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Dynamic thiol/disulfide homeostasis in gestational diabetes mellitus: Is it related with adverse perinatal outcomes?

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Abstract

Objective: To specify the significance of thiol/disulfide homeostasis in the aspect of gestational diabetes mellitus (GDM) and GDM-related complications.

Material and Methods: This study is a prospective review of the data of 61 healthy and non-pregnant women, 58 healthy pregnant women, and 62 pregnant women with GDM.

Results: The patients with gestational diabetes mellitus had significantly higher disulfide/native thiol and disulfide/total thiol concentrations than non-pregnant patients ($p<0.001$ for both) and healthy pregnant patients (p: 0.015 and p: 0.018, respectively). Besides, in GDM group had significantly lower native thiol/total thiol concentrations than non-pregnant patients and healthy pregnant patients ($p<0.001$ and p: 0.016, respectively). There were positive and significant correlations between disulfide levels and HbA1c concentrations (r=0.26, p: 0.042), and between disulfide and oral glucose tolerance test first hour concentrations (r=0.26, p: 0.039). The receiver operating characteristic curve analyses for native thiol, total thiol, and disulfide were unable to predict adverse perinatal outcomes in this cohort.

Conclusion: The significantly higher concentrations of disulfide/native thiol and disulfide/total thiol in women with GDM could be considered as the presence of increased oxidative stress. However, these markers failed to predict adverse perinatal outcomes.

Keywords: gestational diabetes mellitus; oxidative stress; perinatal outcome; pregnancy; thiol/disulfide homeostasis

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first diagnosis in the second or third trimester of pregnancy that is clearly not preexisting diabetes (1). Hyperglycemia caused by GDM is responsible for both maternal, fetal and neonatal complications. In the short term, GDM increases the risk of maternal hypertensive disorders, fetal macrosomia, shoulder dystocia, amniotic fluid anomalies, fetal distress, and cesarean delivery (2). Long term, the risk of developing type 2 diabetes mellitus is increased by 50% in women with GDM (3). Moreover, the offspring of women with GDM are at risk for obesity, glucose intolerance, type 2 diabetes mellitus, and hypertension (4).

Between 24-28th gestational weeks, GDM screening is recommended to all pregnant women who have not been diagnosed with pregestational diabetes after the first antenatal visit.

While only two-step screening tests were used until 2010, after 2010, GDM screening is performed in many centers using single-step 75 g oral glucose tolerance test (OGTT) with the recommendations of 'The International Association of Diabetes and Pregnancy Study Groups (IADPSG)' (5-7). The prevalence of GDM in Turkey varies between 1.2% and 27.9%, depending on the geographic location of the study and the diagnostic tests used (8). Compared with previous years, the prevalence of GDM is increasing due to increased obesity and advanced maternal age all around the world (9).

Previous studies have shown a relationship between oxidative stress and gestational diabetes (3,4). Failure to maintain the balance between the oxidant and antioxidant production of biologic systems results in oxidative stress.

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Pregnancy is one of the situations in which this balance is impaired in favor of oxidants because oxygen consumption in pregnancy increases significantly and excessive amounts of free oxygen radicals are produced in the mitochondrial rich placenta (10). Pregnant women with GDM are exposed to more oxidative stress than healthy pregnant women due to the excess production of reactive oxygen species (ROS) or inadequate protective mechanisms (3,4).

Thiol-disulfide balance is an antioxidant system that protects the cells and minimizes the effects of oxidative damage. In recent years, many studies have reported how thiol/disulfide homeostasis is altered in prediabetes (11), diabetes mellitus (12), and GDM (13). Besides, in the literature, it was reported that decreased native thiol levels predicted adverse pregnancy outcomes in GDM cases diagnosed by two-step diagnostic test (13). In the presence of oxidative stress, thiols react with oxidizing agents and mediate the formation of reversible disulfide bonds between proteins (14). This process mediates the formation of reversible disulfide bonds between proteins. When the oxidative stress is eliminated, the disulfide bonds return to thiol groups again. This cycle preserves dynamic thiol/disulfide homeostasis, which plays an important role in the stabilization of antioxidant defense, apoptosis, and protein structures (15,16).

To the best of our knowledge, there is no study investigating dynamic thiol/disulfide homeostasis in GDM patients diagnosed using a single-step screening test. The aim of this study was to show the changes in dynamic thiol/disulfide homeostasis in GDM cases diagnosed using single-step screening test and to determine whether it was associated with increased adverse pregnancy complications.

Materials and Methods

This multicenter study was performed from July 2018 to November 2018 at the Department of Obstetrics and Gynecology, Izmir Tepecik Training and Research Hospital, Department of Obstetrics and Gynecology, Rize Recep Tayyip Erdogan University Hospital and Department of Clinical Biochemistry, Ankara Yildirim Beyazit University Hospital. The study protocol was undertaken in accordance with the principles of the Declaration of Helsinki and ethical approval was granted (No: 2018/6-13). All participants were asked to sign written informed consent forms.

Women who had diabetes mellitus, multiple pregnancies, fetal anomalies, hypertension, cerebrovascular disease, deep vein thrombosis, pulmonary embolism, hematologic diseases, thyroid or heart disease, chronic liver or renal disease, cancer, autoimmune disorders, inflammatory diseases, and women who smoked and/or consumed alcohol were excluded.

Study design: This is a prospective review of 61 healthy and non-pregnant women, 58 healthy pregnant women, and 62 pregnant women with GDM. All pregnant women admitted to the study centers routinely undergo GDM screening at 24 and 28 weeks using the 75 g OGTT. According to the IADPSG criteria, GDM is diagnosed when at least one of the following conditions are present: fasting glucose concentration higher than 92 mg/dl, 1-hour glucose concentration higher than 180 mg/dl, and/or 2-hour glucose concentration higher than 153 mg/dl (5). The patients in group 1 were consecutively recruited from healthy and non-pregnant women who were admitted to the antenatal polyclinic during the study period. The healthy pregnant women (group 2) and pregnant women with GDM (group 3) were consecutively recruited from patients who underwent the 75 g OGTT for screening gestational diabetes.

Anthropometric measurements were performed with light clothing and no shoes. In each center, all subjects' height and weight measurements were performed by the same qualified researcher using a weekly calibrated weighing scale. Body mass index (BMI) (kg/m2) was calculated by dividing weight (in kilograms) by the square of height (in meters). A calibrated mercury sphygmomanometer was used to measure systolic and diastolic blood pressures. The gestational age of the participants was verified with first trimester ultrasonography. Any adverse pregnancy outcomes, including polyhydramnios, preterm delivery, small for gestational age (SGA), macrosomia, intrauterine growth restriction (IUGR), preeclampsia, need for neonatal intensive care unit, and postpartum hemorrhage were recorded.

Measurement of serum thiol/disulfide homeostasis levels: All peripheral blood samples were collected between 08:00 AM and 10:00 AM after 10-12 hours of fasting, from the antecubital vein using a 20-gauge needle. Blood samples were quickly centrifuged at 1500 rpm for 10 minutes to determine thiol/disulfide hemostasis parameters. Plasma and serum samples were then separated. Serum samples were collected at -80°C until the thiol/disulfide hemostasis measurements were analyzed.

A simple new fully automated colorimetric method was applied to evaluate the serum concentrations of native and total thiol and the ratio of disulfide to native and total thiol. This method is similar to the method developed by Erel and Neselioglu, where dynamic disulfide bonds are reduced by sodium borohydrate to functional thiol groups (17). Serum samples were automatically performed by a clinical chemistry analyzer (Roche, Cobas 501, Mannheim, Germany). The results are given as µmol/L. Using this method, the concentrations of natural thiol, total thiol, and disulfide were determined. Then, the disulfide-natural thiol, disulfide-total thiol, and natural thiol percentages were calculated in all groups.

Statistical analysis: Collected data were analyzed using the Statistical Package for the Social Sciences version 25.0 (SPSS IBM, Armonk, NY, USA). Descriptive statistics are presented as mean ± standard deviations, frequency distributions, and percentages. The Chi-square test was used in the analysis of categorical variables. The normality of distribution of the variables was tested using the Kolmogorov–Smirnov or Shapiro–Wilk test. Equality of variances was checked using the Levene test. One-way analysis of variance, Welch analysis of variance, and the Kruskal–Wallis test were used to determine the significant differences between the three groups. Post hoc tests for

pairwise comparisons were also performed. The Pearson test was used to investigate the correlations among variables. The optimal cut-off points for thiol/disulfide homeostasis parameters in distinguishing the adverse pregnancy outcomes of patients with GDM were further evaluated using receiver operating characteristic curve (ROC) analyses. A probability level of p<.005 was considered to be statistically significant.

Results

The baseline demographic, anthropometric, and biochemical characteristics of the groups are summarized in Table 1. The patients in group 1 were significantly older than those in group 2 and group 3 (p: 0.001 and p: 0.016, respectively). The patients in group 1 had significantly lower BMI than patients in group 3 (p: 0.006). The patients in group 1 had significantly lower waist circumferences than those in group 2 and group 3 (p: 0.001 and p<0.001, respectively).

The patients in group 3 had significantly higher fasting plasma glucose, OGTT first hour, OGTT second hour, HbA1c and CRP concentrations than those of patients in group 2 (p<0.001, p<0.001, p<0.001, p: 0.001, and p: 0.001, respectively). The outcomes related with thiol/disulfide homeostasis are listed in figure 1. Group 1 had significantly higher native thiol and total thiol concentrations than group 2 and group 3 (p<0.001 for all). Group 3 had significantly higher disulfide concentrations than group 1 (p: 0.04). Group 1 had significantly lower disulfide/native thiol and disulfide/total thiol concentrations than group 2 (p: 0.016 and p: 0.009, respectively). Group 1 and group 2 had significantly lower disulfide/native thiol and disulfide/total thiol concentrations than group 3 (p<0.001, p<0.001, p: 0.015, and p: 0.018, respectively). Group 1 had significantly higher native thiol/total thiol concentrations than group 2 (p: 0.013). Group 3 had significantly lower native thiol/total thiol concentrations than group 1 and group 2 (p<0.001 and p: 0.016, respectively).

Table 1. Demographic, anthropometric and biochemical characteristics of groups (mean ± SD)

	Healthy women (n=61)	Healthy pregnant (n=58)	GDM (n=62)	p
Age,years	35.4±10.0	28.7±6.1	31.7±5.0	<0.001* <0.001$^{\alpha}$, 0.016$^{\beta}$, 0.068$^{\Upsilon}$
Body mass index (kg/m^2)	25.20±4.47	25.78±3.73	27.49±3.93	0.006* 0.712$^{\alpha}$, 0.006$^{\beta}$, 0.058$^{\Upsilon}$
Systolic blood pressure (mmHg)	109.3±9.6	109.4±12.6	108.3±10.4	0.834*
Diastolic blood pressure (mm Hg)	66.9±9.1	67.8±9.6	66.3±8.7	0.677*
Waist circumference (cm)	74.7±7.2	79.2±7.4	81.1±5.9	<0.001* 0.001$^{\alpha}$, <0.001$^{\beta}$, 0.299$^{\Upsilon}$
Fasting plasma glucose (mmol/L)	89.4±10.3	78.0±8.95	91.76±16.2	<0.001* <0.001$^{\alpha}$, 0.541$^{\beta}$, <0.001$^{\Upsilon}$
Oral glucose tolerance test 1st hour (mmol/L)	123.2±33.2	119.9±25.1	206.2±29.1	<0.001* 0.807$^{\alpha}$, <0.001$^{\beta}$, <0.001$^{\Upsilon}$
Oral glucose tolerance test 2nd hour (mmol/L)	98.5±23.9	95.7±21.8	167.1±29.2	<0.001* 0.813$^{\alpha}$, <0.001$^{\beta}$, <0.001$^{\Upsilon}$
HbA1c (%)	5.26±0.42	5.10±0.38	5.38±0.42	0.001* 0.074$^{\alpha}$, 0.278$^{\beta}$, <0.001$^{\Upsilon}$
C-reactive protein (mg)	0.37±0.33	0.61±0.60	0.99±0.67	<0.001* 0.051$^{\alpha}$, <0.001$^{\beta}$, 0.001$^{\Upsilon}$
Creatinine (µmol/L)	0.59±0.10	0.54±0.11	0.56±0.12	0.041* 0.034$^{\alpha}$, 0.248$^{\beta}$, 0.614$^{\Upsilon}$
Blood urea nitrogen (mg/dl)	21.02±9.36	15.39±4.35	13.88±4.49	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.416$^{\Upsilon}$
Albumin (g/L)	6.06±1.05	3.75±1.22	3.52±0.99	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.480$^{\Upsilon}$
Total cholesterol (mmol/L)	184.0±33.6	232.8±47.1	234.3±55.4	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, 0.980$^{\Upsilon}$
Triglyceride (mmol/L)	97.6±46.1	184.6±69.3	234.6±84.5	<0.001* <0.001$^{\alpha}$, <0.001$^{\beta}$, < 0.001$^{\Upsilon}$
High density lipoprotein (mmol/L)	17.2±2.2	13.5±1.8	12.1±1.5	<0.001* <0.001$^{\alpha}$, 0.014$^{\beta}$, 0.036$^{\Upsilon}$
Low density lipoprotein (mmol/L)	107.7±27.6	120.8±44.3	128.2±54.3	0.032* 0.231$^{\alpha}$, 0.026$^{\beta}$, 0.615$^{\Upsilon}$

Group 1; healthy women, group 2; healthy pregnant, group 3; pregnant with GDM. α Statistical significance between group 1 and group 2, β Statistical significance between group 1 and group 3, γ Statistical significance between group 2 and group 3, *p<0.05 was accepted to be statistically significant

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The perinatal outcomes of group 2 and group 3 are displayed in Table 2. The weight gain during pregnancy, birthweight, and macrosomia were significantly higher in group 3 than in group 2 (p: 0.006, p<0.001, and p<0.001, respectively). There were no cases of preeclampsia, fetal anomaly or neonatal death in groups.

There were positive and significant correlations between disulfide and HbA1c concentrations (r=0.26, p: 0.042), and between disulfide levels and OGTT first hour concentrations (r=0.26, p: 0.039) (Table 3).

The area under the ROC curve for native thiol was 0.46 (95% CI: (0.304-0.615), p: 0.622). The area under the curve for total thiol was 0.478 (95% CI: (0.322-0.635), p: 0.789). The area under the curve for disulfide was 0.532 (95% CI: (0.362-0.701), p: 0.694). These values were unable to predict adverse perinatal outcomes in this cohort.

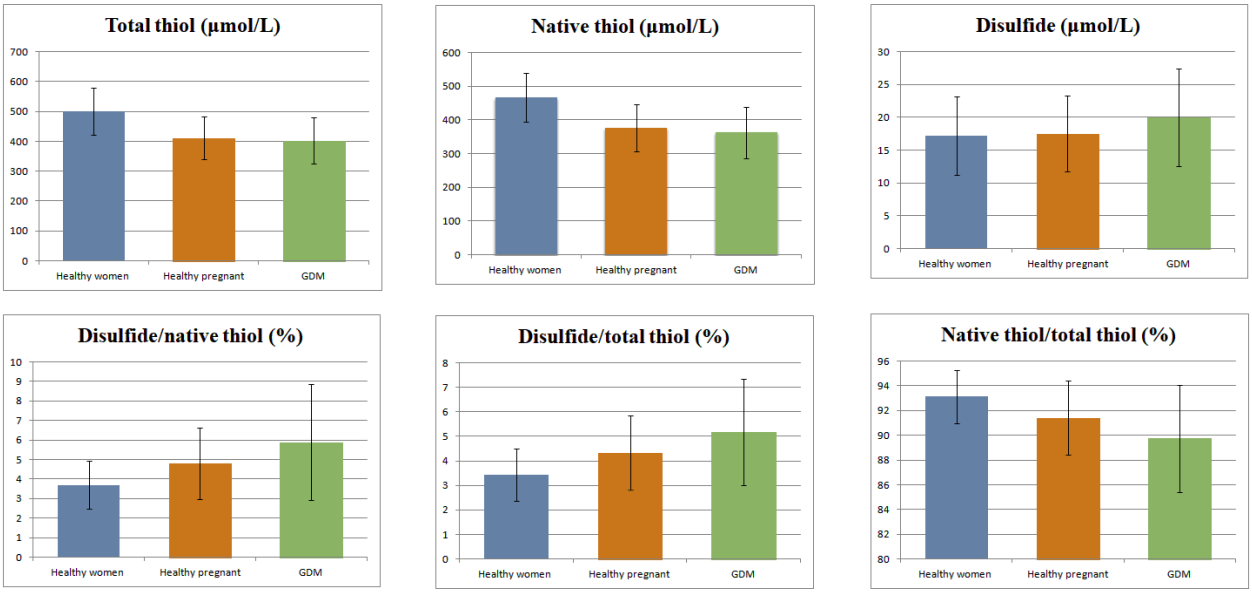

Figure 1. Thiol/disulfide homeostasis of groups (mean ± SD)

Table 2. Perinatal outcomes of GDM and healthy groups (mean ± SD)

	Healthy pregnant (n=58)	GDM (n=62)	p
Gravidity (n)	1.6±0.7	1.7±0.5	0.277
Weight gain (kg)	6.79±4.53	9.24±5.09	0.006
Route of delivery (n, %)			
Vaginal delivery	42 (72.4)	41 (66.1)	0.456
Caesarian section	16 (27.6)	21 (33.9)	
Gestational age at birth (weeks)	38.4±1.3	38.6±1.3	0.528
Birthweight (g)	3184.5±300	3693.6±373.7	<0.001*
Female newborns (n, %)	30 (51.7)	31 (50.0)	0.850
Apgar (1st minute)			
<7	10 (17.2)	9 (14.5)	0.683
≥7	48 (82.8)	53 (85.5)	
Apgar (5th minute)			
<7	6 (10.3)	3 (4.8)	0.252
≥7	52 (89.7)	59 (95.2)	
Polyhydramnios (n, %)	0 (0.0)	5 (8.1)	0.058
Macrosomia (≥4000 g) (n, %)	0 (0.0)	15 (24.2)	<0.001*
Small for gestational age (n, %)	4 (6.9)	3 (4.8)	0.631
Intrauterine growth restriction (n, %)	5 (8.6)	3 (4.8)	0.481
Postpartum hemorrhage (n, %)	7 (12.1)	4 (6.5)	0.352
Postpartum hemorrhage (n, %)	1 (1.7)	3 (4.8)	0.619
Need for neonatal intensive care unit (n, %)	3 (5.2)	2 (3.2)	0.672

*p<0.05 was accepted to be statistically significant

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Table 3. Correlations among variables

	HbA1c	FPG	OGTT 1st hour	Insulin
Native Thiol				
Group 1	r=0.21, p: 0.103	r=0.10, p: 0.437	r= -0.05, p: 0.703	r=0.02, p: 0.855
Group 2	r= -0.04, p: 0.766	r=0.01, p: 0.922	r=0.11, p: 0.406	r=0.01, p: 0.969
Group 3	r= -0.07, p: 0.573	r=0.02, p: 0.910	r=0.05, p: 0.683	r=0.21, p: 0.106
Total Thiol				
Group 1	r=0.19, p: 0.139	r=0.13, p: 0.338	r=0.07, p: 0.159	r=0.04, p: 0.735
Group 2	r= -0.06, p: 0.655	r=0.01, p: 0.969	r=0.08, p: 0.539	r=0.01, p: 0.959
Group 3	r= -0.02, p: 0.860	r=0.03, p: 0.821	r=0.10, p: 0.425	r=0.19, p: 0.903
Disulfide				
Group 1	r= -0.04, p: 0.785	r=0.20, p: 0.129	r=0.16, p: 0.221	r=0.14, p: 0.279
Group 2	r=0.13, p: 0.334	r= -0.04, p: 0.767	r= -0.16, p: 0.228	r=0.01, p: 0.964
Group 3	r=0.26, p: 0.042*	r=0.07, p: 0.565	r=0.26, p: 0.039*	r= -0.11, p: 0.406

FPG; fasting plasma glucose, **HbA1c;** glycated hemoglobin, **OGTT 1.** hour; oral glucose tolerance test first hour. **Group 1;** healthy women, **Group 2;** healthy pregnant**, Group 3;** pregnant with GDM, ***p<0.05** was accepted to be statistically significant

Discussion

The placenta is a source of physiologic oxidative stress in normal pregnancy, but also a rich source of antioxidants (18). Therefore, the placenta plays a major role in maintaining the balance between oxidant and antioxidant systems with enzymatic and non-enzymatic scavengers during pregnancy. These moderate changes in oxidative stress are essential for the maintenance of pregnancy (4, 19). In our study, the women with uncomplicated pregnancies had significantly higher disulfide/native thiol and disulfide/total thiol ratios and significantly lower native thiol/total thiol ratios than healthy women. This finding indicates that oxidative stress is relatively increased in healthy pregnancies.

It has been reported that GDM is associated with excessive oxidative stress, which can be attributed to the overproduction of free radicals and interruption of anti-oxidant defense mechanisms within the placenta. Karacay et al. assessed maternal oxidative damage and anti-oxidant status by measuring lipid peroxidation products, protein oxidation markers, myeloperoxidase and lipid hydroperoxidase between 24-36 weeks of gestation (19). It was found that oxidative markers were significantly increased and anti-oxidant status was significantly reduced in GDM (19). Another study investigated the oxidative stress level during the second and third trimester of pregnancy in patients with GDM (20). It was specified that lipid peroxidation and protein oxidative damage was significantly increased in patients with GDM compared with healthy pregnant women (20). Yildirim et al. observed significantly higher disulfide concentrations and reduced thiol concentrations in patients who were diagnosed as having GDM according to two-step antenatal diabetes screening (21). In our study, disulfide/native thiol and disulfide/total thiol ratios were significantly higher and native thiol/total thiol ratios were significantly lower in patients with GDM.

Moreover, there was a significant and positive correlation between disulfide and HbA1c concentrations and between disulfide and OGTT first hour concentrations. This finding implies that increased oxidative stress might participate in the pathogenesis of GDM.

It is a known fact that increased oxidative stress triggers chronic inflammation through a very complex mechanism consisting of inflammatory mediators such as adhesion molecules and interleukins (22). The pathophysiology of GDM-associated complications is not clearly understood, but the positive feedback cycle involving oxidative stress and chronic systemic inflammation probably plays an important role (23-25). Ozler et al. investigated the predictive power of thiol/disulfide homeostasis parameters for perinatal complications in patients with GDM who were diagnosed with the two-step protocol (13). It was revealed that disulfide concentrations, and disulfide/native thiol and disulfide/total thiol ratios were significantly increased and native thiol/total thiol ratios were significantly decreased in the cord blood of babies born to women with GDM (13). In addition, patients with GDM who had high BMIs before pregnancy were found to have decreased native thiol concentrations at 24-28 weeks of pregnancy and an increased risk for adverse perinatal outcomes (13). Rueangdetnarong et al. assessed an oxidative stress marker (isoprostane) and an inflammatory marker (tumor necrosis factor-α) in GDM (26). Although these markers were increased in patients with GDM during the 24th to 28th weeks of pregnancy, the concentrations of the aforementioned markers in fetal cord blood were statistically similar to those of healthy controls (26). In addition, all perinatal and neonatal outcomes were statistically comparable despite the increase in oxidative stress (26). In our study, thiol/disulfide homeostasis parameters determined during the 24th-28th weeks of gestation failed to predict adverse pregnancy outcomes in patients with GDM, except fetal macrosomia.

This study was designed to evaluate the possible role of thiol/disulfide homeostasis in the occurrence of GDM-related complications. To the best of our knowledge, this is the first study to specify the status of oxidative stress by evaluating thiol/disulfide homeostasis in patients with GDM whose diabetes was diagnosed according to IADPSG criteria. The inclusion of healthy non-pregnant women and healthy pregnant women as control groups might provide an advantage for this study. However, this study has several limitations. First, the study cohort is relatively small, thus serious adverse perinatal outcomes such as preeclampsia, neonatal death or fetal anomalies were not observed. This makes the study inadequate to comment in terms of pregnancy complications. The second limitation is the use of a single-step test for the diagnosis of GDM in this study. In contrast, similar studies in literature used the two-step test for the diagnosis of 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. The third limitation is the lack of data related with good and poor glycemic control. The effects of diabetic diet and/or insulin treatment on oxidative biomarkers have not been evaluated. The fourth limitation is the absence of histopathologic data indicating the severity of oxidative stress and inflammation in placental tissues. The fifth limitation is the lack of longitudinal data related with oxidative stress markers in the first trimester, last trimester, puerperium or cord blood of newborn.

Conclusion

Pregnancies complicated with GDM had significantly higher concentrations of disulfide/native thiol and disulfide/total thiol and lower concentration of native thiol/total thiol than healthy pregnancies. This finding could be considered as the presence of increased oxidative stress in patients with GDM. However, these markers failed to predict adverse perinatal outcomes. Further research is required to understand the role of oxidative stress in the emergence of GDM-related complications.

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