A Study Over Thiol Disulfide Homeostasis in Cord Blood in Women With Gestational Diabetes

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Abstract
Objective: To gain insight into the effect of gestational diabetes mellitus (GDM) on cord blood dynamic thiol/disulfide homeostasis.

Materials and methods: A prospective case-control study was carried out for 132 pregnant women (62 GDM, 70 controls). The cord blood samples were collected from all the participants, and the native thiol-disulfide exchanges were examined by means of an automated method enabling to measure thiol-disulfide balance. In addition to investigating shifts in thiol disulfide balance between GDM and healthy pregnancies, we sought to correlate the thiol / disulfide homeostasis parameters with other clinical and laboratory characteristics of patients with GDM and using insulin (n = 19) and on a diet only (n = 43).

Results: Disulfide amounts, disulfide/native thiol and disulfide/total thiol rates increased (p < 0.001) while native thiol/total thiol decreased in the cord blood of infants born to diabetic mothers (p < 0.001). Furthermore, the patient group administered with insulin and diet only was compared. Disulfide, Disulfide/Native thiol*100, Disulfide/total thiol*100, Native/total thiol*100 differ significantly according to the results (p < 0.05). Disulfide, Disulfide / native thiol * 100, Disulfide/total thiol*100, HbA1c and 75gr are higher than those in patients who do not use insulin. Only Native/total thiol*100 values are higher in patients who use insulin than those who do not.

Conclusion: This study suggests that the infants born to diabetic mothers are exposed to increased oxidative stress. In addition, the patients who use insulin better control their blood glucose, thus reducing the need of newborns for intensive care.

Keywords: Thiol; GDM; Cord Blood

Introduction
Gestational Diabetes Mellitus (GDM) is one of the most common metabolic disorders diagnosed during pregnancy and it refers to carbohydrate intolerance in various levels (1, 2).

GDM screening is often performed in the week 24 to 28 during pregnancy whereas high-risk patients are recommended to be screened at the onset of pregnancy (3).

Fetal and maternal complication risks should be minimized by following treatment and monitoring protocols in patients with GDM (4). The first-line treatment of GDM is medical diet therapy and exercise. However, insulin therapy can be administered if diet and exercise alone are inadequate.

It has been suggested that there is a well-described...
relation between diabetes and oxidative stress (5).

Thiols are organic compounds that contain a sulfhydryl (-SH) group composed of a sulfide bonded with a hydrogen and a carbon (6). Thiol groups are reversibly oxidized under oxidative stress in order to create disulfide bonds, a residual of two cysteine amino acids in proteins. These disulfide bonds may be reduced to thiol groups again in order to sustain the dynamic thiol-disulfide homeostasis, and there is mutual transformation between thiols (reduced state) and disulfide groups (oxidized state). This is how thiol/disulfide homeostasis is sustained (7). Total thiols contain reduced and oxidized thiols while natural thiols contain reduced thiols only (8).

Thiols contribute to substantial part of total antioxidants in body, and play a major role in defense against reactive oxygen species (9). It is a recent fact that the pathogenesis of various acute and chronic diseases offers abnormal thiol/disulfide homeostasis (8, 10). The generation of reactive oxygen species (ROS) is associated with a variety of diseases such as obesity, type II diabetes and cancer (12). Thiol measurement in serum samples provides indirect reflection of antioxidant defense (8, 10, 11-13). It has been found out that the oxidative stress increases in pregnant women with both type I diabetes and GDM caused by excessive generation of ROS and/or lack of antioxidant defenses system, and thus the imbalance in thiol redox homeostasis is associated with diabetes and complications (14, 15).

In this study, we aimed at determining the status of dynamic thiol / disulfide homeostasis as a marker of oxidative stress recently in the cord blood of GDM patients. We also investigated the correlation between thiol / disulfide homeostasis parameters and other clinical and laboratory characteristics of patients with GDM using insulin and diet only.

Materials and methods

Upon the approval of the study ethics committee, Medipol University was projected to serve as a prospective case control study. Between January 2017 and August 2017, a total of 470 pregnant women were screened for GDM with 75 g OGTT in week 24 to 28 of pregnancy at Istanbul Medipol University Hospital’s Outpatient Clinics of Gynecology and Obstetrics. Those who had spontaneous pregnancies between 18-40 years of age followed by our first antenatal visit at our hospital, those who gave birth after > 37 weeks and those who underwent other laboratory tests were included in the study. Those with multiple pregnancy, antenatal hemorrhage, grand multipara (> 4 births), a systemic disease, type 1 and type 2 diabetes, pregnancy with known pre-pregnancy obesity (BMI > 30 kg/m) or known malformation or chromosomal anomaly fetus and those with spontaneous preterm labor and those with diabetes mellitus in first-degree relatives were excluded from the study. The gestational age was calculated by measuring CRL on the first day of the last menstrual period and the first trimester USG. (10, 11). A total of 140 pregnant women (70 GDM, 70 controls) started to work. However, a total of 132 pregnant women (GDM n = 62) (control n = 70) were included in the study as a result of the exclusion of those who could not receive cord blood for technical reasons or who did not deliver at our hospital.

According to IADPSG 75GR OGTT (at least 1 pathological value is enough to diagnose) APG ≥ 92, 1.hr PG ≥ 180, 2.hr ≥ 153 (16, 17).

Those diagnosed with GDM were administered with a diet while those, who failed to be regulated by a diet, were administered with insulin therapy. Those administered with insulin therapy (n = 19) and patients administered solely with medical nutrition therapy were divided into two groups named the diet group (n = 43).

The patients on a diet were followed up for their blood glucose for 15 days. The patients, whose preprandial and pre-course blood glucose was > 95 mg/dl, 2nd-hour Pre-Prandial Blood Glucose ≤ 140 mg/dl, were administered with insulin therapy. The objectives of the therapy were in line with the recommendations set out by American Diabetes Association (ADA) (18, 19).

Immediately after the birth of the neonate, the umbilical cord was clamped and venous blood sample was collected. The samples were processed within 10 minutes after the withdrawal by centrifugation at 5000 revolutions/minute for 10 minutes, and stored at −80°C until an analysis was made.

The cord blood samples of 132 pregnant women giving a full-term birth were taken and compared to thiol/disulfide homeostasis parameters as well as demographic, obstetric and neonatal laboratory results. In addition, out of the patients diagnosed with GDM, those administered with diet only and those on insulin were compared.

Measurement of dynamic thiol/disulfide homeostasis: Thiol/disulfide homeostasis was determined by a novel spectrophotometric method previously described by Erel and Neselioglu (8).
Data Collection Tools and Techniques: The data analyses were tested by means of the package program called IBM SPSS Statistics 24.0 (Statistical Package for Social Science).

Since 75 gr of pre-prandial blood glucose was not normally dispersed as suggested by the analysis, Mann Whitney U-Test was performed. The Chi Square Test was performed in order to gain insight into the relation between the variables of AFI/poli, AFI/olig and AFI/normal, and the use of insulin. The reason why the test results include Fisher's Exact Test scores rather than Pearson Chi-Square test scores is the fact that there were scores fewer than 5 for cross table cells. ROC analysis was conducted for the patients with diabetes to find out their scores of cut-off and sensitive specificity. The results were interpreted in accordance with Youden's Index, a method usually adopted on break points calculated by 2 types of methods.

Results

Given the similarity between gravida and birth style of a total 132 pregnant (62 with GDM and 70 control group), result findings were similar as 77.5% in GDM group and 62.9% in control group were on a diet while only 7.2% of the patients in the GDM group and 51.9% in control group were nulliparous while 58.5% in GDM group and 62.9% in control group were of a total 132 pregnant (62 with GDM and 70 control group), result findings were similar as 77.5% in GDM group while the control group were v

Table 1: Descriptive statistics for the groups: Patient & Control Group Mann Whitney U (MWU) / T-Test Results

| Criterion                                      | GDM           | Control        | p      | Test  |
|-----------------------------------------------|---------------|----------------|--------|-------|
| Native thiol                                   | 343.22 ± 89.44| 347.09 ± 83.55| 0.770  | MWU   |
| Total thiol                                    | 388.82 ± 87.14| 368.70 ± 88.10| 0.060  | MWU   |
| Disulfide                                      | 22.76 ± 9.62  | 10.79 ± 4.61   | 0.000* | MWU   |
| Disulfide/native thiol*100                    | 7.62 ± 6.11   | 3.15±1.16      | 0.000* | MWU   |
| Disulfide/total thiol*100                      | 6.24 ± 3.63   | 2.94±1.03      | 0.000* | MWU   |
| Native/total thiol*100                         | 87.49 ± 7.24  | 94.12±2.06     | 0.000* | MWU   |
| HbA1c                                         | 5.66 ± 0.52   | 5.25 ± 0.26    | 0.000* | MWU   |
| 75gr preprandial blood glucose                 | 90.26 ± 3.71  | 86.66 ± 3.26   | 0.000* | T Test|
| 75gr 1st hour                                  | 197.18 ± 15.57| 144 ± 13.01    | 0.000* | MWU   |
| 75gr 2nd hour                                  | 171.85 ± 14.94| 129.04 ± 3.74  | 0.000* | MWU   |
| Age                                           | 28.11 ± 2.97  | 28.00 ± 3.02   | 0.749  | MWU   |
| Pre-pregnancy BMI                              | 26.33 ± 2.44  | 26.90 ± 2.33   | 0.218  | MWU   |
| Weight gained during pregnancy                 | 13.05 ± 1.64  | 13.15 ± 1.61   | 0.690  | MWU   |
| Weight at birth                                | 3393.55 ± 297.99| 3254.91 ± 141.27| 0.005*| MWU   |

*Statistically significant (0.05) (Mean ± S.S) Mann Whitney U (MWU) / T test

KS: Kolmogorov-Smirnov Test; SW: Shapiro-Wilk Test

The variable 75gr PBG was dispersed normally as it was p = .137 > .05 according to the normality test (Table 1). The variable 75 gr. pre-prandial blood glucose was analyzed by means of parametric tests while the other was analyzed by means of non-parametric tests.

Table 2 compares the patient group by using and not using insulin. The variables including disulfide, disulfide/native thiol*100, disulfide/total thiol*100, native/total thiol*100, HbA1c, 75gr preprandial blood glucose, 75gr 2nd-hour and weight at birth showed significant difference (p < 0.05). The scores of disulfide, disulfide/native thiol*100, disulfide/total thiol*100, HbA1c, 75gr pre-prandial blood glucose, 75gr 2nd-hour and weight at birth were higher in the patient group than the control group. The sole native/thiol*100 score was higher in the control group than the patient group. The comparison of the patient and the control group for variables such as native thiol, total thiol, age, pre-pregnancy BMI and weight gained during pregnancy showed no statistically significant difference (p > 0.05).

Newborns for intensive care was 25.8% for the GDM group and 7% for the control group.

The variables including disulfide, disulfide/native thiol*100, disulfide/total thiol*100, native/total thiol*100, HbA1c, 75gr preprandial blood glucose showed significant difference (p < 0.05). The scores of disulfide, disulfide/native thiol*100, disulfide/total thiol*100, HbA1c, 75gr pre-prandial blood glucose were higher in the patients administered with insulin than those who were administered with such.
The sole native/thiol*100 score is higher in patients on insulin therapy than those who are not. The comparison of the patients using insulin and those not using insulin for variables such as native thiol, total thiol, 75 gr. OGTT, age, pre-pregnancy BMI and weight gained during pregnancy showed no statistically significant difference (p > 0.05).

When the apgar scores are taken into account, the comparison of a total of 62 patients including 3 patients with the apgar score 7 < and 59 patients with the score 7 ≥ showed no statistically significant difference (p > 0.05).

Table 3 shows the sensitivity analyses made for the patients with and without diabetes. The results were interpreted in accordance with Youden’s Index, a method usually adopted on break points calculated by 2 types of methods. According to the results, the ROC analyses for disulfide, disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios were significant (p < 0.05). The best break point for disulfide was 100.00% sensitivity and 4.44% specificity and 11.15 μmol/L while AUC score turned out to be 0.142 (95%CI: 0.053-0.232). The best break point for disulfide/native thiol ratio was 100.00% sensitivity and 0.00 specificity and 1.71 μmol/L while the AUC score turned out to be 0.119 (95%CI: 0.034-0.204). The best break point for native thiol/total thiol ratio was 100.00% sensitivity and 71.11% specificity and 89.86 μmol/L while the AUC score turned out to be 0.867 (95%CI: 0.781-0.954).

**Table 2:** Whitney U (MWU) / T Test Results By the Use of Insulin

| Criterion                        | Not on insulin | On insulin | p      | Test |
|----------------------------------|----------------|------------|--------|------|
| Native thiol                     | 335.96 ± 97.6  | 362.46 ± 61.33 | 0.167  | MWU  |
| Total thiol                      | 387.56 ± 95.10 | 392.15 ± 63.73 | 0.636  | MWU  |
| Disulfide                        | 25.75 ± 9.57   | 14.84 ± 3.02  | 0.000* | MWU  |
| Disulfide/native thiol*100       | 8.93 ± 6.71    | 4.15 ± 0.80   | 0.000* | MWU  |
| Disulfide/total thiol*100        | 7.16 ± 3.88    | 3.82 ± 0.69   | 0.000* | MWU  |
| Native/total thiol*100           | 85.65 ± 7.71   | 92.35 ± 1.37  | 0.000* | MWU  |
| HbA1c                            | 5.82 ± 0.52    | 5.25 ± 0.22   | 0.001  | MWU  |
| Age                              | 27.96 ± 3.06   | 28.53 ± 2.76  | 0.352  | MWU  |
| Pre-pregnancy BMI                | 26.38 ± 2.40   | 26.21 ± 2.61  | 0.893  | MWU  |
| Weight gained during pregnancy   | 12.91 ± 1.61   | 13.41 ± 1.73  | 0.329  | MWU  |
| Weight at birth                  | 3398.89 ± 298.05 | 3379.41±306.52 | 0.570  | MWU  |

*Statistically significant (0.05)

**Table 3:** ROC Tests For Patients With Diabetes Cut-off Levels, Specificity and Sensitivity Test Results

| Cutoff Selection Criterion | Youden’s index | Pathagoras’ theorem | Auc (95%CI) |
|----------------------------|----------------|---------------------|-------------|
| Evaluation Criteria        | Cut off | Spe % | Sen % | Cut off | Spe % | Sen % | P |
| Native thiol               | 288.65 | 94.12 | 31.11 | 338.80 | 64.71 | 55.56 | 0.614 (0.467-0.762) | 0.167 |
| Disulfide                  | 11.15 | 100.00 | 4.44 | 11.15 | 100.00 | 4.44 | 0119 (0034-0204) | 0.000* |
| Total thiol                | 364.10 | 70.59 | 44.44 | 368.85 | 64.71 | 48.89 | 0.539 (0.384-0.695) | 0.636 |
| Disulfide/native thiol ratio | 1.71 | 100.00 | 0.00 | 4.01 | 64.71 | 13.33 | 0.142 (0.053-0.232) | 0.000* |
| Disulfide/total thiol ratio | 1.57 | 100.00 | 0.00 | 3.71 | 64.71 | 13.33 | 0.142 (0.052-0.232) | 0.000* |
| Native thiol/total thiol    | 89.86 | 100.00 | 71.11 | 90.46 | 94.12 | 75.56 | 0.867 (0.781-0.954) | 0.000* |

*Statistically significant (0.05) AUC; area under the curve, CI: Confidence Interval
Discussion

The results show that disulfide, disulfide/native thiol*100 and disulfide/total thiol*100 were higher in the GDM group than the control group. The sole native/thiol*100 score was higher in the control group than the patient group.

Our study suggests that the thiol/disulfide homeostasis in cord blood of mothers with GDM shifts in favor of disulfide, thus causing an increase in thiol oxidation. All the past studies over the balance between the oxidative stress in cord blood and antioxidant defense systems reported an increase in oxidative stress markers (E.g.: MDA and xanthine oxidase) and a significant decrease in antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase) (20, 21). Ates et al. reported in a study over dynamic thiol/disulfide homeostasis that the native thiol levels were significantly low in prediabetic patients (22). This is in compliance with the results of this study as we reduced the native thiol levels in cord blood of diabetic mothers and the native/total thiol ratio. Harper et al. showed in a study that the excess weight gained by patients with GDM has a correlation with macrosomia and negative pregnancy outcomes for newborns (23). Our study showed no significant difference between the patient and the control group in pre-pregnancy BMI, weight gained during pregnancy and weight at birth. Despite this, those with the apgar score < 7 had significantly higher scores of weight gained during pregnancy and weight at birth. We found out that as the patients with higher scores of BMI were excluded, the weight gained during pregnancy and weight at birth were in correlation with the low apgar score for the patients with GDM. This may be associated with the limited number of patients involved in the study. Ozler et al. reported similar results in a study and argued that the thiol/disulfide homeostasis for patients with GDM shifted in favor of homeostasis, thus causing an increase in thiol oxidation (24, 25).

In addition, it was found out that the increased disulfide in cord blood and the decreased native/total thiol rate was significantly in correlation with the low apgar score. This shows that the increased thiol oxidation may be correlated with negative perinatal outcomes in GDM (increased need for NICU). As untreated GDM is risky for perinatal morbidity, it is crucial to make early estimations for potentially negative outcomes of GDM to prevent potential complications. Our study shows a comparison of patients administered and not administered with insulin. The variables including disulfide, disulfide/native thiol*100, Disulfide/total thiol*100 and native/total thiol*100 showed significant difference (p < 0.05). The scores of disulfide, Disulfide/native thiol*100, Disulfide/total thiol*100, HbA1c, 75gr pre-prandial blood glucose were higher in the patients administered with insulin than those who were administered with such. The sole native/thiol*100 score is higher in patients on insulin therapy than those who are not. Our study is in agreement with the results of the previous studies indicating that the probability of need for insulin can be projected based on abnormal OGTT values (26, 27).

Conclusion

In conclusion, we suggested that the offspring born to diabetic mothers are exposed to increased oxidative stress. In this study, we found out that 75gr OGTT were predictive for the future need for insulin therapy in achieving optimal blood glucose regulation in 24–28 weeks of pregnancy. In addition, the patients who use insulin better control their blood sugar, thus reducing the need of newborns for intensive care.

The main restriction on our study was the relatively few number of patients that might limit the applicability of the results on the general population. Therefore, it is necessary to carry out further studies with larger cohorts to confirm the results of this study.

Conflict of Interests

Authors have no conflict of interests.

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References

1. Fauci AS, Braunwald E, Kasper DI, Hauser SL, Longo DL, Jameson JL, et al Harrison's principles of Internal Medicine. 17th ed. New York: McGraw Hill Medical. 2008: 631.
2. Cunningham FG, Leveno KG, Bloom SL, Hauth JC, Gilsrb LC, Wenstrom KD, Williams Obstetrics.22nd ed. New York: McGraw Hill. 2005.; 1170–2.
3. Royal College of Obstetricians and Gynaecologists. Scientific advisory committee opinion paper. Diagnosis and Treatment of Gestational Diabetes 2011; 8, 2011.
4. Saltan İ, İmamoğlu S, Yılmaz C, Salman S, et al. Treatment and follow-up guide of Turkish diagnosis of
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endocrinology and metabolism association of diabetes mellitus and its complications. 2018; 10th Ed. Ankara: Miki:170–5.

5. West IC. Radicals and oxidative stress in diabetes. Diabet Med 2000; 17:171-80.

6. Sen CK., Packer L. Thiols homeostasis and supplements in physical exercise. Am J Clin Nutr 2000; 72:653S–669S.

7. Jones DP., Liang Y. Measuring the poise of thiols/disulfide couples in vivo. Free Radic Biol Med. 2009; 47:1329–38.

8. Erel O, Neselioğlu S. A novel and automated assay for thiols/disulphide homeostasis. Clin Biochem 2014; 47:326–32.

9. Chianeh YR., Prabhu K. Protein thiols as an indicator of oxidative stress. Archives Medical Review Journal. 2014; 23:443–56.

10. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010; 48:749–62.

11. Yuksel M, Ates I, Kaplan M, Alisik M, Erel O, Saygili F et al. The dynamic thiols/disulphide homeostasis in inflammatory bowel disease and its relation with disease activity and pathogenesis. Int J Colorectal Dis 2016; 31:1229–31.

12. Görłach A, Dimova EY, Petry A, Martínez-Ruiz A, Hernansanz-Agustín P, Rolò AP, et al. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? Redox Biol 2015; 6:372-85.

13. Dirican N, Dirican A, Sen O, Aynali A, Atalay S, Bircan HA, et al. Thiols/disulfide homeostasis: a prognostic biomarker for patients with advanced non-small cell lung cancer? Redox Rep 2016; 21:197–203.

14. Dumlu EG, Tokaç M, Bozkurt B, Yıldırım MB, Ergin M, Yalcın A, et al. Correlation between the serum and tissue levels of oxidative stress markers and the extent of inflammation in acute appendicitis. Clinics (São Paulo) 2014; 69:677–82.

15. Matteucci E, Giampietro O. Thiols signaling network with an eye to diabetes. Molecules 2010; 15:8890–903.

16. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33:676–2.

17. Griffin ME, Coffery M, Johnson H, Scanlon P, Foley M, Stronge J, et al. Universal vs., risk factor-based screening for gestational diabetes mellitus: Detection rates, gestation at diagnosis and outcome. Diabet Med 2000; 17:26–32.

18. American Diabetes Association. Gestational diabetes mellitus. Diabetes Care. 2003; 26:S103–S105.

19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2007; 30:S42–7.

20. Biri A, Onan A, Devrim E, Babacan F, Kavutcu M, Durak I. Oxidant status in maternal and cord plasma and placental tissue in gestational diabetes. Placenta 2006; 27:327–32.

21. Madazlı R, Tuten A, Calay Z, Uzun H, Uludag S, Ocak V. The incidence of placental abnormal–ities, maternal and cord plasma malondialdehyde and vascular endothelial growth factor levels in women with gestational diabetes mellitus and non-diabetic controls. Gynecol Obstet Invest 2008; 65:227–32.

22. Ates I, Kaplan M, Inan B, Alisik M, Erel O, Yilmaz N, Guler S. How does thiol/disulfide homeostasis change in prediabetic patients? Diabetes Res Clin Pract 2015; 110:166-71.

23. Harper LM, Tita A, Biggio JR. The institute of medicine guidelines for gestational weight gain after a diagnosis of gestational diabetes and pregnancy outcomes. Am J Perinatol 2015; 32:239–46.

24. Ozler S, Oztas E, Erelb O, Gumus BG, Ergind M, Uygura D, et al. Impact of Gestational Diabetes Mellitus and Maternal Obesity on Cord Blood Dynamic Thiol/Disulfide Homeostasis. Fetal and pediatric Pathology 2017; 36:8-15.

25. Ozler S, Oztas E, Caglar AT, Ergin M, Danisman N. Thiol/disulfide homeostasis in predicting adverse perinatal outcomes at 24-28 weeks of pregnancy in gestational diabetes, The Journal of Maternal-Fetal & Neonatal Medicine 2016; 29: 3699-704.

26. Sapienza AD, Francisco RP, Trindade TC, Zugaib M. Factors predicting the need for insulin therapy in patients with gestational diabetes mellitus. Diabetes Res Clin Pract 2010; 88: 81–6.

27. Wong VW, Jalaludin B. Gestational diabetes mellitus: who requires insulin therapy? Aust N Z J Obstet Gynaecol 2011; 51: 432–6.

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