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Maternal Serum and Fetal Cord Blood Concentrations of Thiol/ Disulfide and Ischemia-Modified Albumin as Predictors of Neural Tube Defects

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ABSTRACT

AIM: To investigate the predictive role of thiol/ disulfide homeostasis and Ischemia-modified albumin (IMA) levels for NTDs.

MATERIAL and METHODS: A total of 71 pregnant women (31 with NTD and 42 healthy controls) were enrolled in this study. This prospective case-control study included pregnant women with NTDs as the study group and randomly selected age-matched pregnant women with healthy fetuses as the control group. The two groups were compared on the basis of thiol/disulfide and IMA levels in the maternal and fetal samples.

RESULTS: No statistically significant difference in native thiol, total thiol, disulfide, and calculated ratios was observed between the groups. However, maternal IMA values were significantly higher in the study group. The IMA was proven to be a predictor with a sensitivity of 77.4% and specificity of 100% for NTDs at a cut-off value of 1.32.

CONCLUSION: The examination of the maternal levels of IMA may be useful in the detection of NTDs.

KEYWORDS: Neural tube defects, Ischemia-modified albumin, Thiol/disulfide, Oxidative stress

ABBREVIATIONS: IMA: Ischemia-modified albumin, **NTD:** Neural tube defects, **ROS:** Reactive oxygen species, **EDTA:** Ischemia-modified albumin

INTRODUCTION

Neural tube defects (NTDs) are a group of malformations due to a multifactorial etiology. This group is the most common abnormality of the nervous system with a reported incidence of 0.2–10/1000 pregnancies (5). The well-defined etiologic factors include folic acid deficiency; genetic and environmental factors, such as maternal fever in the first trimester of pregnancy; drug use; folic acid deficiency; diabetes mellitus; and biochemical factors (8,13). However, the rate of NTDs attributable to a known etiology is low, accounting for one third of all cases (1).

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Oxidative stress can be defined as the imbalance between reactive oxygen species (ROS) activity and antioxidants. It has already been demonstrated that oxidative stress can lead to pathological conditions via the deleterious effects on proteins, lipids, and cell DNA (4). The relationship between increased oxidative stress and NTDs has been found, and vitamin E, which plays an antioxidant role, had a protective effect against NTDs (14). Furthermore, maternal diabetes, which is a known risk factor for NTDs, is reported to be associated with the increased production of ROS, and animal studies have shown that maternal diabetes mellitus causes NTDs through increased oxidative stress and, consequently, increased cell apoptosis (9).

Thiols are organic compounds that contain the sulfhydryl group and account for most of the antioxidant capacity of human metabolism. Disulfides occur in the oxidized state, while thiols occur in the reduced state. In this way, the thiol-disulfide balance maintains most of the oxidation-reduction reactions of the human body (7). Ischemia-modified albumin (IMA) is also a marker of oxidative stress, i.e., an oxidized form of albumin. NTDs, fetal growth restriction, and diabetes are some of the conditions previously studied for IMA (10,21).

Two previous studies have shown that the thiol-disulfide balance had shifted in favor of disulfide, supporting the increased oxidative stress in NTDs (12,14). We aim to investigate whether thiol disulfide and IMA levels might be the early markers of NTDs during pregnancy. We also compared the levels of the above markers in pregnant patients with complicated NTDs and uncomplicated pregnant patients as well as in the cord blood of their fetuses.

MATERIAL and METHODS

This is a prospective case-control study conducted in Ankara City Hospital in the communication of three departments (Perinatology, Neurosurgery, and Neonatology) between October 2019 and May 2020. Ethical approval was obtained from the ethics committee of the institution before data collection (Date: 19.09.2019; No: E2-22-1259). The study protocol was conducted in accordance with the tenets of the Declaration of Helsinki, and written informed consent was obtained from all participants detailing the study.

This study included pregnant women with NTD-affected fetuses in the second trimester of pregnancy as the study group and pregnant women with healthy fetuses corresponding to gestational age as the control group.

Individuals with chronic maternal diseases, such as renal, cardiac, and diabetic diseases, smokers, users of antiepileptic drugs and alcohol, pregnant women whose fetuses had other structural or genetic abnormalities and multiple pregnancies were excluded from the study. Pregnant women with complicated NTDs were given detailed counseling and offered abortion. The subjects who refused abortion were followed until delivery. NTD diagnosis was confirmed by the pediatrician and neurosurgeon after abortion or delivery.

All pregnant women who participated in the study were examined with obstetric ultrasound by maternal-fetal specialists with 11 years of experience. The GE Voluson E10 ultrasound machine was used to perform detailed ultrasound examinations and confirm NTD diagnosis. The participants' age, gravidity, parity, and gestational age were recorded. The ultrasonographic findings of the fetus and fetal biometric calculations were also recorded.

Maternal antecubital venous blood samples were obtained in tubes containing ethylenediaminetetraacetic acid (EDTA) from both groups in the second trimester. The blood samples were centrifuged at 2000xg for 10 minutes within two hours. After the separation of the plasma and serum samples, the plasma was stored in the upper part of the tubes at –80°C until the day of the analysis. Five women with NTD-affected fetuses wanted to terminate the pregnancy at 18, 19, 21, 22, and 22 weeks of gestation, while the other pregnant women remained until delivery. After the delivery of the baby, umbilical cord blood samples were collected to examine thiol disulfide levels and IMA. The age-matched control group of abortions was selected from the women who had spontaneous abortion or preterm delivery. For the umbilical cord samples, the same procedure was performed. A fully automated procedure described by Erel and Neselioglu (7), performed using the Cobas 501 clinical chemistry analyzer (Roche, Mannheim, Germany), was used to evaluate dynamic thiol/disulfide homeostasis in the plasma and IMA values. Sodium borohydride was used to reduce disulfide bonds to thiol groups. Further reduction of DTNB (5,5-dithiobis-(2-nitrobenzoic acid)) was prevented by removing the excess sodium borohydride with formaldehyde. The Ellman reagent and the modified Ellman reagent were used to determine the total and native thiol content. The dynamic disulfide content was calculated as half the difference between the total thiol content and the native thiol content. The results were then expressed in mmol/L.

The IMA content was measured by 0.1% cobalt uptake. After 10 minutes of incubation with cobalt, dithiothreitol was added. Finally, 0.9% sodium chloride solution was added and absorbance was measured using a spectrophotometer. The results were then expressed as absorbance units.

The IBM SPSS Statistics software (22.0, SPSS Inc, Chicago, IL) was used to analyze the data. The demographic characteristics were presented as mean ± standard deviation and median (minimum-maximum) after checking the normality of the variables using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare the variables between the study and control groups. The ROC curve was used to determine the cut-off value of IMA for the prediction of NTDs.

RESULTS

A total of 73 pregnant women (31 with NTD and 42 gestational age-matched controls) were included in this study. Table 1 shows the demographic characteristics of the groups. Accordingly, no statistically significant differences in maternal age, gestational age at the time of blood collection and delivery, gravidity, parity, and BMI ($p>0.05$) were observed in

both groups. Of the cases with NTD, abortion was decided in five of them and medically induced abortion was successfully performed, while the other pregnant women decided to continue the pregnancy until delivery. Only two women from the study group underwent vaginal delivery.

Table II shows the results of the biochemical analyses of the groups based on the maternal and fetal blood samples. The mean maternal serum levels of native thiol in the study and control groups were 194.95 and 198.08, respectively (p>0.05). No statistically significant difference in the total thiol levels was observed between the groups (234.87 vs. 239.56, p>0.05). The mean disulfide levels of the groups were also similar (19.56 vs. 20.13, p>0.05) (Figure 1). Moreover, only the classical IMA

Table I: Demographic Characteristics of the Groups

	NTDs	Control	
	Mean ± SD	**Mean ± SD**	**p***
Age	28.52 ± 5.45	27.73 ± 4.05	0.742
Gravidity	2.21 ± 1.20	1.73 ± 1.27	0.182
Parity	1.30 ± 1.29	0.75 ± 0.69	0.252
BMI	24.41 ± 3.60	24.47 ± 3.65	0.682
GA at birth GA at blood sampling	34.11 ± 3.58 21.4 ± 2.41	34.03 ± 4.50 21.6 ± 2.65	0.548 0.162
Birth weight	2387.80 ± 1135.09	2299.67 ± 1254.67	0.813
Birth height	45.37 ± 7.92	49.45 ± 2.38	0.160

******"Mann-Whitney U" test,* ***GA:*** *Gestational age,* ***BMI:*** *Body mass index.*

Table II: Laboratory Findings of the Groups

	NTDs	Control [n=42]	
	Mean ± SD	**Mean ± SD**	**p***
Classical IMA [ABSU]	1.38 ± 0.15	1.09 ± 0.17	**0.000**
Native thiol [mmol/L]	194.95 ± 43.83	198.08 ± 54.49	0.617
Total Thiol [mmol/L]	234.87 ± 44.58	239.56 ± 59.35	0.597
Disulfide [mmol/L]	19.56 ± 7.88	20.13 ± 14.90	0.423
SS/Native thiol [%]	11.72 ± 7.62	13.45 ± 16.72	0.521
SS/Total Thiol [%]	8.89 ± 4.75	8.69 ± 6.32	0.521
Native Thiol/Total Thiol [%]	83.15 ± 6.99	82.81 ± 12.32	0.394
Albumin [g/dL]	2.95 ± 0.93	3.06 ± 0.99	0.581
UC classical IMA [ABSU]	102.26 ± 174.48	116.59 ± 198.95	0.346
UC native thiol [mmol/L]	291.62 ± 120.36	286.43 ± 135.96	0.882
UC total thiol [mmol/L]	325.99 ± 130.99	321.28 ± 139.14	0.882
UC disulfide [mmol/L]	17.03 ± 7.96	18.32 ± 12.35	0.772
UC SS/Native thiol [%]	8.02 ± 5.58	19.11 ± 47.75	0.976
UC SS/Total Thiol [%]	6.67 ± 4.75	9.12 ± 12.67	0.873
UC Native Thiol/Total Thiol [%]	87.78 ± 4.44	86.02 ± 12.29	0.948
UC Albumin [g/dL]	1.61 ± 1.21	1.75 ± 1.25	0.504
UC ph	7.34 ± 0.07	7.33 ± 0.07	0.623

***** *"Mann-Whitney U" test ,* ***SS:*** *Disulfide,* ***UC:*** *Umbilical Cord.*

values were significantly higher in the study group than in the control group (p=0.000) (Figure 2). The classic IMA was proven to be a significant predictor of NTD with a cut-off value of 1.32, sensitivity of 77.4%, and specificity of 100% (p<0.05) (Figure 3).

The calculated ratios of native thiol/total thiol, disulfide/native thiol, and disulfide/total thiol were not statistically significantly different between the groups (p>0.05).

The analyses of the cord blood samples indicated similar results. The mean native thiol levels of the study and control groups were 291.62 and 286.43, respectively (p>0.05) (Figure 4). No statistically significant difference in the native thiol levels was observed between the groups (p>0.05). The classical IMA values of the groups were also similar (102.26 vs. 116.59, p>0.05).

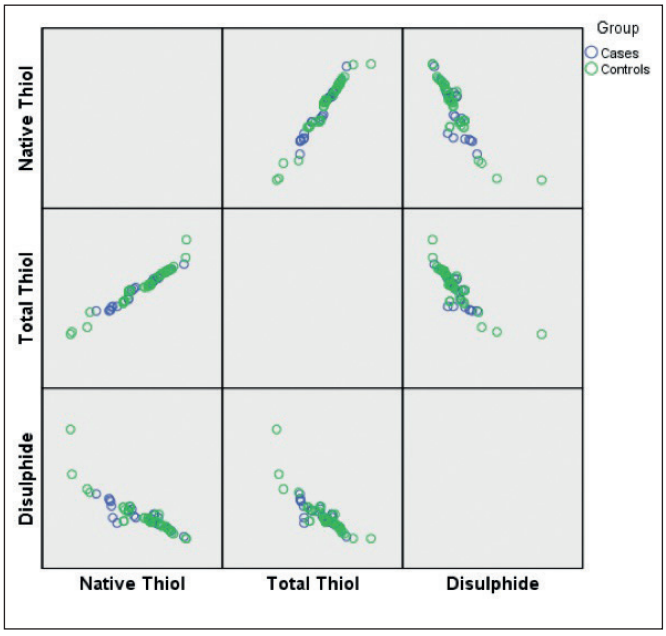

Figure 1: Maternal total thiol, disulfide, and native thiol levels of the groups.

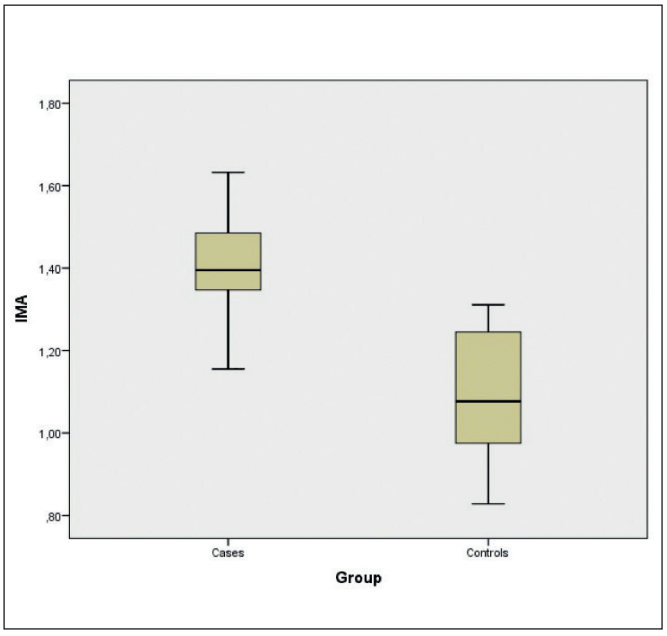

Figure 2: Maternal Ischemia-modified albumin (IMA) levels of the groups.

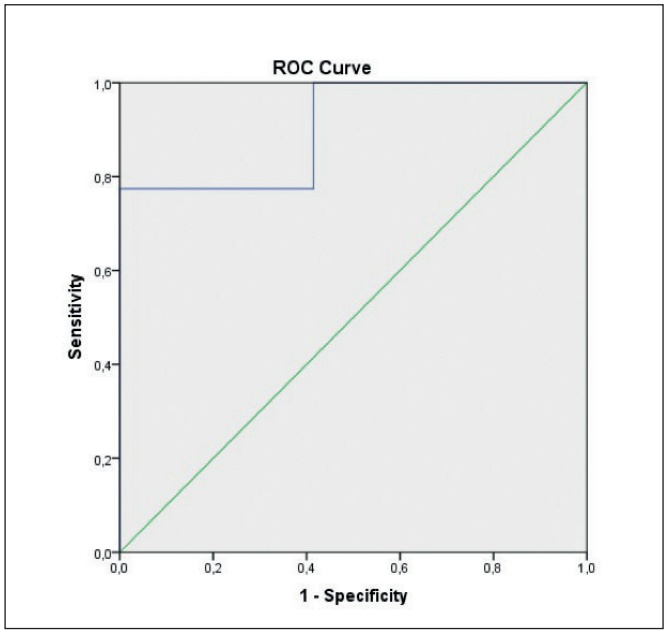

Figure 3: ROC of Ischemia-modified albumin (IMA).

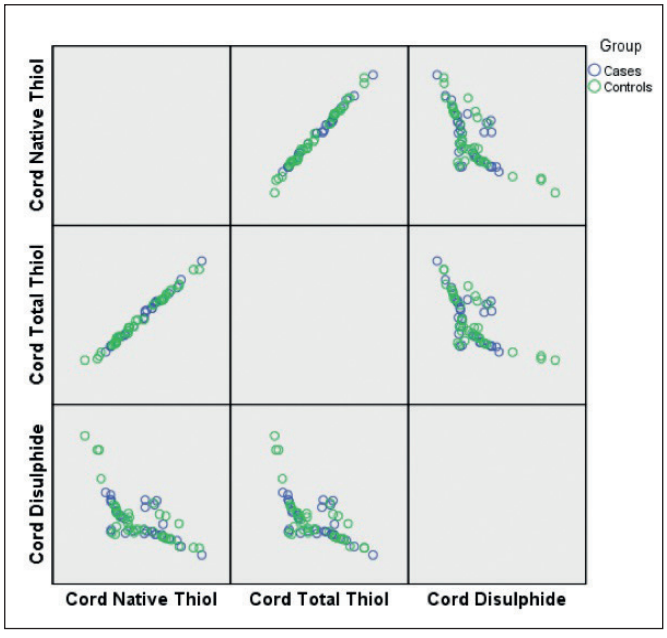

Figure 4: Umbilical cord total thiol, disulfide, and native thiol levels of the groups.

The ratios of native thiol/total thiol, disulfide/native thiol, and disulfide/total thiol were not statistically significantly different between the groups (p>0.05).

DISCUSSION

NTDs are the most common congenital nervous system disorders in fetuses with a reported incidence of 0.2–10/1000 pregnancies (5). Although the exact mechanisms underlying NTDs have not yet been determined, some conditions have been shown to cause NTDs. Uncontrolled maternal diabetes mellitus, a pathological condition associated with increased oxidative stress, is one of the known maternal conditions associated with increased NTD risk. In an experimental animal study, high-concentration oxygen exposure was shown to increase the incidence of NTDs (11). Other animal studies have shown that gestational diabetes mellitus causes NTDs via cell apoptosis due to increased oxidative stress (9). Valproic acid, a known cause of NTDs, has been previously shown to increase ROS levels. In line with this, we aim to determine the possible relationship between oxidative stress and NTDs by evaluating the thiol/disulfide and IMA levels.

Ozyer et al. examined the thiol/disulfide levels in pregnant women whose fetuses had NTDs and found that the balance of thiol-disulfide homeostasis had shifted in favor of disulfide and that the IMA levels were elevated (14). In contrast, Karaman et al. found no association between NTDs and thiol-disulfide homeostasis and the IMA levels (12). In the present study, no significant correlation was observed between the groups in relation to thiol-disulfide homeostasis. However, as expected, the IMA levels were significantly higher in the study group than in the control group. Moreover, we found a cut-off value for IMA to predict NTD with a sensitivity of 77.4% and specificity of 100%. When the cord blood was examined, no statistically significant difference in the thiol/disulfide balance and IMA values was observed.

IMA is a consequence of oxidative changes in the albumin during increased oxidative stress conditions. It has been studied mainly in heart diseases and is considered a sensitive marker for ischemic heart disease (3,6,18). In 2013, Rossi et al. showed that the levels of IMA have increased during pregnancy, especially in pregnancies associated with fetal growth restriction (17). A meta-analysis demonstrated the indicative role of the elevated IMA levels for oxidative stress in noncomplicated pregnancy and preeclampsia (16).

Similar to our results, Guzelmansur et al. found that the serum IMA levels were significantly higher in cases with NTD than in the control group (10).

ROS activities normally occur in human metabolism. However, under conditions, such as hypoxia or inflammation, the ROS levels tend to increase, and if antioxidants cannot compensate for this increase, ROS will harm the organism. Therefore, oxidation-reduction reactions have been the main topic of several studies. To date, many oxidative markers have been studied to determine the etiology of NTDs. Vural et al. studied the oxidative status of fetuses with NTDs and found increased levels of amniotic fluid compared with the control groups. MPO and CAT are the other antioxidant molecules that have been studied in pregnancies complicated with NTDs (20). This study aims to investigate the thiol/disulfide and IMA levels because thiol/disulfide homeostasis maintains most of the oxidation/reduction in the human body.

Similar to our results, Verrotti et al. found no significant difference in the oxidative parameters between the NTD and control groups (19). In addition to thiol/disulfide homeostasis, various oxidative markers have been investigated in the evaluation of the pathology of NTDs. Arslan et al. found an increased incidence of NTDs in infants in whom free radicals such as malondialdehyde were elevated (2). Moreover, the genetic characteristics of the oxidative/antioxidant balance are an important factor in the development of NTDs. It has been shown previously that free radical scavenging activity is genetically determined (15). We believe that these conflicting results demonstrate the multifactorial etiology of NTDs. Thus, it could be suggested that the cause of NTDs cannot be attributed to a single pathology.

The major limitation of this study is that the cord blood samples of the control group were different from those of the age-matched controls of fetuses with aborted NTDs which were obtained in the second trimester. Therefore, the maternal blood samples and the cord blood samples were obtained from different pregnant women and fetuses. Therefore, we could not investigate the correlation between the thiol/disulfide levels of the mothers and fetuses and IMA in the control group. In contrast, in the study group, we followed the maternal blood samples of the pregnant women who had fetuses with NTD until delivery and collected the umbilical cord samples from the same pregnant women. On the other hand, the greatest strength of our study is that we examined both the maternal and fetal thiol/disulfide levels and IMA in all patients with NTDs until delivery. We found that IMA is a significant predictor of NTDs and presented for the first time a cut-off value with high specificity.

CONCLUSION

It can be suggested that maternal IMA level may be a predictor of NTDs. However, the role of the thiol/disulfide level in determining NTDs is still unclear and requires further investigation.

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AUTHORSHIP CONTRIBUTION

Study conception and design: OO, FDO
Data collection: SS, ZD, AES, DD
Analysis and interpretation of results: EFO, OE
Draft manuscript preparation: CT, DS
Critical revision of the article: ADB, AD
All authors (OO, FDO, SS, ZD, AES, DD, EFO, OE, CT, DS, ADB, AD) reviewed the results and approved the final version of the manuscript.

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