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Dynamic Thiol/Disulphide Homeostasis and Ischemic Modified Albumin Levels in Idiopathic Polyhydramnios

İdiyopatik Polihidramniyoz Olgularında Dinamik Tiyol/Disülfit Homeostazı ve İskemik Modifiye Albümin Seviyeleri

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Abstract

Aim: The aim of study was to determine whether idiopathic polyhydramnios is in relation with dynamic thiol-disulfide homeostasis and ischemia modified albumin levels or not.

Material and Method: In this prospective case- control study, a total of 126 participants were included. The patient group consisted of 56 patients who were diagnosed idiopathic polyhydramnios, and the control group consisted of 56 healthy normal pregnant. Native thiol (-SH), total thiol (-SH. -SS), dynamic disulfide (-SS), values from maternal serum were measured and compared between groups.

Results: 112 patients aged between 18-35 years, consisting of 56 idiopathic polyhydramnios and 56 control groups were included in the study. Maternal serum native and total thiol values were significantly higher in study group compared to control group (382.2±78.5 mmol/L vs. 331.8±43.9 mmol/L, p <0.001 and 435.2±76.2 mmol/L vs. 368.2±47.2 mmol/L, p<0.001). Disulphide / native thiol ratio and disulphide / total thiol ratio ratio was found to be statistically significantly higher (7.3±2.2 vs 5.5±0.9, p<0.001 and 6.3±1.7 vs 5.0±0.7, p<0.001), and native thiol/total thiol ratio ratio was significantly lower (87.4±3.4 vs 90.1±1.4, p<0.001) in control group. Mean cord blood ischemia modified albumin (IMA) was 0.69±0.02 Absorbance Unit, cord blood native thiol (SH) level 410.2±80.2, and cord blood total thiol level was 461.1±82.1 µmol/l in study group. All parameters except IMA and Native thiol / total thiol ratio were higher in cord blood samples of study group compared to control group.

Conclusion: The thiol/disulfide balance shifted towards anti-oxidative status in pregnancies complicated with idiopathic polyhydramnios compared to control group.

Keywords: IMA, polyhydramnios, thiol/disulphide homeostasis

Öz

Amaç: Bu çalışmanın amacı, idiyopatik polihidramniosun dinamik tiyol-disülfit homeostazı ve iskemi modifiye albümin düzeyleri ile ilişkisinin olup olmadığını belirlemektir.

Gereç ve Yöntem: Bu prospektif vaka kontrol çalışmasına toplam 126 katılımcı dahil edildi. Hasta grubunu idiyopatik polihidramnios tanısı alan 56 hasta ve kontrol grubunu 56 sağlıklı normal gebe oluşturdu. Native tiyol (-SH), total tiyol (-SH.-SS), dinamik disülfid (-SS), maternal serum değerleri ölçüldü ve gruplar arasında karşılaştırıldı.

Bulgular: Çalışmaya 56 idiyopatik polihidramnios ve 56 kontrol grubu olmak üzere 18-35 yaş arası 112 hasta dahil edildi. Maternal serum nativ ve total tiyol değerleri çalışma grubunda kontrol grubuna göre anlamlı derecede yüksekti (382,2±78,5 mmol/L - 331,8±43,9 mmol/L, p <0,001 ve 435,2±76,2 mmol/L - 368,2±47,2 mmol/ L, p<0.001). Disülfit/doğal tiyol oranı ve disülfür/toplam tiyol oranı istatistiksel olarak anlamlı derecede yüksek bulundu (7,3±2,2'ye karşı 5,5±0,9, p<0,001 ve 6,3±1,7'ye karşı 5,0±0,7, p<0,001) ve doğal tiyol/toplam tiol oranı oranı kontrol grubunda anlamlı olarak daha düşüktü (87,4±3,4'e karşılık 90,1±1,4, p<0,001). Çalışma grubunda ortalama kordon kanı iskemi modifiye albümin (İMA) 0,69±0,02 Absorbans Birimi, kordon kanı nativ tiyol (SH) düzeyi 410,2±80,2 ve kordon kanı total tiyol düzeyi 461,1±82,1 µmol/l idi. Çalışma grubunun kordon kanı örneklerinde İMA ve Native tiyol/toplam tiyol oranı dışındaki tüm parametreler kontrol grubuna göre daha yüksekti.

Sonuç: Kontrol grubu ile karşılaştırıldığında idiyopatik polihidramnios ile komplike olan gebeliklerde tiyol/disülfür dengesi anti-oksidatif duruma doğru kaymıştır.

Anahtar Kelimeler: İMA, polihidramnios, tiyol/disülfit homeostazı

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INTRODUCTION

Polyhydramnios prevalence ranges from 0.2% to 2.0%.[1] It is defined by either an amniotic fluid index (AFI) greater than 24 cm or a deep vertical pocket (DVP) greater than 8 cm.[2] Maternal diabetes mellitus, rhesus iso-immunization, congenital and chromosomal abnormalities and multiple gestation are the main maternal, fetal and placental conditions associated with polyhydramnios.[2] Idiopathic polyhydramnios, in as many as 70.0%, is the entity that is not associated with any etiological factor.[2]

Oxidation is a normal and necessary process that occurs in human metabolism. If there is an imbalance between reactive oxygen species (Oxygen-containing molecules with an unequal number of electrons) and antioxidants, metabolism can be complicated by lipid peroxidation, protein peroxidation and DNA damage.[3] Dynamic thiol-disulfide homeostasis (TDH) is reversal of thiol oxidation in proteins and represents the levels of thiols and disulfides. It is an important parameter associated with regulation of protein function, stabilization of protein structure, protection of proteins against irreversible oxidation of cysteine residues, chaperon function, regulation of enzyme functions and transcription.[4-6] Although, TDH has been studied in many hot topic of women's health, such as reproductive, gynecological pathologies, and obstetric pathologies. A growing body of evidence has demonstrated that TDH is involved in obstetric pathologies with unknown etiology such as preeclampsia, intrauterine growth restriction, oligohydramnios, abortus imminens, hyperemesis gravidarum and gestational diabetes.[7-10]

In this study, we aimed to measure serum dynamic thiol/disulfide hemostasis of third trimester idiopathic polyhydramnios cases whose etiology is unclear and to compare them with healthy pregnancies. This study is the first in the literature to examine the relationship of idiopathic polyhydramnios with thiol/ disulphide hemostasis.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 12.10.2022, Decision No: E2-22-2591). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of one-hundred and twelve, term, single pregnancies, who were followed up in our hospital were enrolled in this prospective case- control study. All of the pregnant women who participated in the study were given detailed information about the study and their informed consent was obtained.

Sample size of our study was calculated as 112 (56 patients in study and 56 patients in control group) with 80% confidence interval and $p<0.05$ significance level. 56 term pregnancies with amniotic fluid >8 cm in the deepest pocket or 24 cm quadrants in ultrasonography were included as the study group. The control group consisted of 56 term pregnancies with normal amniotic index (2-8 cm in one pocket or 5-24 cm in four quadrants). Patients with fetal intrauterine growth retardation, had an abnormality in chromosome screening tests or second trimester obstetric ultrasonography, with a history of maternal systemic disease, smoking, alcohol use, had a membrane rupture and/or a labor pain were not included in the study. Absence of fetal anomaly, absence of intrauterine growth retardation, no history of oligohydramnios and absence of pathology in doppler ultrasonography (patients with umbilical artery doppler systolic/ diastolic ratio >3) during the antenatal follow-up period were accepted as indicators that the fetus was not under chronic stress and chronic hypoxia.

2 ml blood samples were taken from the antecubital vein and 2 ml blood samples were taken from umbilical cord under sterile conditions through a vacutainer to an Ethylenediamine tetra acetic acid (EDTA)-free biochemistry tube at the time of delivery. Samples centrifuged at 4000 rpm for 10 minutes and stored stored at -80 C0 until the analyzing time.

Native thiol (SH), total thiol and disulfide (SS) levels in plasma were measured by a new and automatic method developed by Erel and Neşelioğlu[11] This method is the reduction of dynamic disulfide bonds to functional thiol groups with sodium borohydride (NaBH4). Formaldehyde was used to remove all unused NaBH4. This prevents further reduction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and reduction of disulfide bonds formed by the DTNB reaction. Total thiol content was measured with a modified Ellman reagent. Native thiol content was separated from total thiol content and it was observed that half of the difference obtained gave the amount of disulfide bond. In addition, disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios were also calculated automatically.[11]

Albumin Cobalt Binding Test was used to detect the presence of Ischemia Modified Albumin (IMA). Measurement of IMA levels was obtained using venous blood samples on admittance within 1 hour. Specimens were stored for 30 minutes at room temperature and then centrifuged at 3500 rpm for 5 minutes. Latter samples were transferred to Eppendorf tubes and stored at -80°C until analysis. This test was performed by adding 50 mL 0.1% cobalt (II) chloride ($CoCl_2,6H_2O$) (Sigma-Aldrich Chemie GmbH Riedstrasse 2, Steinheim, Germany) to the patient serum. After mixing, followed by 10 minutes of incubation to allow for albumin cobalt binding, 50 mL 1.5 mg/mL dithiothreitol was added. After mixing followed by 2 minutes of incubation, 1.0 mL of a 0.9% sodium chloride solution was added in order to reduce the binding capacity. The absorbance of samples was measured at 470 nm using a spectrophotometer. The results were expressed as absorbance units (ABSU).[12]

SPSS (Statistical Package for Social Sciences) for Windows version 22.0 software was used for the statistical analysis of the data obtained in our study. Distrubition was checked

by using the Shapiro Wilk and Kolmogorov-Smirnov test. Comparisons between groups of demographic and laboratory data of all participants in those with normal distribution were made with Student's t test. Non-normally distributed data were compared between groups using the Mann-Whitney U test. Categorical data were presented as numbers and percentages and compared with the Chi-square test. Pearson correlation analysis was used to determine the relationship between native and total thiol and some other continuous variables. Data are presented as mean±standard deviation and median (minimum-maximum) or number (percentage). The statistical significance level was accepted as p<0.05.

RESULTS

112 patients aged between 18-35 years, consisting of 56 idiopathic polyhydramnios and 56 control groups were included in the study. The mean age of 56 pregnant women in our study was 30.7±5.3 in the patient group and 27.8±4.9 in the control group. 17(31%) of patients in study group and 16(29.6%) of patients in control group were nulliparous (p=0.835). Total weight gained during pregnancy was 12.3±3.9 kg in the patient group; 11.1±3.2 kg in the control group (p=0.069). The characteristic features of the patients were shown in **Table 1**.

Table 1. Demographic characteristics of the subjects

	Polihidramnios (n=54)	Control group (n=54)	p
Maternal age	30.7±5.3	27.8±4.9	0.003
Nulliparity (n, %)	17 (31.5)	16 (29.6)	0.835
Maternal BMI (kg/m²)	31.2±4.7	26.6±4.2	<0.001
Gestational weight gain (kg)	12.3±3.9	11.1±3.2	0.069
Deepest vertical pocket (mm)	91±14	58±16	<0.001
Total AFI (mm)	275±37	164±52	<0.001
Gastational weeks at delivery	37.9±1.3	39.1±1.0	<0.001
Birthweight (g)	3503±575	3416±365	0.351
Apgar score 1st min <7 (n, %)	4 (7.4)	0	
Apgar score 5st min <7 (n, %)	0	0	

Maternal serum native and total thiol values were significantly higher in the idiopathic polyhydramnios group than control group (382.2±78.5 mmol/L vs. 331.8±43.9 mmol/L, p <0.001 and 435.2±76.2 mmol/L vs. 368.2±47.2 mmol/L, p<0.001). In the idiopathic polyhydramnios group when disulphide / native thiol ratio and disulphide / total thiol ratio ratio was found to be statistically significantly higher (7.3±2.2 vs 5.5±0.9, p<0.001 and 6.3±1.7 vs 5.0±0.7, p<0.001), native thiol / total thiol ratio ratio was significantly lower (87.4±3.4 vs 90.1±1.4, p<0.001). Maternal serum disulphide level in polyhydramnios group were higher compared to control group (26.5±6.1 vs 18.2±3.1, p<0.001). The IMA value was significantly lower in the IO group than control group (0.76±0.10 ABSU vs 0.68±0.06, p < 0.01) (**Table 2**).

Mean cord blood IMA was 0.69±0.02 Absorbance Unit (ABSU), cord blood native thiol (SH) level 410.2±80.2, and cord blood total thiol level was 461.1±82.1 µmol/l in study group. In the control group, cord blood IMA was 1.04±0.14 ABSU, cord blood native thiol (SH) level was 359.4±54.5 µmol/l and cord blood total thiol level was 397.6±57.9 µmol/l. All parameters except IMA and Native thiol / total thiol ratio were higher in cord blood samples of study group compared to control group (**Table 2**).

DISCUSSION

In this prospective case-control study, we examined the patients with idiopathic polyhydramnios who were in third trimester of pregnancy whether the etiology is in relation with TDH or not. The results of study revealed that the thiol/ disulfide balance shifted towards anti-oxidative status in cases with idiopathic polyhydramnios compared to healthy pregnant women with normal AFI. The high levels of antioxidant markers (native and total thiol) and low levels of oxidative markers (-SS/-SH and -SS/-SH, -SS and IMA levels) prove the presence of compensator mechanisms in idiopathic polyhydramnios. This is the first study which evaluates the thiol/ disulphide homeostasis in pregnancies complicated with idiopathic polyhydramnios.

Pregnancy itself is a state of oxidative stress. Increased metabolic activity, increased placental mitochondrial activity, and increased production of ROS during fetal growth can be listed as the main causative factors of oxidative status in normal pregnancy.[13] Superoxide anions produced by placental mitochondria are the most important source of ROS and lipid peroxidation.[14] Free

Table 2. Oxidative/ anti-oxidative markers in maternal serum and cord blood

	Maternal serum			Cord blood		
	Polyhydramnios (n= 54)	Control group (n= 54)	p	Polyhydramnios (n= 46)	Control group (n= 54)	p
Native Thiol (mean±SD)	382.2±78.5	331.8±43.9	<0.001	410.2±80.2	359.4±54.5	<0.001
Total Thiol (mean±SD)	435.2±76.2	368.2±47.2	<0.001	461.1±82.1	397.6±57.9	<0.001
Disulphide (mean±SD)	26.5±6.1	18.2±3.1	<0.001	25.5±4.2	19.1±3.1	<0.001
Native thiol / total thiol ratio	87.4±3.4	90.1±1.4	<0.001	88.7±2.5	90.3±1.4	<0.001
Disulphide / native thiol ratio	7.3±2.2	5.5±0.9	<0.001	6.4±1.6	5.4±0.8	<0.001
Disulphide / total thiol ratio	6.3±1.7	5.0±0.7	<0.001	5.7±1.2	4.8±0.7	<0.001
Ischemia modified albumin (mean±SD)	0.71±0.03	1.06±0.13	<0.001	0.69±0.02	1.04±0.14	<0.001

radicals originating from the placenta pass into the mother's circulation and undergo detoxification, and if there is not enough antioxidant activity, the placental ROS level rises. ROS amount rising in the placenta causes lipid, protein and DNA destruction, causing placental cell death and placental insufficiency. As a result of endothelial insufficiency induced by oxidative stress, pre-eclampsia, IUGR, preterm delivery, and recurrent pregnancy loss are more common.[15]

Antioxidant capacity, which increases as the gestational week increases, reaches its highest level in the third trimester. However, intense and continuous exposure to oxidative stress affects the placental antioxidation capacity and causes the consumption and reduction of antioxidants.[15] Oxidative stress is an important factor in many complications during the second and third trimester of pregnancy. Oxidative stress in pregnancy complicated with oligohydramnios has been explained based on two main mechanisms. According to the first hypothesis, inadequate extravillous trophoblast invasion could result in an imbalance of oxidant/antioxidant activity when antioxidant capacity does not keep pace with increased oxygen tension leading to a chronic state of oxidative stress. Second hypothesis explains the oxidative stress by intermittent maternal blood flow in the intervillous space resulting in ischemic-reperfusion damage. An extensive production of ROS leads to irreversible cellular dysfunction and tissue damage.[16] Intrestingly, TDH homeostasis in cases complicated with polyhydramnios without any etiological factor has not been questioned to date.

Our results showed clearly that the thiol/disulfide balance shifted towards anti-oxidative status in pregnancies complicated with idiopathic polyhydramnios compared to cases with normal AFI. The results raise the following question The principal theoretical implication of this study is that because of the predominance of antioxidants at the end of the 3rd trimester can be a result of regeneration phase following placental damage, and fetal renal perfusion switching to the compensatory polyuric/diuretic phase.

The generalisability of these results is subject to certain limitations. For instance, current study was limited by its sample size and designed in a single center. Randomised controlled trials with larger sample sizes could provide more definitive evidence.

CONCLUSION

All TDH parameters except disulphide levels and native thiol/total thiol ratio were higher in cases with idiopathic polyhydramnios. The evidence so far has proposed that thiol-disulfide homeostasis is an important issue and needs to be elucidated wholly. More research with larger sample sizes is needed to better understand the dynamic thiol/disulphide homeostasis and its role in pathophysiology of idiopathic polyhydramnios.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 12.10.2022, Decision No: E2-22-2591).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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