Dysregulation of tumor necrosis factor-α and interleukin-6 as predictors of gestational disorders

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Background
Approximately 7% of all pregnancies are complicated by gestational diabetes mellitus (GDM). Maternal complications pertaining to GDM are associated with a variety of complications in pregnancy, most notably preeclampsia (PE), which is characterized by an exaggerated systemic inflammatory response. The study aimed to examine the clinical significance of detection of the cytokine tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in pregnant women with GDM and PE.

Patients and methods
A total of 60 pregnant women, comprising 25 diagnosed with GDM, 15 with PE, and 20 controls normoglycemic and normotensive, were examined. TNF-α and IL-6 were estimated.

Results
IL-6 concentration was significantly higher in the pregnant females who developed GDM and PE later during pregnancy compared with the control group ($P < 0.0001$ and 0.003, respectively). TNF-α concentration showed only significant difference in the women who developed GDM later during pregnancy when compared with the control group ($P < 0.0001$).

Conclusion
TNF-α and IL-6 have been proposed to play an important role in the prediction of the pathogenesis of GDM and PE.

Keywords:
gestational diabetes mellitus, interleukin-6, preeclampsia, tumor necrosis factor

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Introduction

Gestational diabetes mellitus (GDM) is one of the most common medical disorders in pregnancy (Khorasani *et al.*, 2018). Nearly 7% of all pregnancies are complicated by GDM; the incidence ranges from 1 to 14% of all pregnancies, depending on the studied population (American Diabetes Association, 2004).

There is a marked reduction in insulin sensitivity late in pregnancy comparable to that found in type 2 diabetes and obesity (Tarasenko *et al.*, 2018). The postreceptor mechanisms contributing to the insulin resistance in normal pregnancy appear to be multifactorial (Metzger *et al.*, 2007). Gestational diabetes was diagnosed in women with a preexisting glucose intolerance revealed by routine glucose tolerance screening during pregnancy (Robitaille and Grant, 2008). The strong family link of GDM with type 2 diabetes and polymorphisms reflects an inherent malfunction of the β-cell exposed by the insulin resistance that occurs during pregnancy (Lindsay, 2009).

Maternal complications related to GDM comprise increased risk of cesarean section, hypertensive disorders of pregnancy, and preeclampsia (PE) (Schmidt *et al.*, 2001). PE is a hypertensive disorder of pregnancy that considerably contributes to maternal and fetal/neonatal morbidity and mortality (American College of Obstetricians and Gynecologists Committee on Obstetric Practice, ACOG Practice Bulletin, 2002). Although PE complicates 6–10% of pregnancies in the United States, the incidence is higher in developing countries (Sibai, 2003). PE occurs in 12% of diabetic women compared with 8% of nondiabetics (Duley, 2009). Moreover, the risk of PE is related to maternal age and the duration of preexisting diabetes. The rate of PE is associated with the level of glycemic control (Banerjee *et al.*, 2004). PE comprises excessive inflammatory response and increased secretion of inflammatory cytokines. The pro-inflammatory and anti-inflammatory cytokines appear to be part of the maternal inflammatory response (Duley, 2009). Increased serum levels of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) in women with PE have been postulated to be involved in the pathogenesis and maternal vascular dysfunction

in PE. These consequently could have serious effect on the developing embryo.

Patients and methods

A total of 60 pregnant women enrolled from the Prenatal Diagnosis Clinic in the National Research Center were included according to the family history of diabetes. Gestational age was based on the date of the last menstrual period or by ultrasound examination if the last menstrual period date was unknown or ultrasound demonstrated an incongruity of more than 10 days. Almost all patients had an ultrasound scan former to or at the time of sampling. The exclusion criteria were diabetes, hypertension, multiple pregnancies, fetal congenital abnormalities, chronic inflammatory disorders, current use of corticosteroid, polycystic ovarian syndrome, and collagen vascular disease. Of the 60 pregnant women, 25 were diagnosed with GDM, 15 with PE, and 20 were normoglycemic and normotensive controls. Women were followed up till delivery. Those who developed GDM or PE during the study had a thorough counseling concerning diet, daily exercise, and regular evaluation of blood sugar or blood pressure. The oral glucose tolerance test was performed for all studied women. They were advised to fast for 12 h before the test. Plasma glucose concentrations were detected by the glucose oxidase method, based on the American Diabetes Association (2003). Diagnosis of hypertension was established on systolic blood pressure more than or equal to 140 mmHg and/or diastolic more than or equal to 90 mmHg after the 20th week of pregnancy based on the average of at least two measurements, taken using the same arm. Protein was detected in urine using dipsticks, and the diagnosis of proteinuria was based on protein concentration of more than or equal to 30 mg (≥1 + on dipstick) in two arbitrary urine samples in the absence of a urinary tract infection. Fasting blood glucose and insulin were measured at 16 weeks of gestation and insulin resistance was calculated using the homeostasis model assessment of insulin resistance. The value for insulin resistance was calculated from the product of the fasting concentration of insulin and glucose divided by a constant using the following formula: fasting serum insulin (μU/ml)×fasting plasma glucose (mg/dl)/405.

All participants granted informed consent, and the study was approved by the Ethical Committee of the National Research Center number 09035. A maternal venous fasting blood sample was attained and collected over sodium fluoride for immediate glucose determination, and heparin for insulin and cytokine measurement, and then centrifuged for the separation of plasma using (Sigma Laborzentrifugen, DRG International, Quantikine R and D systems is Saint Diego, California, USA) centrifuge. The obtained plasma was collected frozen at −20°C. Maternal glucose in plasma was analyzed spectrophotometrically using a kit from Stanbio (Boerne, Texas, USA). Fasting plasma insulin was measured using a kit from DRG International (USA). TNF-α was evaluated by Orgenium Finland Laboratories' human TNF-α ELISA kit, and IL-6 was assayed using Quantikine R and D systems, ELISA kit.

Statistical analysis

Data analysis was performed with statistical package for the social sciences, version 16. Results are expressed as mean ± SD. Means of the groups were compared with the Student *t* test. For comparisons, *P* value less than 0.05 was accepted as significant.

Results

Statistical analysis was performed using Graph Pad Prism 5.00. Student's *t* test was used for normally distributed values. It was done to determine the significance of difference between the control and each of the GDM and the PE group with respect to maternal age at delivery, weight, blood pressure, and cytokine level. When the variance was significantly different, Welch correction was done. The nonparametric Mann–Whitney *U* test determined the significant difference between the control group and each of the GDM and the PE groups with respect to their glucose level, insulin level, and insulin resistance. *P* values less than or equal to 0.05 were considered statistically significant.

Women who developed gestational diabetes were older at expected date of delivery, had higher body weight, and 48% of them were aged more than or equal to 30 years. None of the control groups had previous GDM. On the contrary, 52% with gestational diabetes had family history of diabetes and five of them had previous gestational diabetes (Table 1). The maternal age of women who developed PE at delivery slightly differed from the normotensive pregnant females (Table 2).

Fasting glucose, fasting insulin, and homeostasis model assessment of insulin resistance were significantly

Table 1 Gestational diabetes mellitus group versus control group

	Control (*n*=20) (mean±SD)	GDM (*n*=25) (mean±SD)	*P*
Maternal age at delivery (years)	26.1±6.21	29.0±4.83	0.085
Age ≥30 years [*n* (%)]	6 (30)	12 (48)	
Body weight (kg)	78±17.6	83.2±10.2	0.221
Family history of DM [*n* (%)]	5 (25)	13 (52)	
Previous GDM	None	5	

GDM, gestational diabetes mellitus. *P*<0.05 is considered significant.

higher in women who subsequently developed GDM compared with controls (Table 3). Figs. 1–3 demonstrated the difference in fasting glucose, fasting insulin levels, and the degree of insulin resistance in the control group and the GDM group.

When IL-6 was measured at 16–18 weeks of gestation, its concentration was significantly higher in GDM group and PE late in pregnancy as compared with the control group, which remained of normal glucose tolerance and normotensive throughout pregnancy, with *P* value less than 0.0001 and 0.003, respectively (Fig. 4).

Unlike IL-6, TNF-α when measured at 16 weeks of gestation showed only significant difference in the GDM group that developed later during pregnancy when compared with the control group, which remained of normal glucose and normotensive during pregnancy, with a *P* value less than 0.0001. As for the group that developed PE later on during pregnancy, the TNF-α concentration was not significantly different when compared with the control group (Fig. 5 and Table 4). The adverse outcomes in neonates of pregnant affected females with GDM and PE are demonstrated in Tables 5 and 6, respectively.

Table 2 Preeclamptic group versus control group

	Control (*n*=20) (mean±SD)	Preeclampsia (*n*=15) (mean±SD)	*P*
Maternal age at delivery (years)	26.1±6.21	29.4±5.19	0.105
Age ≥30 years [*n* (%)]	6 (30)	2 (13.3)	
Body weight (kg)	78±17.6	80±16.5	0.734
Systolic BP	115±6.7	142±14.8	<0.0001
Diastolic BP	74.7±4.3	96.9±9.1	<0.0001
Previous PE	None	3	

BP, blood pressure; PE, preeclampsia. *P*<0.05 is considered significant.

Table 3 Glucose, insulin, and insulin resistance in control versus gestational diabetes mellitus group

	Control (*n*=20) (mean±SD)	GDM (*n*=25) (mean±SD)	*P*
Fasting glucose (mg/dl)	82.6±3.8	89±5.2	0.0019
Fasting insulin (μU/ml)	9.71±3.6	14±1.9	0.0006
HOMA-IR	1.9±0.9	2.91±1.7	<0.0001

GDM, gestational diabetes mellitus; HOMA-IR, homeostasis model assessment of insulin resistance. *P*<0.05 is considered significant.

Table 4 Comparison of interleukin-6 and tumor necrosis factor alpha in control, gestational diabetes mellitus, and preeclampsia groups

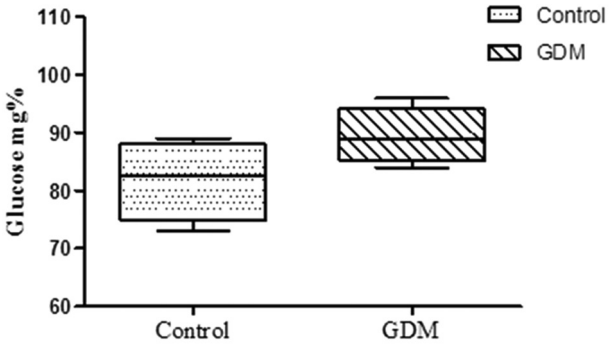
	Control (*n*=20)	GDM (*n*=25)	Preeclampsia (*n*=15)	*P*
IL-6 (pg/ml)	4.95±1.27	7.23±1.77	6.83±1.45	<0.0001* 0.003**
TNF-α (pg/ml)	4.08±0.837	6.57±1.51	4.07±0.961	<0.0001* 0.9653**

GDM, gestational diabetes mellitus; IL-6, interleukin-6; PE, preeclampsia; TNF-α, tumor necrosis factor alpha. *P*<0.05 is considered significant. **P* of control versus GDM. ***P* of control versus PE.

Discussion

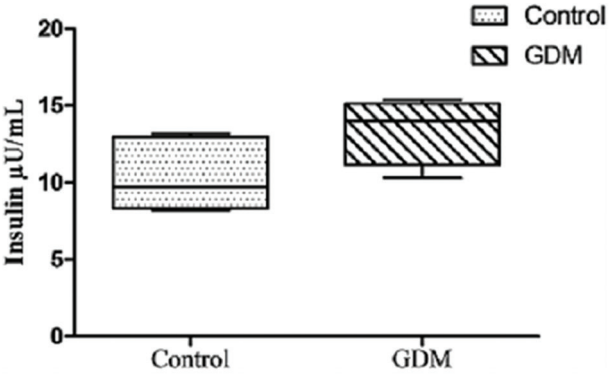
Prepregnancy obesity is associated with increased risk of adverse pregnancy outcome such as GDM, gestational

Figure 1



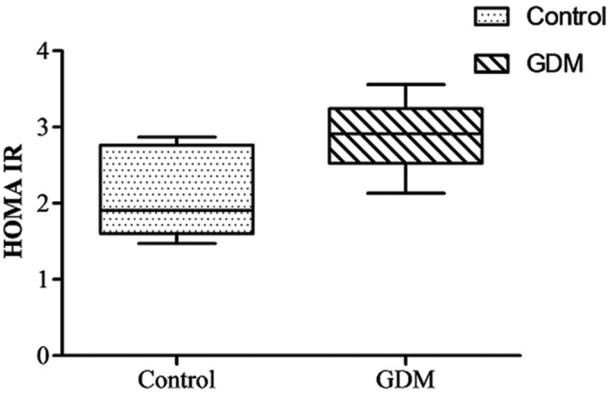
Fasting glucose level in control and GDM groups. GDM, gestational diabetes mellitus.

Figure 2



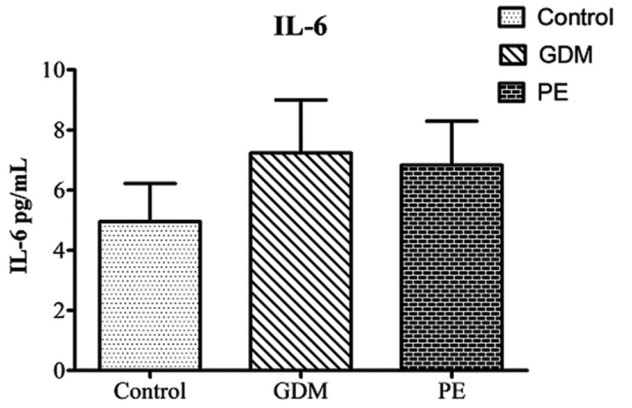
Fasting plasma insulin level in control and GDM groups. GDM, gestational diabetes mellitus.

Figure 3



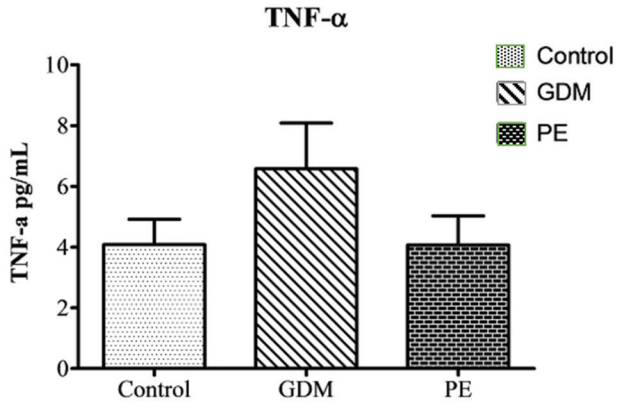
Insulin resistance in control and GDM groups. GDM, gestational diabetes mellitus.

Figure 4



IL-6 concentration in the three studied groups. IL-6, interleukin-6.

Figure 5



TNF-α in the three studied groups. TNF-α, tumor necrosis factor alpha.

Table 5 Neonatal outcomes of gestational diabetes mellitus-affected women

Total number of newborns	25
Number of negatively affected newborns [*n* (%)]	16 (64)
Newborns with macrosomia (>4 kg)	10
Newborns with respiratory distress	2
Newborns with congenital anomalies	2
Newborns with jaundice	2

Table 6 Neonatal outcomes of preeclampsia-affected females

Total number of newborns	15
Number of negatively affected newborns [*n* (%)]	10 (66.66)
Newborns with low birth weight	6
Newborns with intrauterine growth retardation	4

hypertension, PE, fetal macrosomia, and the need of cesarean delivery (Singh and Rastogi, 2008).

GDM and type 2 diabetes mellitus have similar pathophysiology of increased insulin resistance. It has been shown that GDM and type 2 diabetes mellitus share similar genetic backgrounds (Khambalia *et al.*, 2013). In our study, 52% of the women who developed gestational diabetes had a family history of diabetes. The recurrence rate of gestational diabetes in a second consecutive pregnancy was 41.2% (Hernandez-Díaz *et al.*, 2009).

The association between primiparity and PE was at the core of several pathophysiological theories (Lappas *et al.*, 2004). The risk recurrence was approximately 15% for women who had PE in one previous pregnancy, and approximately 30% were complicated with PE when two affected consecutive previous pregnancies occurred (Morisset *et al.*, 2011). On the contrary, Wikstrom *et al.* (2011) found that women with previous preterm PE have increased risks of adverse pregnancy outcomes in a second pregnancy despite the absence of PE. In our current study, 35% of the pregnant females who developed PE experienced a previous pregnancy complicated with PE.

Our study revealed significant difference in the level of IL-6 in the GDM compared with the control group, during 15–20 weeks of gestation. This is in contrast to the study of previous investigators who demonstrated that the release of IL-6 was not different in patients with GDM versus women with normal glucose tolerance (Lain *et al.*, 2008). However, others verified that serum IL-6 levels were higher in GDM mothers compared with control women (Ategbo *et al.*, 2006; Yang *et al.*, 2018). The discrepancy may be explained by the presence of study differences in gestational time at IL-6 measurement (Morisset *et al.*, 2011).

In the study of McLachlan *et al.* (2006), TNF-α correlated inversely with insulin secretion in pregnancy and was significantly higher in the GDM group. Xue-Lian *et al.* (2008) found significant difference in the values of TNF-α among different groups (control and GDM) and gestational impaired glucose intolerance both at 14–20 and 24–32 weeks of gestation. In a cross-sectional study involving 53 pregnant women in the early third trimester of pregnancy, TNF-α was significantly higher in GDM compared with controls (Salmi *et al.*, 2012). TNF-α inhibits insulin secretion and regulates glucose uptake in GDM (Vrachnis *et al.*, 2012). This agrees with the findings of our study where TNF-α was significantly higher in GDM groups compared with the level of the control group. In contrast, Eschler *et al.* (2018) concluded that the TNF-α levels in the plasma of GDM and control mothers were not significantly different.

In pregnancy, IL-6 has been proposed to aggravate insulin resistance and participate in the pathogenesis of GDM (Heinrich *et al.*, 2003).

Our study revealed elevation in IL-6 but not TNF-α in women with PE. Xiao *et al.* (2012) elucidated increased levels of IL-6 in women with PE compared with healthy pregnant women regardless the onset of PE, supporting a generalized inflammatory condition

in PE. Similarly, previous studies delineated that IL-6 was significantly increased in women with PE at more than or equal to 36th weeks. Before delivery, the values of IL-6 and TNF-α in preeclampatic pregnant women were found to be significantly higher when compared with normotensive pregnant women (Kronborg *et al.*, 2010). However, other results do not support an increase in IL-6 levels in patients with early- and late-onset PE (Kalinderis *et al.*, 2011). The conflicting data on cytokine levels are most likely to be the result of the timing of sampling, but most importantly it reflects the diversity that lies within the pathogenesis of PE.

Several adverse neonatal outcomes were associated with GDM and PE, such as the pathogenesis of congenital malformations, which are four to ten times higher in pregnant women with diabetes. The precise mechanism by which it occurs has not been completely clarified. It is supposed that hyperglycemia could cause damage to the developing embryo, an increased production of free oxygen radicals, deficiency of myoinositol and arachidonic acid, and a disruption in signal transduction (Roca-Rodríguez *et al.*, 2017).

In this study, it was found that 36% of the neonates of GDM women and 33.33% of preeclampetic ones were affected. Neonates of GDM mothers experienced macrosomia, respiratory distress, congenital anomalies, and jaundice with an incidence of 40, 8, 8, and 8%, respectively. According to a study done by Gasim (2012), neonates born to women with GDM had a significantly higher rates of macrosomia compared with the neonates born to mothers from the control group. Additionally, the study of Bener *et al.* (2011) showed that the neonates of GDM mothers were at increased risk of preterm birth (12.6%), macrosomia (10.3%), and birth trauma (8%).

PE is a significant risk factor in the development of intrauterine growth retardation (IUGR) and represents the most common cause of IUGR in the nonanomalous infants (Plaks *et al.*, 2013). PE is characterized by placental hypoperfusion and shallow trophoblast invasion of uterine blood vessels, putting the fetus at risk for IUGR and low birth weight (Marseglia *et al.*, 2016). According to the findings of our current study, 40% of the neonates of the group with PE experienced low birth weight and 26.6% experienced IUGR. This indicates the negative effect on the health of the neonates of mothers with gestational disorders.

Conclusion

The results suggest that TNF-α and IL-6 play an important role in the prediction of the pathogenesis of GDM and PE.

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Conflicts of interest

There are no conflicts of interest.

References

American College of Obstetricians and Gynecologists Committee on Obstetric Practice, ACOG Practice Bulletin (2002). Diagnosis and management of preeclampsia and eclampsia. *Obstet Gynecol* **99**:159–167.

American Diabetes Association (2004). Gestational diabetes mellitus. *Diabetes Care* **27:**S88–S90.

Ategbo J, Grissa O, Yessoufou A, Hichami A, Dramane L, Moutairou K, *et al.* (2006). Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab* **91**:4137–4143.

Banerjee S, Ghosh U, Banerjee D (2004). Effect of tight glycemic control on fetal complications in diabetic pregnancies. *J Assoc Physicians India* **52**:109–113.

Bener A, Saleh N, Al-Hamaq A (2011). Prevalence of gestational diabetes and associated maternal and neonatal complications in a fast-developing community: global comparisons. *Int J Womens Health* **3**:367–373.

Duley L (2009). The global impact of pre-eclampsia and eclampsia. *Semin Perinatol* **33**:130–137.

Eschler DC, Kulina G, Garcia-Ocana A, Li J, Kraus T, Levy CJ (2018). Circulating levels of bone and inflammatory markers in gestational diabetes mellitus. *Biores Open Access* **7**:123–130.

Gasim T (2012). Gestational diabetes mellitus: maternal and perinatal outcomes in 220 Saudi women. *Oman Med J* **27**:140–144.

Heinrich P, Behrmann I, Haan S, Hermanns H, Müller-Newen G, Schaper F (2003). Principles of interleukin (IL)-6-type cytokine signaling and its regulation. Biochem J **374**:1–20.

Hernandez-Díaz S, Toh S, Cnattingius S (2009). Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. *Br Med J* **338**:2255.

Kalinderis M, Papanikolaou A, Kalinderi K, Loannidou E, Giannoulis C, Karagiannis V, *et al.* (2011). Elevated serum levels of interleukin-6, interleukin-1β and human chorionic gonadotropin in pre-eclampsia. *Am J Reprod Immunol* **66**:468–475.

Khambalia A, Ford J, Nassar N, Shand A, McElduff A, Roberts C (2013). Occurrence and recurrence of diabetes in pregnancy. *Diabetic Med* **30**:452–456.

Khorasani ZM, Bonakdaran S, Rafieie HP (2018). The relationship between vitamin D deficiency and insulin resistance in pregnant women with gestational diabetes. *Curr Diabetes Rev. doi: 10.2174/1573399814666181102100816*. [Epub ahead of print].

Kronborg C, Gjedsted J, Vittinghus E, Hansen T, Allen J, Knudsen U, *et al.* (2010). Maternal and umbilical serum levels of interleukin-6, interleukin-8, and tumor necrosis factor-alpha in normal pregnancies and in pregnancies complicated by preeclampsia. *J Matern Fetal Neonatal Med* **23**:880–886.

Lain K, Daftary A, Ness R, Roberts J (2008). First trimester adipocytokine concentrations and risk of developing gestational diabetes later in pregnancy. *Clin Endocrinol* **69**:407–411.

Lappas M, Permezel M, Rice G (2004). Release of proinflammatory cytokines and 8-isoprostane from placenta, adipose tissue, and skeletal muscle from normal pregnant women and women with gestational diabetes mellitus. *J Clin Endocrinol Metab* **89**:5627–5633.

Lindsay R (2009). Gestational diabetes mellitus causes and consequences. *Br J Diabetes Vasc Dis* **9**:27–31.

Marseglia L, D'Angelo G, Manti S, Reiter J, Gitto E (2016). Potential utility of melatonin in preeclampsia intrauterine fetal growth retardation, and perinatal *Asphyxia. Reprod Sci* **23**:970–977.

McLachlan K, O'Neal D, Jenkins A, Alford F (2006). Do adiponectin, TNF alpha, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy. *Diabetes Metab Res Rev* **22**:131–138.

Metzger B, Buchanan T, Coustan D, De Leiva A, Dunger D, Hadden D, *et al.* (2007). Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care* **30**:S251–S260.

Morisset A, Dubé M, Côté J, Robitaille J, Weisnagel S, Tchernof A (2011). Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. *Acta Obstet Gynecol Scand* **90**:524–530.

Plaks V, Rinkenberger J, Dai J, Flannery M, Sund M, Kanasaki K, *et al*. (2013). Matrix metalloproteinase-9 deficiency phenocopies features of preeclampsia and intrauterine growth restriction. *Proc Natl Acad Sci U S A* **110**:11109–11114.

Robitaille J, Grant A (2008). The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. *Genet Med* **10**:240–250.

Roca-Rodríguez MDM, López-Tinoco C, Fernández-Deudero Á, Murri M, García-Palacios MV, García-Valero MDA, *et al*. (2017). Unfavorable cytokine and adhesion molecule profiles during and after pregnancy, in women with gestational diabetes mellitus. *Endocrinol Diabetes Nutr* **64**:18–25.

Salmi A, Zaki N, Zakaria R, Nor Aliza A, Rasool A (2012). Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus. *VASA* **41**:96–104.

Schmidt M, Duncan B, Reichelt A, Branchtein L, Matos M, Costa e Forti A, *et al*. (2001). Gestational diabetes mellitus diagnosed with a 2-h 75-g oral glucose tolerance test and adverse pregnancy outcomes. *Diabetes Care* **24**:1151–1156.

Sibai B (2003). Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol* **102**:181–192.

Singh S, Rastogi A (2008). Gestational diabetes mellitus. Diabetes and metabolic syndrome. *Clin Res Rev* **2**:227–234.

Tarasenko KV, Gromova AM, Pikul KV, Lysenko RB, Nesterenko LA (2018). Pathogenesis of insulin resistance in pregnant women with obesity. *Wiad Lek* **71**:801–806.

Vrachnis N, Belitsos P, Sifakis S, Dafopoulos K, Siristatidis C, Pappa K, *et al*. (2012). Role of adipokines and other inflammatory mediators in gestational diabetes mellitus and previous gestational diabetes mellitus. *Int J Endocrinol* **2012**:1–6.

Wikstrom AK, Stephansson O, Cnattingius S (2011). Previous preeclampsia and risks of adverse outcomes in subsequent nonpreeclamptic pregnancies. Am J Obstet Gynecol **204**:148.

Xiao J, Yin Y, Gao Y, Lau S, Shen F, Zhao M, *et al*. (2012). The increased maternal serum levels of IL-6 are associated with the severity and onset of preeclampsia. *Cytokine* **60**:856–860.

Xue-Lian G, Hui-xia Y, Yi Z (2008). Variations of tumor necrosis factor-alpha, leptin and adiponectin in mid-trimester of gestational diabetes mellitus. *Chin Med J Engl* **121**:701–705.

Yang Y, Liu L, Liu B, Li Q, Wang Z, Fan S, *et al*. (2018). Functional defects of regulatory T cell through interleukin 10 mediated mechanism in the induction of gestational diabetes mellitus. *DNA Cell Biol* **37**:278–285.

American Diabetes Association (2003). Medical identification products. Diabetes Forecast 56:123-6.