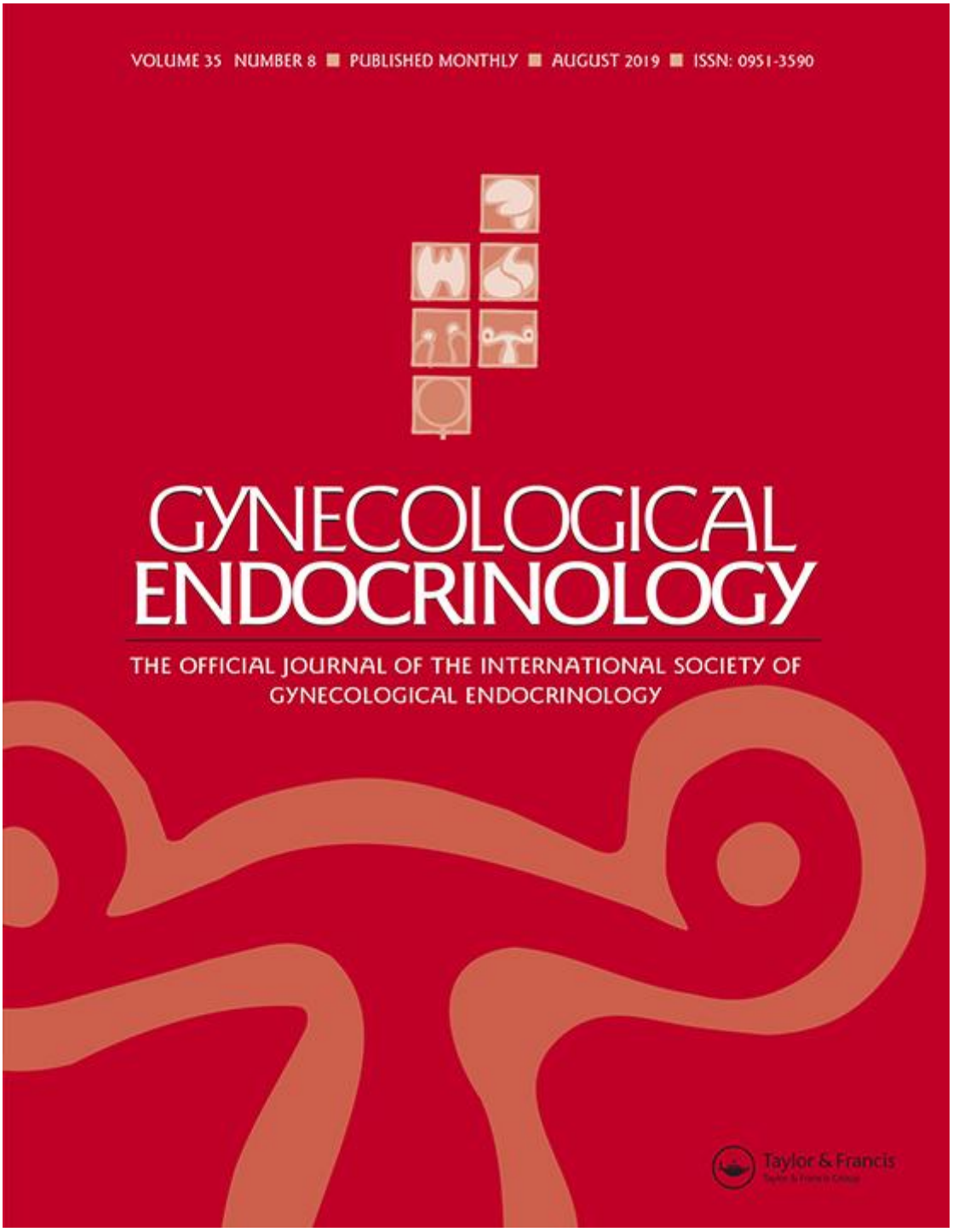
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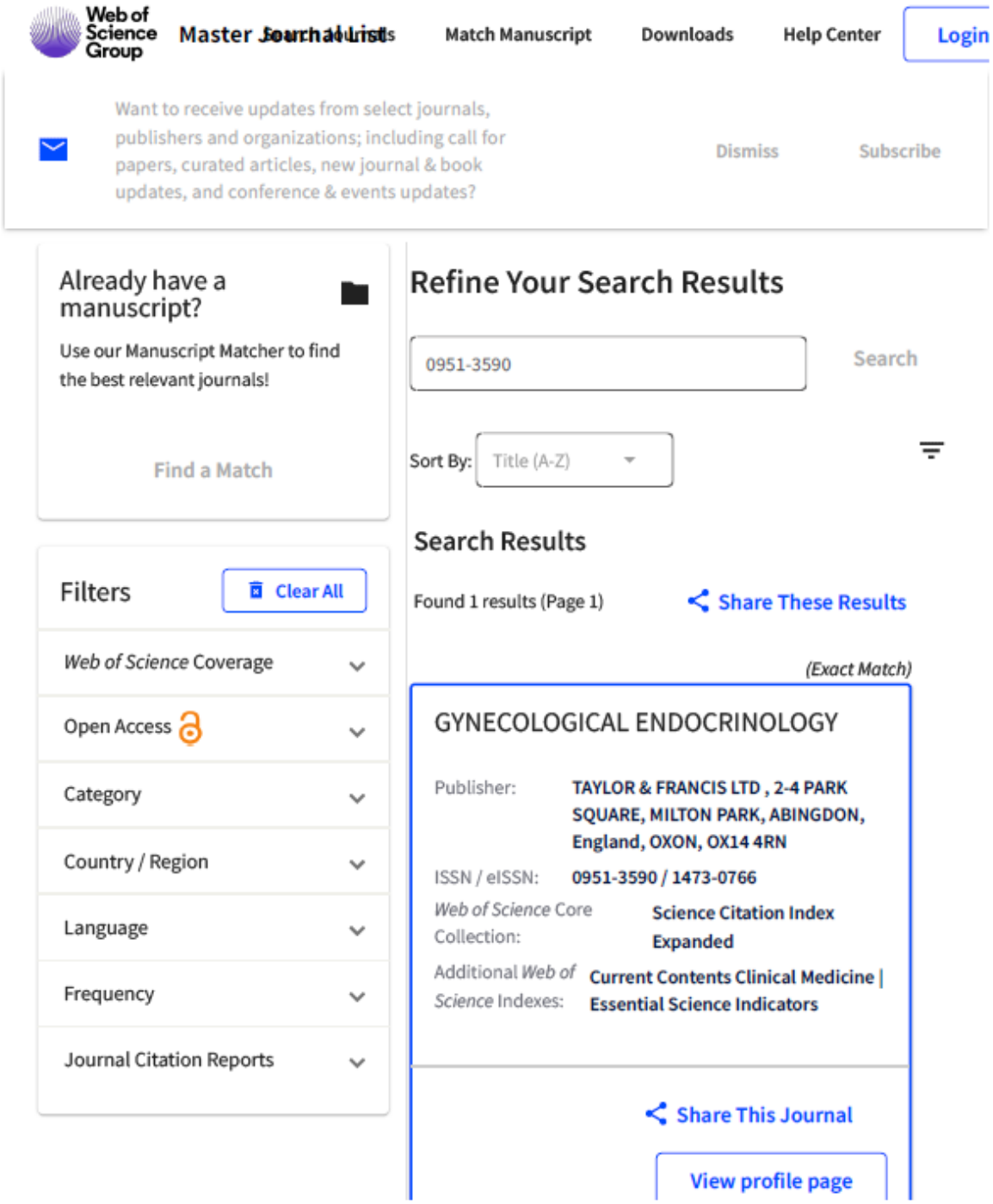
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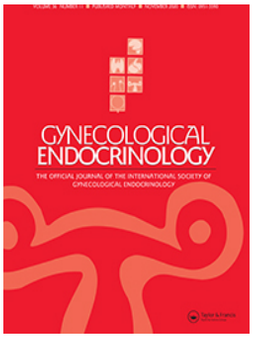
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Evaluation of thiol/disulfide homeostasis in patients with gestational diabetes mellitus

Suat Cakina[a], Buket Aydın[b] and Fatma Beyazit[b]

[a]Health Service Vocational College, Canakkale 18 Mart University, Canakkale, Turkey; [b]Faculty of Medicine, Department of Obstetrics and Gynecology, Canakkale 18 Mart University, Canakkale, Turkey

ABSTRACT

This study aimed to study the dynamic thiol/disulfide homeostasis in pregnant women with gestational diabetes mellitus (GDM). Forty-five pregnant women with GDM and 45 age-matched healthy pregnancies were included in this study. Thiol/disulfide homeostasis was measured using a commercial kit (Rel Assay Diagnostics). The patients with GDM had significantly higher disulfide concentrations than healthy pregnant patients ($p = .001$). Besides, the GDM group had significantly higher disulfide/total thiol, disüplhide/native thiol, and native thiol/total thiol ratio than healthy pregnant patients ($p = .001$, $p = .001$ and $p = .001$, respectively). The significantly higher concentrations of disulfide, disulfide/total thiol, disüplhide/native thiol, and native thiol/total thiol ratio in women with GDM could be considered as the increased oxidative stress.

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Oxidative stress; thiol/disulfide homeostasis; gestational diabetes mellitus

Introduction

Gestational diabetes mellitus (GDM) has been classically defined as the variable glucose intolerance occurring at time of pregnancy [1–3]. The prevalence of GDM is rapidly increasing in developed and developing countries despite population, different screening methods, and diagnostic criteria. One of the main factors which cause mortality and morbidity among both the mother and the infant all over the world is GDM. There is a high risk of obesity, cardiovascular disease, neonatal hypoglycemia, and type 2 diabetes mellitus in babies born to GDM women throughout their lives [4–6].

Although the pathophysiology of GDM is not clearly understood, it is associated with oxidative stress caused by free radicals. Most free radicals are composed of oxygen atoms and are called Reactive Oxygen Species (ROS). The constant generation of cellular ROS may cause irreversible damage to protein, lipid and DNA. GDM are associated with the overproduction of ROS, which will cause oxidative stress [7,8].

Thiols that play a major role in the prevention of any oxidative stress condition development among the cells are the organic compounds containing sulfhydryl (–SH) groups. When oxidative stress increases, reversible disulfides are formed between protein thiol groups and thiols. There can be a reduction in the connective structures of reversible disulfide to thiol groups, keeping the balance between thiol and disulfide. There is an association between abnormal thiol-disulfide balance levels and oxidative stress. Any disturbance of the balance between antioxidant defenses and production of the species of reactive oxygen (free radicals) called oxidative stress. Antioxidants are substances that protect the organism against the deleterious effects of free radicals in tissues. thiol-disulfide homeostasis is identified as an oxidative stress marker in recent years and shown to play a vital role in cellular functions [9,10].

In recent years, many studies have reported the alteration of thiol/disulfide homeostasis in prediabetes, diabetes mellitus, and GDM. Besides, in the literature, it was reported that decreased native thiol levels predicted adverse pregnancy outcomes in GDM cases [9,11]. This study aimed to investigate thiol-disulfide homeostasis, among the patients who had GDM and to compare them with healthy pregnancies based on the results.

Materials and methods

Study population

In this case-control study, 45 women who were diagnosed to have GDM, and 45 healthy pregnant controls were included. The study was conducted in the Department of Obstetrics and Gynecology, Çanakkale 18 Mart University Hospital between February 2019 and January 2020. All pregnant women admitted to the study centers routinely undergo GDM screening in 24 and 28 weeks with the 75 g OGTT. Based on the IADPSG criteria, GDM is diagnosed when at least one of the following conditions is present (Figure 1): fasting glucose concentration higher than 92 mg/dl, 1-h concentration of glucose higher than 180 mg/dl, and 2-h concentration of glucose higher than 153 mg/dl.

All participants who had preexisting diabetes (type 1 or type 2), preeclampsia, chronic systemic diseases, and multiple pregnancies, who underwent treatment with insulin or oral antihyperglycemic agents, used drugs or supplements and had smoked, and alcoholism were excluded from the study (Figure 1).

The principles of the Declaration of Helsinki were applied, and ethical approval was granted to perform study protocol (No: 2011-KAEK- 7/2019- E.1900020037). All participants were asked to sign written informed consent forms.

CONTACT Suat Cakina suatcakina@comu.edu.tr Health Service Vocational College, Çanakkale 18 Mart University, Çanakkale, Turkey.
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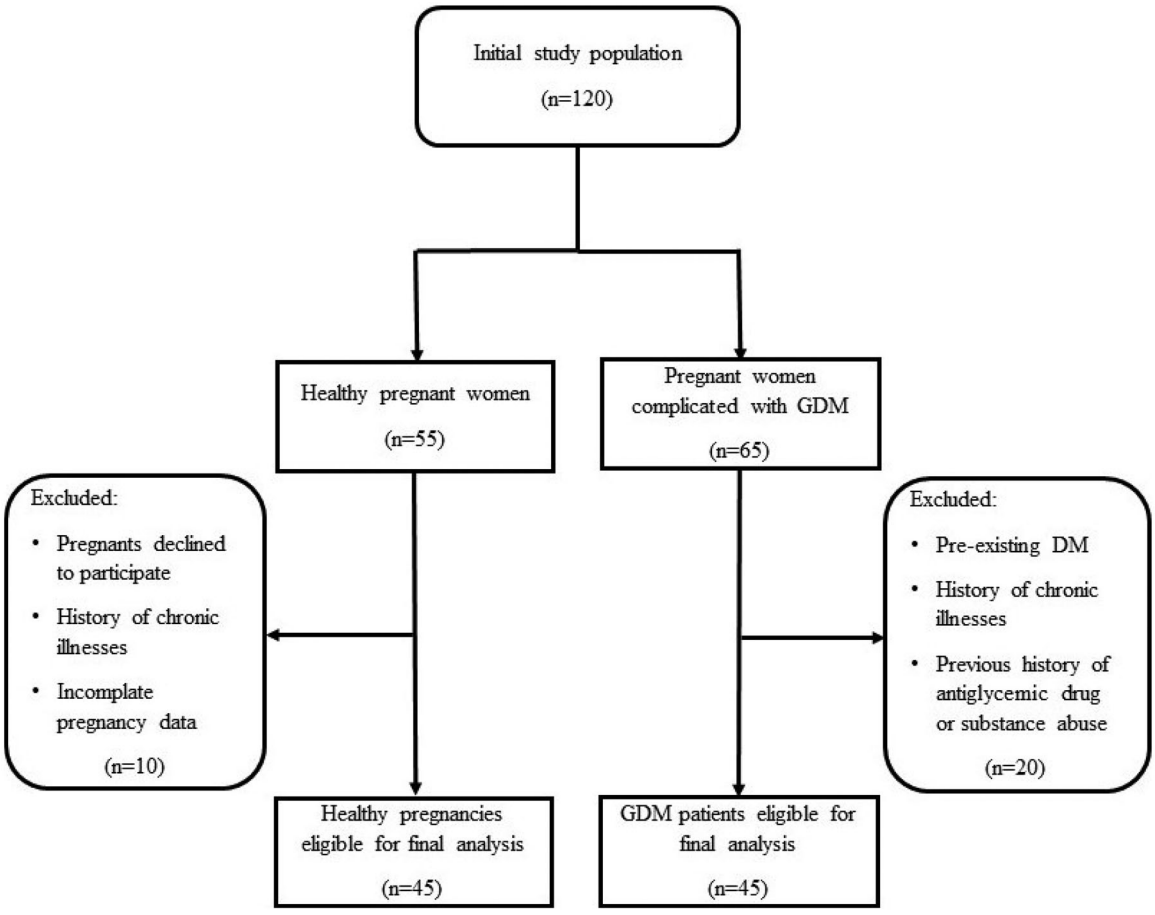


Figure 1. Flow diagram of inclusion procedure of gestational diabetes mellitus (GDM) patients.

Measurement of serum thiol/disulfide homeostasis levels

A 20-gauge needle was used to take all peripheral blood samples from the antecubital vein between 08:00 AM and 10:00 AM after 10–12 h of fasting. Quick centrifugation of blood samples was done at 1500 rpm for 10 min to determine thiol/disulfide hemostasis parameters. Serum and plasma samples were then isolated. Serum samples were collected at −80 °C until the thiol/disulfide hemostasis measurements were analyzed.

A new spectrophotometric and automatic method which Neselioglu and Erel developed and is available commercially, was used to measure tests (Rel Assay Diagnostics, Turkey) [12]. In this method, sodium borohydride was used for reduction of reducible and dynamic disulfide bonds in the samples to free functional thiol groups. The unused reduced-sodium borohydride was prevented from being reduced to dithionite-2 nitrobenzoic (DTNB) by removing NaBH4 with formaldehyde. After the ultimate measurement of reaction with DTNB and their levels, total thiol levels and native thiol were determined. The disulfide level was indicated by half of the result difference obtained by subtracting amount of native thiol from content of total thiol. The results are given in μmol/L. Also, these parameters were used to calculate ratios of disulfide/total thiol, native thiol/total thiol, and disulfide/native thiol.

Statistical analysis

SPSS, version 19.0. (SPSS, IBM Company) was used for statistical analysis. The data distribution normality was examined by the Kolmogorov-Smirnov normality test. Results are presented as mean ± standard deviation(SD) and were compared using the Student's t test. All statistical analyses had significant $p < .05$.

Results

Table 1 shows the brief demographic and biochemical properties of the groups. The average age of patients with GDM was 29.76 ± 6.94 years, while the healthy control individuals' average age was 28.62 ± 5.50 years ($p = .390$). The mean gestational age was 28.31 ± 5.87 weeks for GDM groups ($p = .067$). The GDM patients had significantly higher BMI than the control group had ($p = .040$). The patient's group had significantly higher fasting plasma glucose, 1 h OGTT, 2 h OGTT, and HbA1c concentrations than the control group had ($p = .001$).

While total serum thiol and natural thiol concentrations were lower than those of the control group, the disulfide concentration was found to be higher in the GDM group ($p = .072$, $p = .260$, and $p = .001$, respectively). Average values of disulfide/total thiol, disüplhide/native thiol, and native thiol/total thiol were significantly higher in the group with GDM ($p = .001$, $p = .001$, and $p = .001$, respectively).

Discussion

Placenta is both a source of physiological oxidative stress and a rich source of antioxidants in normal pregnancy [13,14]. Therefore, the placenta is highly effective in keeping oxidative stress, i.e. the balance between oxidant and antioxidant systems during pregnancy. There may be slight changes in the oxidative

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Table 1. Demographic and clinical details of study subjects.

	GDM ($n = 45$)	Control ($n = 45$)	p Value[a]
Age (years)	29.76 ± 6.94	28.62 ± 5.50	.390
Gestational age (weeks)	28.31 ± 5.87	26.48 ± 3.01	.067
Body mass index (kg/m^2)	28.51 ± 3.70	26.78 ± 3.99	.040
Fasting plasma glocose (mg/dL)	92.41 ± 9.68	85.07 ± 7.82	.001
Glucose at 60 min (mg/dL)	172.68 ± 3.21	120.41 ± 2.87	.001
Glucose at 120 min (mg/dL)	132.98 ± 4.01	107.96 ± 2.53	.001
HA1C (g/dL)	5.33 ± 0.41	4.87 ± 0.41	.001
Total cholesterol (mmol/L)	247.91 ± 3.90	219.13 ± 4.76	.002
Triglyceride (mmol/L)	228.94 ± 10.54	185.40 ± 8.44	.033
High density lipoprotein (mmol/L)	76.28 ± 2.07	76.05 ± 1.76	.950
Low density lipoprotein (mmol/L)	170.27 ± 3.77	143.55 ± 4.14	.020
Total thiols (μmol/L)	494.78 ± 4.78	516.98 ± 2.61	.072
Native thiols (μmol/L)	268.98 ± 5.24	281.84 ± 5.41	.260
Disulfide (μmol/L)	19.58 ± 3.25	13.52 ± 2.38	.001
Disulfide/total thiol ratio	3.99 ± 0.77	2.63 ± 0.42	.001
Disulfide/native thiol ratio	7.62 ± 2.33	4.95 ± 1.20	.001
Native/ total thiol ratio	53.63 ± 8.37	50.84 ± 7.29	.001

[a]Student's t-test for continuous variables (variables with normal distribution).

stress to protect pregnancy [15–18]. In our study, pregnant women with GDM had disulfide/natural thiol, disulfide/total thiol, and natural thiol/total thiol rates significantly higher than the healthy pregnancies had. This finding shows that oxidative stress relatively increased in GDM pregnancies.

It has been reported that GDM is associated with excessive oxidative stress, due to the overproduction of free radicals and interruption of anti-oxidant defense mechanisms within the placenta [19–21]. Karacay et al. [22] reported the role of oxidative damage and antioxidant among pregnant women, and it was found that oxidative markers increased significantly, and antioxidant status decreased significantly in GDM. Another study reported that protein oxidative damage and lipid peroxidation significantly increased in GDM patients compared to healthy pregnant women [23]. Yildirim et al. [24] observed significantly higher disulfide concentrations and reduced thiol concentrations in patients who were diagnosed with GDM based on two-step OGTT diabetes screening. In our study, disulfide/natural thiol, disulfide/total thiol, and natural thiol/total thiol ratios were significantly higher in patients with GDM. This finding implies that the pathogenesis of GDM may be caused by increased oxidative stress.

It is known that it triggers chronic inflammation through a very complex mechanism of inflammatory mediators such as adhesion molecules and interleukins due to increased oxidative stress. Despite the failure to completely understand GDM-related complications pathophysiology, the oxidative stress is thought to be effective [25,26]. Ozler et al. [27] reported that disulfide concentrations, ratios of disulfide/native thiol, and disulfide/total thiol significantly increased, and there was a significant reduction in native thiol/total thiol ratios in the babies' cord blood for the women with GDM. Also, patients with GDM who had high BMIs before pregnancy were found to have lower native thiol concentrations at pregnancy 24–28 weeks. Rueangdetnarong et al. [28] showed that an oxidative stress marker (isoprostane) and an inflammatory marker (tumor necrosis factor-α) increased in patients with GDM during the 24th–28th weeks of pregnancy. Also, all perinatal and neonatal outcomes were statistically comparable despite the increase in oxidative stress.

Although in the present study we focused on thiol/disulfide homeostasis alterations in patients with GDM, a growing number of evidence suggests significant associations between thiol/disulfide homeostasis and obstetric complications during pregnancy. In this context, a recent study by Cetin et al. [29] successfully demonstrated a decrease in maternal serum native thiol and total thiol concentrations in idiopathic intrauterine growth restriction (IUGR) patients compared to healthy pregnant controls. Similarly, Korkmaz et al. [20] studied thiol-disulfide homeostasis in preeclampsia patients. They showed that thiol-disulfide homeostasis was impaired in women with preeclampsia, and this impairment increased with the disease severity. Thiol/disulfide homeostasis was also explored in several studies in patients with recurrent pregnancy loss in which impaired serum thiol/disulfide homeostasis was suggested as an underlying mechanism explaining this obstetric complication [14,30]. In our study, thiol/disulfide homeostasis parameters determined during the 24th–28th weeks of gestation failed to predict outcomes of adverse pregnancy in patients with GDM.

This study was to evaluate the possible effect of thiol/disulfide homeostasis on the occurrence of GDM-related complications. This study cohort is relatively small. Thus serious adverse perinatal outcomes such as preeclampsia, neonatal death, or fetal anomalies were not observed. This leads the study inadequate to mention the pregnancy complications. Also, in this study, a one-step test was used to diagnose the GDM. In contrast, similar studies in the literature used the two-step test to diagnose the GDM. The adoption of different diagnostic tests may lead to variations in the description of perinatal outcomes and their probable relationship with oxidative stress markers. In the study group, the effects of diabetic diet and insulin therapy on oxidative biomarkers have not been evaluated. Another limitation of the study is the absence of histopathological data on the severity of oxidative stress and inflammation in placental tissues.

In conclusion, pregnancies complicated with GDM had disulfide/total thiol, disulfide/natural thiol, and natural thiol/total thiol rates significantly higher than healthy pregnancies had. Further studies should be done to determine the relationship between GDM and oxidative stress and to understand the oxidative stress effect on GDM.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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