with pregnancy. The present study evaluates the changes in maternal adipocytokines (leptin, adiponectin, resistin, visfatin and tumor necrosis factor-alpha; TNF- α) in pregnancy complicated with GDM compared to normal pregnancy at 2nd and 3rd trimesters. The study included total number of 142 pregnant women classified into 4 groups: normal pregnancy (n = 33) and pregnancy with GDM (n = 24) both at 2nd trimester and normal pregnancy (n = 38) and GDM (n = 47) at 3rd trimester. Both GDM groups were significantly presented with elevated body mass index, fasting blood sugar and abnormal oral glucose tolerance test compared to their matched control. Results indicated reduction in maternal serum leptin and adiponectin in GDM compared to normal pregnancy at 3rd trimester. Elevated resistin and TNF- α were evident among pregnancy complicated with GDM at both tested trimesters. On the other hand, significant elevation in maternal visfatin was noted between GDM and matched control at 2nd trimester only. Significant increase in maternal leptin and visfatin and resistin was noted by advances in gestational period in healthy pregnancy. On the other hand, reduced adiponectin and elevated visfatin mean values were noticed in GDM at 3rd compared to 2nd trimester. It could be concluded that increased insulin resistance accompanies

GDM is associated with suppressed leptin and adiponectin and increased resistin and TNF- α which might suggest their involvement in the development of GDM.

Keywords Diabetes · Adipocytokines · Pregnancy

Introduction

During pregnancy, changes in maternal metabolism occur in response to the growing fetus and placenta and their increasing metabolic needs. Pregnancy is associated with alterations in the regulation of glucose metabolism caused by the actions of human placental growth hormone, prolactin, cortisol and progesterone. These hormones antagonize the action of insulin, particularly during the 2nd and 3rd trimesters, leading to a state of relative insulin resistance as pregnancy progresses [1]. In early pregnancy, insulin secretion increases, while insulin sensitivity is unchanged, decreased, or may even increased [2]. However, in late gestation, maternal adipose tissue depots decline, while postprandial free fatty acid (FFA) levels increase and insulin-mediated glucose disposal worsens by 40–60 % [2]. The ability of insulin to suppress whole-body lipolysis is also reduced during late pregnancy [3], and further reduced in gestational diabetes mellitus (GDM) subjects [4], contributing to greater postprandial increases in FFAs, increased hepatic glucose production, and severe insulin resistance [5].

Gestational diabetes mellitus is a common medical complication associated with pregnancy. GDM is defined as any degree of glucose intolerance that occurs with pregnancy or is first discovered during pregnancy [6]. GDM imposes risks on both mother and fetus. Some of these risks continue throughout the lifetime of mother and child. Mothers with GDM have a 40–50 % chance of developing type 2 diabetes

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mellitus for the 20 years following their diagnosis of GDM [7]. In Saudi Arabia, The prevalence of GDM ranges from 3.8 to 12.5 % according to American Diabetes Association and World Health Organization criteria, respectively [8]. Maternal hyperglycemia causes increased glucose delivery to the fetus, resulting in fetal hyperinsulinemia and increased fetal growth [9]. This plethora of risks demonstrates the importance of early risk stratification with appropriate screening and diagnosis and of therapeutic interventions that maintain optimal glycemic control.

A large body of evidence has supported the role of adipose tissue in the regulation of insulin resistance in both non-pregnant and pregnant participants. In this respect, adipocytokines, which are adipocyte-derived hormones, have been implicated in the regulation of maternal metabolism and gestational insulin resistance [10]. The placenta is also an endocrine organ producing hormones such as human placental lactogen, placental growth hormone, and corticotrophin-releasing hormone, as well as cytokines involved in the process of insulin resistance, fetal growth, and labor induction in non-diabetic pregnancies and in pregnancies complicated by GDM [11]. As during pregnancy adipocytokines are produced not only by the adipose tissue but also by the placenta. Dysregulation of human placental function in pregnancies with GDM was previously reported by many authors [12].

The present study was motivated to evaluate the extent to which maternal adipocytokines (leptin, adiponectin, resistin, visfatin and tumor necrosis factor- α) are associated with the risk of developing GDM. The levels of these adipocytokines were assessed at second and third trimesters in pregnancy complicated with GDM and were compared with their matched values in normal pregnancy with matched gestational period.

Materials and Methods

This study was approved by Directorate of Health Affairs, and by Unit of Biomedical Ethics, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Saudi Arabia. Blood samples were collected from Maternity and Children's Hospital, Al-Mesadiah, Jeddah and from Gynecology Department, King Abdul-Aziz University Hospital. GDM was diagnosed according to World Health Organization, fasting plasma glucose ≥ 7.0 mmol/l or plasma glucose after 2 h ≥ 7.8 mmol/l, [13]. Each participant was asked to sign an informed consent.

Subjects

142 pregnant women (71 normal pregnant women and 71 pregnant women with GDM) with no history of diabetes;

type 1 or type 2; were included. All normal and GDM pregnant women had a regular follow up visits and were diagnosed by hospital physicians. Each participant was provided with a questionnaire included: name, age, weight, height, maternal (MA) and gestational (GA) ages, number of pregnancies and miscarriage. Body mass index (BMI) was calculated at sampling time. The exclusion criteria included: hypertension, preeclampsia, urinary tract infection, fever, fetal/placental abnormalities, alcohol intake and remarkable previous medical-surgical and gynecological maternal history. Almost all included pregnant women with GDM were treated with nutrition therapy except 3 and 6 cases in 2nd and 3rd trimesters respectively were treated with insulin therapy. Normal pregnant women and women with GDM were classified into 4 groups based on GA: normal pregnant women at 2nd trimester ($n = 33$) with a range of age from 18 to 38 years, pregnant women with GDM at 2nd trimester ($n = 24$) with a range of age from 24 to 45 years, normal pregnant women at 3rd trimester ($n = 38$) with a range of age from 18 to 43 years and pregnant women with GDM at 3rd trimester ($n = 47$) with a range of age from 21 to 45 years.

Blood Sample Collection

Each participant was subjected to a single withdrawal of fasting blood sample during one of her pregnancy follows up visits either at 2nd or 3rd trimester. Each blood sample was subjected to determination of fasting blood glucose, glycated hemoglobin and adipocytokines levels. Another blood sample was collected following oral administration of 100 % glucose solution after 1, 2 and 3 h to perform oral glucose tolerance test (OGTT 1, 2, 3 h).

Biochemical Assays

- Determination of blood glucose (FBS, OGTT at 1, 2 and 3 h) was carried out using glucose hexokinase method, employing a kit provided by Siemens Healthcare Diagnostic Limited, UK.
- Glycated hemoglobin (HbA_{1C}): was determined using kit purchased from Siemens Healthcare Diagnostic Limited, UK.
- Hormonal assay: Specific ELISA kits purchased from Assaypro Company were employed for determination of: Serum leptin (catalog no. EL2001-1). The assay sensitivity was ~ 0.12 ng/ml—serum adiponectin (catalog no. EA2500-1). The assay sensitivity was ~ 0.5 ng/ml—serum resistin (catalog no. ER1001-1). The assay sensitivity was 0.2 ng/ml—serum TNF- α (catalog no. ET2010-1). The assay sensitivity was ~ 0.015 ng/ml—ELISA kit purchased from Phoenix Pharmaceuticals, INC. Company was used for determination of serum

Table 1 Patients' characteristics, OGTT and glycated hemoglobin (HbA1C) in 2nd and 3rd trimesters for normal pregnancy and GDM

Group parameter	2nd control	2nd GDM	<i>P</i> value	3rd control	3rd GDM	<i>P</i> value
MA (years)	27.65 ± 1.041	36.38 ± 1.007	0.0001 ^a	31.16 ± 1.022	33.51 ± 0.872	NS ^a 0.013 ^b NS ^c
BMI (kg/m ²)	27.65 ± 1.284	31.10 ± 1.066	0.025 ^a	27.13 ± 0.817	30.08 ± 0.736	0.020 ^a NS ^b NS ^c
SBP (mmHg)	109.69 ± 2.08	111.52 ± 2.255	NS ^a	106.91 ± 1.764	109.56 ± 1.309	NS ^a NS ^b NS ^c
DBP (mmHg)	62.63 ± 1.361	62.48 ± 1.920	NS ^a	60.40 ± 1.251	64.76 ± 1.636	NS ^a NS ^b NS ^c
GA (weeks)	20.59 ± 0.694	21.25 ± 0.862	NS ^a	32.16 ± 0.582	33.85 ± 0.509	0.042 ^a 0.0001 ^b 0.0001 ^c
FBS (mg/dl)	85.42 ± 1.581	102.39 ± 5.381	0.001 ^a	85.19 ± 1.719	95.94 ± 3.150	0.007 ^a NS ^b NS ^c
OGTT 1 h (mg/dl)	139.73 ± 7.461	203.12 ± 21.365	0.0001 ^a	141.22 ± 4.927	189.0 ± 7.286	0.0001 ^a NS ^b NS ^c
OGTT 2 (mg/dl)	121.23 ± 5.461	174.04 ± 14.365	0.0001 ^a	125.53 ± 5.346	154.16 ± 7.071	0.011 ^a NS ^b NS ^c
OGTT 3 h (mg/dl)	98.00 ± 4.481	156.33 ± 29.096	0.001 ^a	109.65 ± 4.640	138.76 ± 7.844	0.008 ^a NS ^b NS ^c
HbA1C (%)	5.13 ± 0.191	6.48 ± 0.337	0.0001 ^a	5.51 ± 0.082	5.97 ± 0.116	NS ^a NS ^b NS ^c

P value is significant at <0.05 and *P* ≥ 0.05 is considered Non significant (NS)

^a GDM at 2nd or at 3rd versus matched control; ^b control at 2nd versus control at 3rd; ^c GDM at 2nd versus GDM at 3rd

visfatin (catalog no. EK-003-80). The assay sensitivity was 1.9 ng/ml.

which. For all comparisons, *P* < 0.05 were considered statistically significant.

Statistical Analysis

All statistical analysis was performed using SPSS statistical software package (SPSS Inc, USA). Numerical data presented in this study were expressed as mean value for each parameter ± its corresponding standard error of the mean. One way analysis of variance (ANOVA) was carried out to test the significance of difference between groups mean values for each parameter. In case of significant *P* value, multiple comparisons using Latin square design was carried out to test which group's mean value differs from

Results

At 2nd trimester, significant increase in MA and BMI were noted in GDM group compared to normal pregnant women (*P* = 0.0001 and 0.025 respectively), while other characteristics (SBP, DBP and GA) remained unchanged. At 3rd trimester, both mean values of BMI and GA were significantly elevated in pregnant women with GDM compared to their matched control group (*P* = 0.020 and 0.042 respectively), Table 1.

Table 2 Maternal adipocytokines in 2nd and 3rd trimesters for normal pregnancy and GDM

Group parameter	2nd control	2nd GDM	<i>P</i> value	3rd control	3rd GDM	<i>P</i> value
Leptin (ng/ml)	3.72 ± 0.315	3.12 ± 0.303	NS ^a	7.25 ± 0.819	4.25 ± 0.426	0.0001 ^a 0.0001 ^b NS ^c
Adiponectin (ng/ml)	34.19 ± 3.893	25.61 ± 3.423	NS ^a	32.93 ± 2.579	15.71 ± 1.112	0.0001 ^a NS ^b 0.019 ^c
Resistin (ng/ml)	5.33 ± 0.613	11.27 ± 1.378	0.0003 ^a	8.68 ± 0.576	14.75 ± 1.54	0.0001 ^a 0.0002 ^b NS ^c
Visfatin (ng/ml)	5.56 ± 0.331	6.66 ± 0.426	0.048 ^a	8.38 ± 0.517	9.01 ± 0.405	NS ^a 0.0001 ^b 0.001 ^c
TNF- α (ng/l)	10.52 ± 0.561	13.21 ± 0.556	0.001 ^a	11.01 ± 0.379	13.99 ± 0.409	0.001 ^a NS ^b NS ^c

P value is significant at <0.05 and $P \geq 0.05$ is considered Non significant (NS)

^a GDM at 2nd or 3rd versus matched control; ^b control at 2nd versus control at 3rd; ^c GDM at 2nd versus GDM at 3rd

Significantly increased mean values of all blood glucose indices (FBS, OGTT 1 h, OGTT 2 h, OGTT 3 h and HbA_{1C}) were noted in pregnant women with GDM at 2nd trimester compared to matched normal pregnant women. Meanwhile, at 3rd trimester, same results were obtained between GDM and matched control except for HbA_{1C} (Table 1).

At 2nd trimester, pregnancy complicated with GDM showed no significant changes in leptin and adiponectin and significant elevation in the mean values of resistin, visfatin and TNF- α compared to normal pregnant group. However at 3rd trimester, mean values of both leptin and adiponectin were significantly reduced, meanwhile, mean values of resistin and TNF- α were significantly increased in pregnant women with GDM compared to their matched control group. Results obtained from the present study indicated significant elevation in the mean values of leptin ($P = 0.0001$), resistin ($P = 0.0002$) and visfatin ($P = 0.0001$) in normal pregnancy by advanced gestational period. However, in pregnancies complicated with GDM, significant reduction in serum adiponectin and elevation in visfatin were obtained at 3rd trimester compared to 2nd trimester (Table 2).

Discussion

Data presented in this study demonstrated no significant differences in patients characteristics between GDM and control groups, however, women with GDM were more likely to be of advanced maternal age and overweight

compared to their matched healthy pregnant women. Mean values of OGTT indices were significantly elevated in GDM than healthy control, indicating increased insulin resistance in pregnant women with GDM group, either at 2nd or 3rd trimester. Thus, it is the defect in insulin secretion which differentiates those women who develop GDM than those who maintain normal glucose tolerance [14].

In this study, no significant change in maternal serum leptin was noted between GDM and normal pregnancy at 2nd trimester; however, at 3rd trimester significantly decreased mean value was noted among pregnant women with GDM compared to their matched control value. The available literatures regarding maternal leptin in GDM are characterized by conflicting data. Leptin levels were increased [15], decreased [16] or unchanged [17] in GDM compared to normal pregnancy. Differences in maternal leptin between GDM and control subjects might be due to differences in the expression of leptin receptors signals between normal and diabetic women. Challier et al. [18], reported that, in contrast with the transmembrane leptin receptor, expression of the soluble receptor is increased in placentas of women with GDM. Soluble leptin receptor is capable of binding leptin with a high affinity. Increased expression of soluble leptin receptor was suggested to act as a leptin binder, therefore limiting its accessibility to the transmembrane receptor. This, in turn, may modulate the release of leptin from GDM placentas, which might account for the decreased leptin observed in our and in previous studies in GDM patients [11].

We observed elevated maternal serum leptin at 3rd trimester compared to 2nd trimester in women with normal

pregnancy and in pregnancies complicated with GDM, being only significant in normal pregnancy. Although, different studies had documented that serum leptin elevated during pregnancy with maximum concentrations at 22 and 27 weeks of gestation parallel with the peak of maternal fat accumulation [19], however, others reported elevated leptin concentrations during the 3rd trimester of pregnancy which decreases to pre-pregnancy concentrations around parturition [20, 21]. An upward trend in leptin levels has been reported through the three trimesters which seems to parallel the increase in percent fat mass through the same three trimesters [22]. In addition, the human placenta expresses high amounts of leptin messenger RNA (mRNA) and protein, while leptin receptors are abundant in the placenta, as well as the chorion and amnion [20]. There is evidence to suggest that placenta, rather than maternal adipose tissue makes a substantial contribution to the increase of maternal leptin concentrations [21]. The human placental leptin gene has a specific promoter region suggesting that placental leptin is differentially regulated as compared to leptin originating from adipose tissue. Furthermore, the fetus itself contributes to leptin production starting early in the second trimester although to a much lesser extent than the placenta [23]. Approximately, 95 % of total placental leptin is released to the maternal circulation [24], where leptin modulates maternal energy expenditure and metabolism [25]. Placental leptin mRNA production is upregulated by TNF- α and IL-6 [26]. In addition, leptin regulates placental growth, nutrient transfer, angiogenesis, and trophoblast invasion [27]. Therefore, one possible function of the increased maternal leptin levels by advance in pregnancy may be to enhance the mobilization of maternal fat stores, to increase availability and trans-placental transfer of lipid substrates to the fetus [25].

Noticeable decrease in adiponectin in pregnancy with GDM at 2nd (25 %) and 3rd (52 %) trimesters compared to their matched healthy pregnant females groups was noted, being only significant in the 3rd trimester. In agreement with this finding, lower adiponectin gene expression was noted in GDM placenta [28]. Different studies had reported decreased maternal adiponectin levels in GDM independent of maternal BMI and insulin sensitivity [11, 29]. One study [11] however reported no significant difference in the release of adiponectin from human placenta and maternal adipose tissue between GDM and normal pregnant women.

It is interesting to point out that the risk of GDM was found to be increased by 20 % for each 1 ng/ml decrease in maternal adiponectin concentration. At 13 weeks of gestation, 4-sixfold increased risk of subsequent GDM was developed in women with adiponectin concentrations below 6.4 mg/ml as compared to pregnant women with higher concentrations of adiponectin [30]. Adiponectin

expression and secretion was shown to be affected by a myriad of factors. States that increase insulin sensitivity, such as weight loss, female gender, Caucasian race, and insulin-sensitizing drugs (thiazolidinedione), have elevated circulating adiponectin levels [31]. Conversely, states that decrease insulin sensitivity, such as diabetes, obesity, androgens, and inflammatory cytokines appeared to suppress adiponectin expression and secretion [32]. Therefore, the inconsistent results between the different reports including ours regarding maternal adiponectin may be related to differences in obesity and ethnicity which play a role in circulating adiponectin level [33]. Maeda et al. [34] reported that adiponectin gene expression is up-regulated and that plasma adiponectin concentrations are increased in obese mice and in insulin resistance obese humans by thiazolidinediones. Also, racial differences in adiponectin levels have been observed where African-American women display lower adiponectin level than Caucasians. Non-Caucasians had lower adiponectin levels than Caucasians. Significantly lower differences were still found between non-Caucasian subjects when grouped [33, 35]. Other studies had examined adiponectin in GDM and healthy pregnant. Adiponectin was noticeably but not statistically lower in GDM compared to normal pregnancy [36]. The pathophysiology of hypo adiponectinemia in GDM still not well documented. Beltowski [37] speculated that the insulin-sensitizing effect of adiponectin may result from four different mechanisms: (1) increased lipid oxidation; (2) direct improvement of insulin signaling at the receptor/post-receptor level; (3) inhibition of gluconeogenesis, and (4) inhibition of TNF- α signaling in adipose tissue. As adiponectin has insulin sensitizing effect, levels of adiponectin might further aggravate insulin resistance. Thus insulin might be able to suppress plasma adiponectin concentrations.

Our results indicated unchanged adiponectin levels by advancing gestational period in normal pregnancy, while hypo adiponectinemia was more pronounced in GDM at 3rd compared to 2nd trimester. A previous study reported progressive decline in maternal adiponectin secretion during the course of pregnancy which was associated with a 60 % reduction in adiponectin mRNA in white adipose tissue [38]. Reduced adiponectin levels in second and third trimesters compared to first trimester in healthy pregnant subjects was reported by many authors [38, 39], although others reported unchanged values [22, 29]. In the present study, unchanged maternal circulating adiponectin in normal pregnancy between 2nd and 3rd trimesters may represent an independent protective mechanism against the gradual increase in insulin resistance. While hypo adiponectinemia in GDM by advancing gestational period may be consistent with decreased insulin sensitivity by 3rd trimester.

In our study, maternal serum resistin was significantly elevated in GDM women compared to their matched control at 2nd and 3rd trimesters. These results are in agreement with many studies. Serum resistin was reported to be higher in GDM compared to normal pregnancy at their 2nd trimester [40]. Other researchers indicated that despite markedly higher glucose and insulin levels, serum resistin levels did not differ significantly in pregnant women with GDM and healthy pregnant women at term [11]. The discrepancy in these findings may be related to differences in studied population or sampling time. It is worth mentioning to indicate that our studied subjects with GDM were almost gestational age matched with normal pregnancy, however they were more obese than control pregnant women at 2nd and 3rd trimesters, beside insulin resistance was more marked in GDM pregnant women than control subjects. It was reported that resistin is up-regulated in both genetic and diet-induced obesity in vivo and down-regulated by the anti-diabetic agents [41]. In human, it was suggested that resistin mRNA expression in adipocytes is increased in obese subjects [42], but other report was unable to detect any association between obesity and resistin expression [43]. According to this finding it seems that both obesity and increased resistin concentrations may be important factors in the increased insulin resistance accompanying GDM. In our study, maternal resistin was significantly elevated in normal pregnancy; but not in GDM; by advances in gestation. Placental resistin gene expression was found to be more prominent as pregnancy advances [44]. Moreover, resistin up-regulation was observed in the 3rd trimester, which has been postulated to play a role in the regulation of maternal energy metabolism during pregnancy [44, 45]. Administration of recombinant resistin was found to impair glucose tolerance, whereas immune-neutralization of resistin in diet-induced obese mice lowered glucose levels [46]. A number of studies have found insulin to be a key regulator of resistin gene expression [47, 48]. In another study [17] insulin stimulated the release of resistin from human placenta. Since normal pregnancy and pregnancy complicated with GDM are considered a state of hyperinsulinemia and increased insulin resistance, beside the previously reported increased resistin secretion by insulin support the role of resistin in the induction of the known increased insulin resistance by advanced gestational age and also elevated insulin resistance associated with GDM compared to normal pregnancy.

The present study indicated significantly increased maternal visfatin by 3rd compared to 2nd trimester in normal pregnancy. Increased mean value was also observed in GDM at 3rd compared to 2nd gestational period. In normal pregnancy, significantly higher mean visfatin concentrations were obtained during 2nd and 3rd compared to 1st trimester and during 3rd compared to 2nd

trimester in parallel with β -cell secretion indices [47]. Visfatin concentrations have been documented to be gradually increased between non-pregnant women and women in the third trimester of pregnancy, probably compensating for the gradual increase in insulin resistance [48, 49]. However, other study demonstrated a sevenfold increase in visfatin gene expression and protein in omental fat of pregnant women, as compared to controls, but only a small increase in serum visfatin levels, suggesting that visfatin may act locally as a paracrine/autocrine agent and not as a hormone [50]. On the other hand, another report [48] indicated that serum visfatin concentrations in the first trimester of pregnancy were positively predict insulin sensitivity in the second trimester, however, this close association disappears later, possibly due to an increase in visfatin secretion by an additional source other than adipose tissue, namely the placenta [48].

Our results demonstrated significantly higher maternal visfatin concentrations in GDM at 2nd ($P = 0.048$, border line significant) but not at 3rd trimester compared to matched control group. Some authors reported lower maternal visfatin in GDM [49], while others reported increased concentration [51]. The number of participants, gestational age at enrolment, BMI, and the specificity of the immunoassays applied differ between all the above studies. Gestational diabetes mellitus is a state of temporary insulin resistance, and elevated visfatin concentrations might counteract high glucose and insulin levels [52]. Thus, elevated maternal visfatin concentrations in GDM at 2nd trimester may reflect an impairment of visfatin function in target tissues, dysregulation of biosynthesis, or a response to hyperglycemia [52]. On the other hand, TNF- α is known to enhance visfatin expression in human placental cells [53]. Thus, the placenta could be a source of increased visfatin in GDM. However, no differences were observed in visfatin mRNA expression in fat and placental tissues between GDM and normal participants at term [49]. In the present study, obesity has no effect on maternal visfatin either in normal pregnancy or in pregnancy complicated with GDM at both tested gestational period. Beside, no significant correlations between BMI and maternal serum visfatin in normal pregnancy or in GDM at 2nd and 3rd trimesters were obtained. These results are supported by Mazaki-Tovi et al. [47] who obtained a comparable median visfatin concentration between normal and overweight pregnant women. On the other hand, lack of association between circulating visfatin and BMI was previously reported [49], although others reported positive [54] and negative [55] association between BMI and maternal visfatin. Other investigators could not demonstrate an association between visceral fat mass and plasma visfatin concentrations [56]. In this study, the comparable maternal visfatin concentration between overweight and normal

weight pregnancy challenge the suggested association between visfatin and obesity.

Significantly elevated serum TNF- α level was demonstrated in pregnant women with GDM compared to normal pregnancy both at 2nd and 3rd trimesters. Elevated TNF- α in GDM had been reported earlier by many studies [15, 28]. Winkler et al. [57] indicated that insulin treatment of GDM patients may contribute to the elevated TNF- α levels. However, in the present study three cases in 2nd trimester and six cases in 3rd trimesters complicated with GDM were treated with insulin and had lower TNF- α values than those under diet control. The authors also suggested that higher BMI and thus the increased adipose tissue volume in GDM may be responsible for the observed elevated TNF- α concentration in GDM. In our study, significantly higher BMI was observed in pregnant women with GDM compared to their matched control at 2nd and 3rd trimesters. Oxidative stress and inflammatory changes induced by hyperglycemia may also result in increased TNF- α secretion [58]. Increased secretion of TNF- α was found to exert an inhibitory effect on insulin secretion and insulin regulated glucose uptake in GDM, thus contributing to sustained hyperglycemia [16]. Coughlan et al. [59] hypothesized that TNF- α released from gestational tissues during the third trimester may represent an additional diabetogenic factor exacerbating insulin resistance. Moreover, exposure of placental tissue to high glucose in healthy women resulted in decreased TNF- α levels in the medium. In contrast, the accumulation of TNF- α in media from placentas obtained from women with GDM was significantly greater under high glucose conditions when compared with placentas incubated in normal glucose concentrations. As glucose metabolism deteriorates in women with GDM, hyperglycemia results in the elevation of TNF- α ; this contributes to insulin resistance, hyperlipidemia, and decreased peripheral glucose uptake, which in turn exacerbates the diabetic state [59].

From the present study, it could be concluded that all studied adipocytokines, except visfatin, showed remarkable variations in their levels between GDM and control subjects. Increased insulin resistance that accompanies pregnancies complicated with GDM is associated with suppressed leptin and adiponectin and increased resistin and TNF- α compared to normal pregnancy, suggesting strongly their association with insulin resistance in GDM. On the other hand, maternal visfatin showed weak association at 2nd and no association at 3rd trimester with insulin resistance in GDM. The most important limitation in this study was smaller sample size, especially in GDM at 2nd trimester, resulting in high SE, that masks significance between GDM and control group for some parameters as adiponectin. Beside, the present study measured the levels of maternal adipocytokines after GDM had been clearly

diagnosed. It is recommended to extend the present study to explore the relationship between levels of the studied adipocytokines and the onset of glucose metabolism disorder. Also, it might be possible that changes in the levels of adipocytokines may precede the onset of abnormal glucose level during pregnancies complicated with GDM which may be of predictive value in the diagnosis of this pregnancy complication. Although TNF- α showed significant change in GDM compared to healthy pregnancy, however we do not recommend using this marker as a predictive or as a diagnostic marker for GDM because of low blood level, beside it is an inflammatory marker which is affected by many diseases.

Acknowledgments The authors are grateful to the Deanship of Scientific Research, King Abdul Aziz University, Jeddah, Saudi Arabia, for the generous financial support of the Project No. 17-026/430. The authors also thank Prof. Hassan S Abduljabbar, Consultant and Professor of Obstetrics and Gynecology, King Abdul Aziz University Hospital and Dr. Nabeela Y. Qusti, Senior Specialist in Maternity and Children Hospital, Jeddah, for their assistance in diagnosing and collecting samples.

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