Endocrinología, Diabetes y Nutrición 71 (2024) 12–18

Endocrinología, Diabetes y Nutrición

www.elsevier.es/endo

ORIGINAL ARTICLE

Evaluation of serum placenta-specific gene 8 protein, total antioxidant capacity, interleukin-10, interleukin-17A, interleukin-21 and interleukin-33 levels in Turkish women with gestational diabetes mellitus

Enver Ciraci[a], Tugba Elgun[b,*], Asiye Gok Yurttas[c], Hazel Cagin Kuzey[d], Yagmur Ekenoglu Merdan[e], Muhammed Sait Toprak[f], Sermin Tetik[g]

[a] *Biruni University, Faculty of Pharmacy, Department of Biochemistry, Zeytinburnu, Istanbul, Turkey*
[b] *Biruni University, Faculty of Medicine, Department of Medical Biology, Zeytinburnu, Istanbul, Turkey*
[c] *Istanbul Health and Technology University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkey*
[d] *Memorial Şişli Hospital, Gynaecology and Obstetrics, Istanbul, Turkey*
[e] *Biruni University, Faculty of Medicine, Department of Medical Microbiology, Zeytinburnu, Istanbul, Turkey*
[f] *Demiroglu Bilim University, Vocational School of Health Services, Department of Medical Laboratory, Istanbul, Turkey*
[g] *Lefke European University, Faculty of Pharmacy, Department of Biochemistry Lefke, Northern Cyprus*

Received 28 September 2023; accepted 24 November 2023

KEYWORDS
Gestational diabetes mellitus;
Cytokines;
Biomarkers;
PLAC8;
Total antioxidant capacity

Abstract
Purpose: Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance that begins or is diagnosed during pregnancy. Our study aimed to establish a correlation between proinflammatory and anti-inflammatory response in order to be able to develop treatment strategies and determine early diagnosis biomarkers in the sera of cases diagnosed with GDM. Moreover, we aimed to investigate interleukin (IL), placenta-specific gene 8 protein (PLAC8) and total antioxidant capacity (TAC) in patients with GDM.
Methods: A total of 121 patients were included in the study. These were divided into four patient groups: pregnant and diagnosed with DM (P-GDM, $n = 30$); pregnant and not diagnosed with DM (P-NGDM, $n = 32$); non-pregnant diagnosed with DM (NP-DM, $n = 29$) and non-pregnant and not diagnosed with DM (NPNDM, $n = 30$). IL-10, IL-17A, IL-21, IL-33, PLAC8 and TAC determinations from patients were evaluated by ELISA (Enzyme-Linked ImmunoSorbent Assay) method.
Results: IL-10 and IL-33 concentrations were found to be significantly higher in P-GDM and NP-DM patient groups compared to P-NGDM and NP-NDM groups ($p < 0.001$). The PLAC8 level in the P-GDM patient group (20.38 ± 5.37) was determined to be significantly higher than in the P-NGDM

* Corresponding author.
E-mail address: telgun@biruni.edu.tr (T. Elgun).

patient group (3.41 ± 2.17, $p < 0.001$). TAC in the P-NGDM and NP-NDM groups (12.42 ± 2.31 vs. 12.96 ± 3.78, $p < 0.001$) was determined to be significantly higher than in the P-GDM and NP-DM groups (4.8 ± 0.52 vs. 2.21 ± 0.71, $p < 0.001$).
Discussion: The fact that the importance of PLAC8 level and TAC in the diagnosis and follow-up of GDM in pregnancy is demonstrated for the first time in this study shows that it is unique.

PALABRAS CLAVE
Diabetes mellitus gestacional;
Citocinas;
Biomarcadores;
PLAC8;
Actividad antioxidante total

Evaluación de los niveles séricos de proteína del gen 8 específico de la placenta, capacidad antioxidante total, interleucina-10, interleucina-17A, interleucina-21 e interleucina-33 en mujeres turcas con diabetes mellitus gestacional

Resumen
Objetivo: La diabetes mellitus gestacional (DMG) se define como una intolerancia a los carbohidratos que comienza o se diagnostica durante el embarazo. El objetivo de nuestro estudio consistió en proporcionar una correlación entre la respuesta proinflamatoria e inflamatoria para desarrollar estrategias de tratamiento y determinar biomarcadores séricos diagnósticos tempranos en las mujeres con DMG. Además, pretendíamos investigar la interleucina (IL), la proteína del gen 8 específica de la placenta (PLAC8) y la actividad antioxidante total (AAT) de las pacientes con DMG.
Métodos: En el estudio se incluyó a un total de 121 pacientes. Hubo cuatro grupos: embarazadas y con diagnóstico de DMG (E-DMG, n = 30), embarazadas y sin DMG (E-NDMG, n = 32); no embarazadas con DMG (NE-DMG, n = 29) y no embarazadas y sin DMG (NE-NDMG, n = 30). Las determinaciones de IL-10, IL-17A, IL-21, IL-33, PLAC8 y la AAT de las mujeres se evaluaron mediante el método ELISA (prueba de inmunoadsorción enzimática).
Resultados: Se observó que las concentraciones de IL-10 e IL-33 eran significativamente superiores en los grupos de pacientes E-DMG y NE-DMG en comparación con los de E-NDMG y NE-NDMG (p < 0,001). La concentración de PLAC8 en el grupo de E-DMG (20,38 ± 5,37) era significativamente superior que en el de E-NDMG (3,41 ± 2,17) (p < 0,001). La AAT en los grupos de E-NDMG y NE-NDMG (12,42 ± 2,31 vs. 12,96 ± 3,78, p < 0,001) era significativamente mayor que en los de E-DMG y NE-DMG (4,8 ± 0,52 vs. 2,21 ± 0,71, p < 0,001).
Discusión: En este estudio se ha demostrado por primera vez la importancia de la concentración de PLAC8 y de la AAT en el diagnóstico y seguimiento de la DMG en el embarazo, lo que lo convierte en un trabajo sin precedentes.

Introduction

The prevalence of maternal obesity is increasing rapidly worldwide and is reported as a major gynaecological problem, due to increased mother and child mortality and morbidity. It has been shown that while obese women have a 10% tendency to develop pregnancy complications such as gestational diabetes mellitus (GDM), their children also develop cardiovascular and metabolic diseases in later life.[1] Maternal obesity and GDM may be associated with a chronic condition called ''meta-inflammation'' that develops against low-grade inflammation and an acute inflammatory response. Developing cardiovascular and metabolic disorders in addition to pregnancy complications such as pre-eclampsia, thromboembolism and gestational diabetes in obese women is one of the risks that obese women face during the process of their pregnancy.[2] In this group, the risk of developing GDM was found to be 1.3–3.8 times higher in obese women compared to women with normal body mass index. It is also known that 70% of women with GDM have the risk of developing type 2 diabetes (T2DM).[1,3–5]

GDM is defined as glucose intolerance that begins during the second or third trimester of pregnancy. Many experimental and clinical studies have proven that metabolic syndrome components such as impaired glucose tolerance, dyslipidaemia and hypertension are associated with low-grade systemic inflammation. Metabolic syndrome is a metabolic disorder characterised by biochemical and clinical findings in which some or all of the parameters of insulin resistance, impaired glucose balance, dyslipidaemia, obesity and hypertension are present.[4] Inflammatory signalling pathways are activated in metabolic syndrome, changing the release of proinflammatory and anti-inflammatory cytokines, and causing biochemical and clinical disorders attributed to metabolic syndrome. Despite advances in this area, the

pathogenesis and pathophysiology of GDM has not been fully clarified.[1,4,6]

It has been reported that proinflammatory and anti-inflammatory cytokines are necessary for the development and continuation of pregnancy from the beginning to the end of pregnancy.[7] It has been shown that gestational diabetes mellitus constitutes a metabolic disorder that needs to be explained and followed up because the balance between the proinflammatory system and the anti-inflammatory system tends towards proinflammation, when compared to non-pregnant women.[8,9]

There are studies showing that hyperglycaemia initiates inflammation and causes the secretion of cytokines, which are protein molecules that function as immune mediators and regulators expressed by various cell types, and the formation of the acute phase response.[9,10] In addition to the physiological role in the fetoplacenta during pregnancy, the expression of cytokines at abnormal levels has a role in the pathophysiology of GDM.[10,11]

In recent years, there have been increasing studies on the role of the inflammatory system in the pathogenesis of T2DM and GDM.[12,13] Because of the similarity between T2DM and GDM and the clear relationship between type II diabetes and inflammation, it has been hypothesised that inflammation may also be involved in the pathophysiology of GDM.[14,15] During pregnancy, the increase in cytokines such as interferon-gamma (IFN-G) and tumour necrosis factor-alpha (TNF-A) synthesised by Th1, the producer of proinflammatory cytokines, jeopardises the pregnancy, while IL-4, which is synthesised by Th2, is one of the anti-inflammatory cytokines. Increased synthesis of IL-6 and IL-10 ensures the continuation of a normal pregnancy period. IL-10 production is related to genetic variations in its promoter region, and this region controls transcription and contains SNPs that are related to diabetes and its complications.[16,17] In addition, inflammation caused by secreted cytokines is thought to be associated with increased insulin resistance in pregnant women with gestational diabetes.[18]

Th1 cells initiate inflammation by acting on the cell-mediated immune system and are therefore considered cytotoxic agents. However, Th2 cells are effective in humoral immunity and regulate the inflammatory response.[19] Th1/Th2 changes have been observed not only in normal pregnancies, but also in pregnancies complicated by diabetes. According to the results of clinical and experimental studies, it has been suggested that while the Th1 response decreases during pregnancy, the Th2 response increases.[20] Studies have shown that Th2 response predominates with a successful pregnancy, while Th1 activity is prominent in spontaneous abortions and pre-eclampsia. However, since it is associated with Th2 immunity in recurrent miscarriages, the Th1/Th2 balance was insufficient to explain the mechanism preventing foetal allograft rejection.[21,22]

Placenta-specific gene 8 (PLAC8) is one of the highly conserved placental regulatory genes among placental mammals. It promotes invasion and migration of human trophoblast cells during implantation. It is highly expressed in serumcytoid dendritic cells and tissues of the immune system such as bone marrow, lymph nodes, and spleen in adult mammals.[23]

Materials and method

The study was reviewed and approved by the Ethics Committee of Biruni University (2019/30-10) and was conducted in accordance with the World Medical Association Declaration of Helsinki. Between 2021 and 2022, female patients between the ages of 18 and 40 who were followed up routinely at Biruni University Hospital and at internal medicine and obstetrics and gynaecology outpatient clinics were included in the study. The 75 g oral glucose tolerance test (OGTT) was performed on the pregnant group between 24 and 28 weeks of pregnancy.

A total of 121 volunteer patients were included in the study. These were divided into four patient groups: diagnosed with GDM (P-GDM, $n = 30$) not diagnosed with GDM (P-NGDM, $n = 32$); non-pregnant diagnosed with DM (NP-DM, $n = 29$) and not diagnosed with DM (NP-NDM, $n = 30$).

For the diagnosis of GDM and DM, 75 g glucose load and 0 hour $\geq$95 mg/dl on OGTT; 1st hour $\geq$180 mg/dl; 2nd hour $\geq$153 mg/dl is considered as a critical value according to IADPSG (International Association of Diabetes and Pregnancy Study Groups).

Anthropometric measurements (BMI), demographic data (age, gestational age) and biochemical parameters (fasting blood glucose, HbA1c, insulin, lipid) of the individuals included in the study were examined. Venous blood samples were collected from all subjects after an eight-hour overnight fasting period. Blood samples (serum) were collected in tubes without anticoagulants, centrifuged at 3000 rpm for 10 min, and stored at $-80\,^{\circ}C$ until analysis.

IL-21, IL-33, IL-10, IL-17A, PLAC8 and total antioxidant capacity determinations from patients were evaluated by ELISA (Enzyme-Linked ImmunoSorbent Assay) method. Reagents in ELISA kits (BT-LAB, Shanghai Korain Biotech) were prepared and studied in accordance with the procedure IL-10 (Cat. No: E0102Hu), IL-17A (Cat. No: E0047Hu), IL-21 (Cat. No: E6614Hu), IL-33 (Cat. No: E0044Hu), PLAC-8-onzin (Cat. No: E6632Hu) and total antioxidant capacity assays (Cat. No: E4350Hu). The calibration standard curve graph was drawn from the absorbance values obtained as a result of the study. IL-21, IL-33, IL-10, IL-17A, PLAC8 and total antioxidant concentrations of patient serum samples were calculated using the calibration standard curve graph formula.

Statistical analyses

Prism 9.1.1 (GraphPad Software, La Jolla, CA) was used for all statistical analyses and significance was noted when the p value was <0.05. Differences between the groups were compared by Mann–Whitney U test. Relationships between variables were tested by Spearman's rank correlations. The two-way ANOVA test was used to compare the differences in cytokine, PLAC8 and total antioxidant activity (TAC) concentrations.

Results

Demographic characteristics, anthropometric measurements and biochemical parameters of the study subjects

Table 1 Clinical characteristics of the study participants.

Variable	P-GDM (m ± SD)	P-NGDM (m ± SD)	NP-DM (m ± SD)	NP-NDM (m ± SD)	*p* value
Age (year)	27.38 ± 4.68	26.96 ± 5.42	26.25 ± 3.17	27.66 ± 5.18	>0.061
Gestational age (weeks)	25.31 ± 1.96	26.02 ± 1.38	N/A	N/A	>0.076
BMI (kg/m²)	25.41 ± 4.33	23.21 ± 3.87	22.25 ± 3.61	26.35 ± 4.52	<0.05
OGTT 0 h (mg/dl)	102.35 ± 17.38	80.26 ± 8.52	119.6 ± 19.8	76.42 ± 7.93	<0.001
OGTT 2 h (mg/dl)	188.4 ± 38.97	116.28 ± 9.45	180.51 ± 17.31	108.73 ± 72	<0.001
HbA1c (%)	5.96 ± 1.36	N/A	5.17 ± 1.36	N/A	<0.0009
Insulin (mIU/ml)	15.62 ± 3.28	34.12 ± 6.21	18.45 ± 4.41	31.87 ± 5.27	<0.001
Triglycerides (mg/dl)	2.73 ± 0.96	2.83 ± 0.68	2.63 ± 0.79	2.81 ± 0.82	<0.05
Total cholesterol (mg/dl)	5.76 ± 0.69	5.62 ± 0.91	5.68 ± 0.89	5.74 ± 0.986	>0.05
LDL-cholesterol (mg/dl)	3.5 ± 0.59	3.29 ± 0.91	3.46 ± 0.72	3.43 ± 0.78	>0.05
HDL-cholesterol (mg/dl)	1.88 ± 0.41	1.77 ± 0.5	1.98 ± 0.36	1.79 ± 0.53	<0.001
Ultrasonography results					
Foetal weight (kg)	2.35 ± 0.84	1.86 ± 0.57	N/A	N/A	<0.001
Abdominal circumference (cm)	29.3 ± 6.45	26.21 ± 3.25	N/A	N/A	<0.001

m: mean; SD: standard deviation; P: pregnancy; NP: non-pregnancy; DM: diabetes mellitus; GDM: gestational diabetes mellitus; OGTT: oral glucose tolerance test.

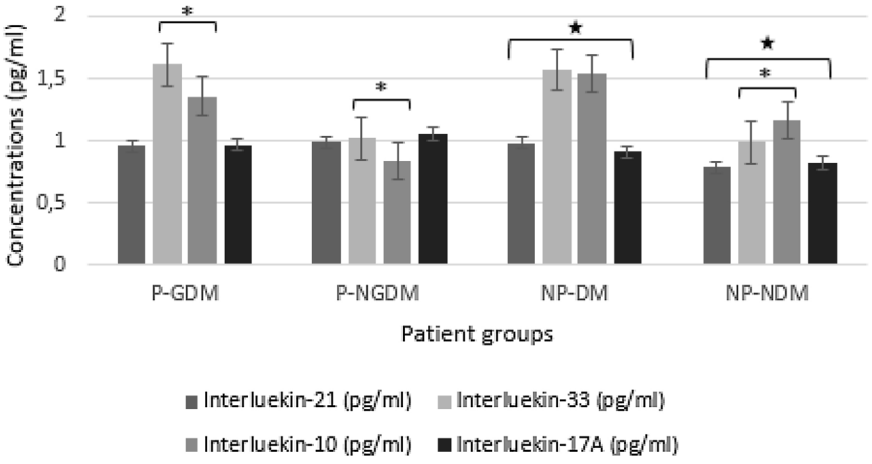

Figure 1 Serum levels of cytokines (IL-10/IL-17A/IL-21 and IL-33) for all groups. *$p < 0.001$; ⋆$p < 0.05$.

are presented in Table 1. The P-GDM, P-NGDM, NP-DM and NP-NDM groups were of similar maternal age (27.38 ± 4.68; 26.96 ± 5.42; 26.25 ± 3.17 vs. 27.66 ± 5.18 years, $p = 0.061$), and the P-GDM and P-NGDM groups were of similar gestational age (25.31 ± 1.96 vs. 26.02 ± 1.38 weeks, $p = 0.076$), respectively. The BMI of the P-GDM group was significantly higher than in the P-NGDM group (25.41 ± 4.33 vs. 23.21 ± 3.87 kg/m², $p < 0.05$). The BMI of the NP-DM group was significantly lower than in the NP-NDM group (22.25 ± 3.61 vs. 26.35 ± 4.52, $p < 0.05$). The patients from the P-GDM and NP-DM groups had significantly higher glucose levels (102.35 ± 17.38 vs. 119.6 ± 19.8 and 188.4 ± 38.97 vs. 180.51 ± 17.31) at 0 h and 2 h of the OGTT (mg/dl) ($p < 0.001$ and p 0.001, respectively). The level of HbA1c (%) was found to be significantly higher in the P-GDM and NP-GDM groups (5.96 ± 1.36 vs. 5.17 ± 1.36, $p < 0.0009$) compared to the P-DM and NP-DM groups. The level of insulin (mIU/ml) was found to be significantly lower in the P-GDM and NP-GDM groups (515.62 ± 3.28 vs. 18.45 ± 4.41, $p < 0.001$) compared to the P-DM and NP-DM groups (34.12 ± 6.21 vs. 31.87 ± 5.27, $p < 0.001$). HDL-cholesterol (mg/dl) levels were found to be significantly higher in the P-GDM and NP-DM groups (1.88 ± 0.41 vs. 1.98 ± 0.36, $p < 0.05$) compared to the P-NGDM and NP-NDM groups (1.77 ± 0.5 vs. 1.79 ± 0.53, $p < 0.05$). No significant changes were detected in total cholesterol (mg/dl) and LDL-cholesterol (mg/dl) levels for each group ($p > 0.05$) (Table 1 and Fig. 1).

When the results were evaluated in terms of ultrasonography results, foetal weight (kg) and abdominal circumference (cm) were found to be significantly higher in GDM patients (2.35 ± 0.84 vs. 29.3 ± 6.45) compared to NGDM patients (1.86 ± 0.57 vs. 26.21 ± 3.25) ($p < 0.001$) (Table 1).

In the study, DM (gestational and non-gestational) and cytokine (IL-10/IL-17A/IL-21 and IL-33), TAC and change in PLAC8 levels were evaluated (Table 2).

Table 2 Serum levels of cytokines, PLAC8 and TAC of patients with and without DM.

Variable	P-GDM	P-NGDM	NP-DM	NP-NDM	*p*
Interleukin-21 (pg/ml)	0.96 ± 0.54	0.99 ± 0.41	0.98 ± 0.52	0.79 ± 0.51[#]	<0.05
Interleukin-33 (pg/ml)	1.61 ± 0.62	1.02 ± 0.39	1.57 ± 0.8	0.99 ± 0.48	<0.001
Interleukin-10 (pg/ml)	1.36 ± 0.42	0.84 ± 0.21	1.54 ± 0.38	1.17 ± 0.4	<0.001
Interleukin-17A (pg/ml)	0.97 ± 0.36	1.06 ± 0.52	0.91 ± 0.52	0.82 ± 0.31	>0.05
PLAC8 (ng/ml)	20.38 ± 5.37	3.41 ± 2.17	N/A	N/A	<0.001
TAC (ng/ml)	4.8 ± 0.52	12.42 ± 2.31	2.21 ± 0.71	12.96 ± 3.78	<0.001

m: mean; SD: standard deviation; P: pregnancy; NP: non-pregnancy; DM: diabetes mellitus; GDM: gestational diabetes mellitus; PLAC8: placenta-specific gene 8 protein; TAC: total antioxidant capacity.
[#] $p < 0.05$.

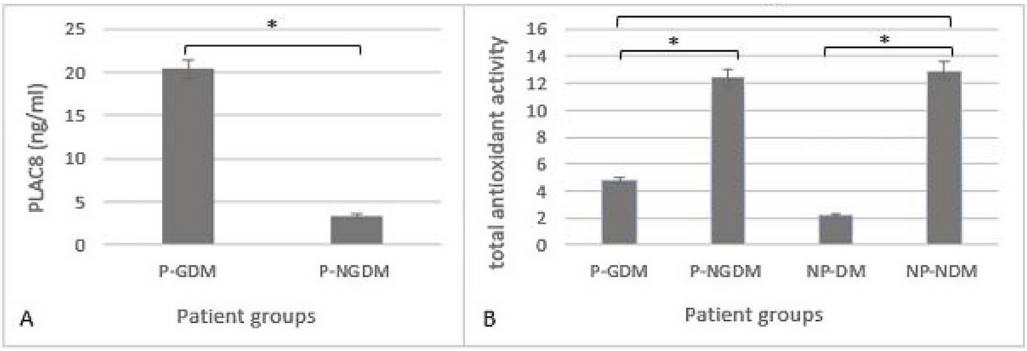

Figure 2 Serum levels of PLAC8 and TAC. The graph in (A) shows the change in PLAC8 concentration of the P-GDM and P-NGDM groups. The graph in (B) shows the change in TAC concentration of the all groups. *$p < 0.001$.

The levels of IL-10/IL-17A/IL-21 and IL-33 associated cytokines in patients with DM (P-GDM and NP-DM) and without DM (P-NGDM and NP-NDM)

IL-17A and IL-21 concentration levels in the NP-NDM patient group (0.79 ± 0.51, $p < 0.05$) were determined to be lower than in the P-GDM, P-NGDM and NP-DM groups (0.96 ± 0.54, 0.99 ± 0.41 vs. 0.98 ± 0.52, $p < 0.05$). IL-33 and IL-10 concentration levels in the P-GDM and NP-DM patient groups (1.61 ± 0.62 vs. 1.57 ± 0.8, $p < 0.001$) were determined to be higher than in the NP-GDM and NP-NDM groups (1.02 ± 0.39 vs. 0.99 ± 0.48, $p < 0.001$) (Fig. 1).

The levels of PLAC8 in patients with and without DM

The PLAC8 level in the P-GDM patient group (20.38 ± 5.37) was determined to be significantly higher than in the P-NGDM patient group (3.41 ± 2.17, $p < 0.001$) (Fig. 2). As expected, there was no pregnancy in the NP-DM and NP-NDM groups, so PLAC8 level could not be determined (Table 2).

The levels of TAC in patients with and without DM

TAC in the P-NGDM and NP-NDM groups (12.42 ± 2.31 vs. 12.96 ± 3.78, $p < 0.001$) was determined to be significantly higher than in the P-GDM and NP-DM groups (4.8 ± 0.52 vs. 2.21 ± 0.71, $p < 0.001$). When the P-GDM and NP-DM groups were compared to each other, TAC in the P-GDM group (4.8 ± 0.52) was determined to be significantly higher than in the NP-DM group (2.21 ± 0.71, $p < 0.001$). When the P-NGDM and NP-NDM groups were compared to each other, no significant difference was observed ($p > 0.05$) (Fig. 2).

Discussion

In previous studies, it has been shown that changes in inflammatory factors due to GDM cause irreversible pancreatic islet damage. Women with GDM are at higher risk of developing type 2 diabetes mellitus in the future.[24,25] The role of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), IL-6 and IL-1 and anti-inflammatory cytokines such as IL-10 have been previously evaluated in GDM, but few studies have investigated the role of pro-inflammatory cytokines such as IL-21, IL-17A in the pathogenesis of GDM. The role of newly discovered anti-inflammatory cytokines IL-33, PLAC8 and TAC level in the pathogenesis of GDM has not been investigated yet. This study has shown that pregnant women with GDM have higher serum IL-10, IL-33, PLAC8 and TAC levels than pregnant women with normal gestation.

The relationship between IL-10 concentration and GDM has yet to be concluded, while both decreased[24] and increased IL-10 levels have been reported in type 2 DM and GDM patients.[25] IL-10 has been extensively studied for its role in GDM and it has been suggested that IL-10 gene polymorphism could potentially alter network pathways of genes that result in a vulnerability to GDM in at-risk populations.[26] In our study, it was determined that IL-10 was significantly higher in the P-GDM and NP-DM patient groups compared to the P-NGDM and NP-NDM groups ($p < 0.001$).

In their study of interleukin-33 and its receptor soluble suppression of tumorigenicity 2 in the diagnosis of gestational diabetes mellitus, Fan et al. found that, compared to women with normal glucose tolerance pregnancies, patients with GDM had increased secretions of IL-33, soluble suppression of tumorigenicity 2 (sST2), IL-6 and TNF-α in their plasma with elevated homeostatic model assessment (HOMA). Moreover, they found that IL-33/sST2 was positively correlated with HOMA, IL-6 and TNF-α levels in the plasma of patients with GDM, respectively.[27] In our study, we found that IL-33 concentration was significantly higher in the P-GDM and NP-DM patient groups compared to the P-NGDM and NP-NDM groups ($p < 0.001$).

Tagoma et al. investigated plasma immune mediators during gestational weeks 23–28 in 213 women at risk of GDM, aiming to find associations between GDM and its complications, and they compared the results with clinical data from pregnancy and post-partum follow-up. They found that lower levels of adiponectin and higher levels of CCL2 were found in women with GDM. IL-27 levels were associated with lower likelihood of GDM, and showed a risk association with glutamic acid decarboxylase autoantibody positivity. Similarly, they reported that higher IL-22 levels increased the odds of glutamic acid decarboxylase autoantibody positivity. They found that TGF-β1 was associated with post-partum fasting glucose levels, and CCL4 with post-partum C-peptide levels. Finally, they showed that women who developed pregnancy complications had higher levels of CXCL10 and CCL4. But they did not find any difference between groups in terms of IL-10, IL-17A and IL-21.[28]

Despite the fact that the precise endogenous biochemical function of PLAC8 is unclear, more recent data shows that it regulates the innate immune response, adipocyte differentiation and cell proliferation/survival processes. Adjunct studies indicated a role for PLAC8 in promoting tumorigenesis. It is possibly via mechanisms involving proliferation, survival, autophagy and epithelial-to-mesenchymal transition.[29] For these studies, endothelial progenitor cells were obtained and cultured from isolated umbilical cord blood cells. Rogulski et al.[30] found that PLAC8 overexpression functioned as a protective mechanism for endothelial colony-forming cells to avoid senescence. Given that hyperglycaemia of GDM enhances endothelial colony-forming cells senescence and impairs vasculogenesis, their findings demonstrated an adaptive response of foetal endothelial colony-forming cells to circumvent the negative effects of a hyperglycaemic environment. Besides, differential methylation was explored in endothelial colony-forming cells from GDM mothers in the first intron of an isoform of PLAC8.

In our study, while the PLAC8 level was found to be significantly higher in the P-GDM group, it was significantly lower in the P-NGDM, NP-DM and NP-NDM groups ($p < 0.001$).

We demonstrated that TAC increased significantly in the P-NGDM and NP-NDM groups compared to the P-GDM and NP-DM groups ($p < 0.001$). In addition, when the P-GDM and NP-DM groups were compared to each other, TAC in the P-GDM group was found to be significantly higher than in the NP-DM group ($p < 0.001$).

Conclusion

In this study, it was determined that PLAC8 level is a determining factor for the diagnosis and follow-up of GDM in pregnant women. The fact that the importance of PLAC8 level and TAC in the diagnosis and follow-up of GDM in pregnancy is shown for the first time in this study demonstrates that it is unique. With our study, it has been shown that there may be new parameters that can be easily measured from the blood for pregnant women who do not prefer sugar overload. New studies are needed to detect whether there are significant differences in circulating levels of cytokines in patients with GDM.

Ethical approval

Ethics committee approval was received for this study from the Ethics Committee of Biruni University (2019/30-10).

Conflict of interest

The authors report no conflict of interest.

Acknowledgements

This work was supported by the Research Fund of the Biruni University. Project Number: Biruni-BAP-2020-01-30 and Tubitak_1002 Project Number: 123S102.

References

1. Wendland EM, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, et al. Gestational diabetes and pregnancy outcomes – a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. BMC Pregnancy Childbirth. 2012;12:23.
2. McMeekin P, Geue C, Mocevic E, Hoxer CS, Ochs A, McGurnaghan S, et al. The cost of prevalent and incident cardiovascular disease in people with type 2 diabetes in Scotland: data from the Scottish Care Information-Diabetes Collaboration. Diabet Med. 2020;37:1927–34.
3. Ferrara A. Increasing prevalence of gestational diabetes mellitus: a public health perspective. Diabetes Care. 2007;30 Suppl. 2:S141–6.
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012;35 Suppl. 1:S64–71.
5. Reece EA. The fetal and maternal consequences of gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2010;23:199–203.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2006;29 Suppl. 1:S43–8.
7. Gomes Fagundes DL, Luzia França E, da Silva Fernandes RT, de Castro Pernet Hara C, Morceli G, Honorio-França AC, et al. Changes in T-cell phenotype and cytokines profile in maternal blood, cord blood and colostrum of diabetic mothers. J Matern Fetal Neonatal Med. 2016;29:998–1004.
8. Zhao X, Liu J, Shen L, Wang A, Wang R. Correlation between inflammatory markers (hs-CRP, TNF-α, IL-1β, IL-6, IL-18),

glucose intolerance, and gestational diabetes mellitus in pregnant women. Int J Clin Exp Med. 2018;11:8310–6.
9. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans. Role of oxidative stress. Circulation. 2002;106:2067.
10. Sudharshana Murthy KA, Bhandiwada A, Chandan SL, Gowda SL, Sindhusree G. Evaluation of oxidative stress and proinflammatory cytokines in gestational diabetes mellitus and their correlation with pregnancy outcome. Indian J Endocrinol Metab. 2018;22:79–84.
11. Peltier MR, Drobek CO, Bhat G, Saade G, Fortunato SJ, Menon R. Amniotic fluid and maternal race influence responsiveness of fetal membranes to bacteria. J Reprod Immunol. 2012;96:68–78.
12. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11:98–107.
13. Richardson AC, Carpenter MW. Inflammatory mediators in gestational diabetes mellitus. Obstet Gynecol Clin North Am. 2007;34:213–24.
14. Gomes CP, Torloni MR, Gueuvoghlanian-Silva BY, Alexandre SM, Mattar R, Daher S. Cytokine levels in gestational diabetes mellitus: a systematic review of the literature. Am J Reprod Immunol. 2013;69:545–57.
15. Salmi AA, Zaki NM, Zakaria R, Nor Aliza AG, Rasool AH. Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus. Vasa. 2012;41:96–104.
16. Daher S, de Arruda Geraldes Denardi K, Blotta MH, Mamoni RL, Reck AP, Camano L, et al. Cytokines in recurrent pregnancy loss. J Reprod Immunol. 2004;62:151–7.
17. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63:601–10.
18. Hauguel-de Mouzon S, Guerre-Millo M. The placenta cytokine network and inflammatory signals. Placenta. 2006;27:794–8.
19. Gomes Fagundes DL, França EL, da Silva Fernandes RT, Hara Cde C, Morceli G, Honorio-França AC, et al. Changes in T-cell phenotype and cytokines profile in maternal blood, cord blood and colostrum of diabetic mothers. J Matern Fetal Neonatal Med. 2016;29:998–1004.
20. Matarese G, Procaccini C, de Rosa V. At the crossroad of T cells, adipose tissue, and diabetes. Immunol Rev. 2012;249:116–34.
21. Piccinni MP, Beloni L, Livi C, et al. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat Med. 1998;4:1020–4.
22. Sıfnaıos E, Mastorakos G, Psarra K, Panagopoulos N-D, Panoulıs K, Vıtoratos N, et al. Gestational diabetes and T-cell (Th1/Th2/Th17/Treg) immune profile. In vivo. 2019;33:31–40.
23. Crome SQ, Wang AY, Levings MK. Translational minireview series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. Clin Exp Immunol. 2010;159:109–19.
24. Ledford JG, Kovarova M, Koller BH. Impaired host defense in mice lacking ONZIN. J Immunol. 2007;178:5132–43.
25. Van Exel E, Gussekloo J, de Craen AJM, et al. Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. Diabetes. 2002;51:1088–92.
26. Mohebbatikaljahi H, Menevse S, Yetkin I, et al. Study of interleukin-10 promoter region polymorphisms (−1082A/G, −819T/C and −592A/C) in type 1 diabetes mellitus in Turkish population. J Genet. 2009;88:245–8.
27. Kang J, Liu C-H, Lee C-N, et al. Novel interleukin-10 gene polymorphism is linked to gestational diabetes in Taiwanese population. Front Genet. 2019;10:89.
28. Fan W, Kang W, Li T, Luo D, Huang L, Yang Y, et al. Interleukin-33 and its receptor soluble suppression of tumorigenicity 2 in the diagnosis of gestational diabetes mellitus. Int J Clin Pract. 2021;75:e14944.
29. Tagoma A, Haller-Kikkatalo K, Oras A, Roos K, Kirss A, Uibo R. Plasma cytokines during pregnancy provide insight into the risk of diabetes in the gestational diabetes risk group. J Diabetes Investig. 2022;13:1596–606.
30. Rogulski K, Li Y, Rothermund K, Pu L, Watkins S, et al. Onzin, a c-Myc-repressed target, promotes survival and transformation by modulating the Akt-Mdm2-p53 pathway. Oncogene. 2005;24:7524–41.