ORIGINAL ARTICLE

Is Serum Antioxidant Status Impaired in Pregnant Women at High Risk for Carrying a Down Syndrome-affected Fetus?

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ABSTRACT

Objective: *To establish the oxidant/antioxidant status in serum samples from pregnant women above the threshold for Down syndrome (DS) risk, according to the quadruple test.*

Methods: *Thirty maternal serum samples that were above the threshold for DS risk (study group) were chosen from pregnant women whose quadruple tests were studied at Ankara University İbni Sina Hospital Central Laboratory. They were matched with the control group consisting of 30 pregnant women whose DS risk were below threshold. Malondialdehyde level, glutathione peroxidase and non-enzymatic superoxide radical scavenger activities (NSSAs) were analyzed in the study and control groups.*

Results: *It was found that NSSA was significantly decreased in the study group as compared to the control group (*$p = 0.006$*). Malondialdehyde levels had a tendency to increase with gestational week in both groups (*$p = 0.042$ *in the study group and* $p < 0.001$ *in the control group).*

Conclusion: *There is a significant decrease in non-enzymatic antioxidant capacity in pregnant women that were above the threshold for DS risk, as compared to the control group. In the context of these results, dietary antioxidant supplementation might be a useful approach during early gestation, especially around the time of conception, possibly to prevent bearing a DS fetus.*

Keywords: Down syndrome, oxidant stress, pregnancy, prenatal screening.

INTRODUCTION

Down syndrome (DS) is a chromosomal disorder caused by the trisomy, translocation or mosaicism of chromosome 21 in humans. It appears in about 1 of every 700 live births and is the most frequent genetic cause of mental retardation (1, 2). Down syndrome patients often have cardiac malformations, premature aging, cataracts and growth retardation (3). Although the exact mechanism underlying DS is far from clear, over-expression of genes located in the 21st chromosome, resulting in the loss of chromosomal balance, is considered to be a determinant for DS phenotype (3, 4). The gene for Cu/Zn superoxide dismutase (SOD, EC 1.15.1.1), as an antioxidant enzyme, is known to be located on the long arm of the 21st chromosome (4). It has been demonstrated that SOD activity is increased in the serum samples of women carrying a DS-affected fetus (5). It has also been found that oxidant stress markers, including increased protein and lipid peroxidation, decreased glutathione (GSH) and thioredoxin levels, and induction of heat shock protein response, are increased in the amniotic fluids of pregnant women carrying DS-affected fetuses (2). Increased SOD activity, not compensated by glutathione peroxidase (GSH-Px, EC 1.11.1.9) and catalase (EC 1.11.1.6) activities, may lead to oxidative imbalance (6).

There are prenatal screening tests to determine the risk for carrying DS-affected fetuses. The quadruple test, one of the prenatal screening tests, calculates the fetal DS risk at term from maternal age at term and the concentration of four markers in maternal serum, namely alpha-fetoprotein (AFP), unconjugated estriol (uE_3), human chorionic gonadotropin (hCG) and inhibin-A, at 14–22 weeks of gestation (7).

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DOI: 10.7727/wimj.2015.178

Oxidative stress occurs when there is an imbalance between oxidant agents and antioxidant mechanisms. Free radical is a species which has one or more unpaired electrons and is known as an oxidant agent (8). Malondialdehyde (MDA), the lipid peroxidation end product, is one of the markers for oxidative stress (9). Superoxide, a free radical, is converted to H_2O_2 by SOD, and GSH-Px is an enzyme that plays a role in the reduction of H_2O_2 to water (10). There are also non-enzymatic factors playing a role in the scavenging of superoxide, known as non-enzymatic superoxide radical scavenger activity (NSSA) (11).

To the best of our knowledge, there is no study yet in the literature investigating the relation between maternal oxidant/antioxidant status and DS risk provided by the prenatal screening tests. For this reason, this study aimed to establish oxidant/antioxidant status in serum samples from pregnant women above the threshold for DS risk at term according to the quadruple test. It also aimed to determine the differences in oxidant/antioxidant status between low- and high-risk pregnancies for DS.

SUBJECTS AND METHODS

This study was approved by the Ethics Committee of Ankara University Faculty of Medicine (Decision date and number: December 26, 2011 and 42-896). All the pregnant women admitted to Obstetrics clinics of the Ankara University Faculty of Medicine for prenatal quadruple screening test between February 2012 and June 2012 were evaluated after taking their informed consent. Sample size was calculated *a priori* with the G*Power v.3.1.3 statistics program (12). There was a two-tailed α error probability value of 0.05 and assumption of 0.8 effect size with 0.8 power (1-β error probability), and sample size was calculated as 27 samples in each group with a total of 54 samples. The sample size was increased approximately 10% and accepted as 30 per group as a precaution for unpredicted losses. Patient age, body weight and gestational week were obtained from the quadruple test order forms. Inclusion criteria for the study were being between 18 and 37.1 years of age and having a singleton pregnancy. All of the others and the pregnant women who were smokers, diabetics using insulin, were pregnant by *in vitro* fertilization and with a risk found for trisomy 18 and neural tube defect above the threshold were excluded from the study. A total of 764 pregnant women were enroled during this period and 143 of them were excluded according to the mentioned criteria. Down syndrome risk at term above 1/270 according to the quadruple test was considered as a positive risk, and the test results of 30 pregnant women among all were above this level. These 30 pregnant women were chosen as the study group (high risk for having DS-affected fetus). For the control group (low risk for having DS-affected fetus), another 30 pregnant women whose age, body weight and gestational week matched the study group were chosen among the pregnant women given negative result for DS risk. Blood samples obtained in plain tubes were centrifuged to separate the serum. After separation of the serum they were divided into three different tubes, one for the routine quadruple test and the other two tubes were stored at −80 °C for the oxidant/antioxidant assays and for routine. For the quadruple test AFP, total hCG, uE_3 and inhibin A levels were measured in the serum by Beckman Coulter DxI 800 Immunoassay autoanalyser (13). The risk for DS was calculated by using Benetech Prenatal Risk Assessment (Benetech PRA) program (14).

For the evaluation of the oxidant/antioxidant status, MDA levels (oxidant parameter) and GSH-Px enzyme activities and NSSA (antioxidant parameters) were measured in the stored serum samples from the pregnant women. Malondialdehyde level (μmol/L) was measured by the thiobarbituric acid reactive substances (TBARS) method (15). Glutathione peroxidase activity (IU/L) was measured by following the changes in reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) absorbance at 340 nm (16). The extinction coefficient of NADPH was used for GSH-Px enzyme activity calculation. Non-enzymatic superoxide radical scavenger activity (mU/L) was measured by the method based on the nitro blue tetrazolium (NBT) reduction. One unit of NSSA was expressed as the substance amount causing 50% inhibition in the NBT reduction rate (11).

In the statistical evaluation of the results, SPSS 15.0 (Chicago, IL, USA) for Windows program was used (17). Independent two-sample *t*-test was used to determine the differences between the groups for age, body weight and gestational weeks. Mann–Whitney *U*-test was used to determine the differences between the groups for DS risk, MDA, GSH-Px and NSSA. In correlation analysis, Spearman test was used. *p*-Values < 0.05 were considered as significant.

RESULTS

Thirty-nine pregnant women were found to be at a high risk for carrying a DS-affected fetus, nine of which were then kept out of the study according to the exclusion criteria. Study group consisted of the remaining 30 pregnant women. Control group (n = 30) was randomly

chosen among the DS risk negative pregnant women (n = 591) after matching them to the study group for age and gestational week to eliminate the effects of these parameters on biochemical parameters.

The study and control groups consisted of 5 pregnant women between ages 18 and 25, 15 pregnant women between ages 26 and 33 and 10 pregnant women between ages 34 and 37. As for gestational week, the groups consisted of 4 pregnant women in 15th gestational week, 10 in 16th gestational week, 6 in 17th gestational week and 8 in 18th gestational week and 2 in the 19th gestational week. Comparison of demographic data between the groups is presented in Table 1. As shown in Table 1, there were no statistically significant differences in age, body weight and gestational week between the groups.

Median DS risk ratios of the study and control group were 1 in 113 and 1 in 17 500, respectively ($p < 0.001$). There were no statistically significant differences between the groups for MDA and GSH-Px. Non-enzymatic superoxide radical scavenger activity values of the study group were lower than that of the control group (2.224 vs. 4.786, respectively, $p = 0.006$; see Table 2).

The distribution of NSSA values is also presented in box plot graphic in the Figure.

Correlation analyses between DS risk, test parameters and demographic data in the study and control groups are shown in Table 3. Malondialdehyde levels were found to increase significantly in both study and control groups with gestational week ($p = 0.042$, $r = 0.37$ and $p < 0.001$, $r = 0.71$, respectively).

Table 1: Age, body weight and gestational week values of the pregnant women in the study and control groups (mean ± SD)

Parameters	Control group (n = 30)	Study group (n = 30)	Student's *t*-test
Age (year)	30.2 ± 4.5	30.4 ± 4.5	$p > 0.05$
Body weight (kg)	63.4 ± 9.8	62.9 ± 9.5	$p > 0.05$
Gestational week	17.2 ± 1.1	17.2 ± 1.2	$p > 0.05$

SD = standard deviation.

Table 2: Down syndrome risks of groups and oxidant/antioxidant parameters measured in serum samples obtained from the pregnant women in the study and control groups (median − lowest and highest values)

Parameters	Control group (n = 30)	Study group (n = 30)	Mann–Whitney *U*-test
DS risk[#]	1/17 500 (1/50 000–1/375)	1/113 (1/253–1/8)	$p < 0.001$*
MDA (µmol/L)	0.874 (0.618–1.559)	0.807 (0.645–1.479)	$p > 0.05$
GSH-Px (IU/L)	76.8 (33.6–124.8)	74.4 (28.8–192)	$p > 0.05$
NSSA (mU/L)	4.786 (1.869–9.923)	2.224 (0.148–12.207)	$p = 0.006$*

[#] Threshold value for high DS risk = 1/270 at term. * Statistically significant. DS = Down syndrome; MDA = malondialdehyde; GSH-Px = glutathione peroxidas; NSSA = non-enzymatic superoxide radical scavenger activity.

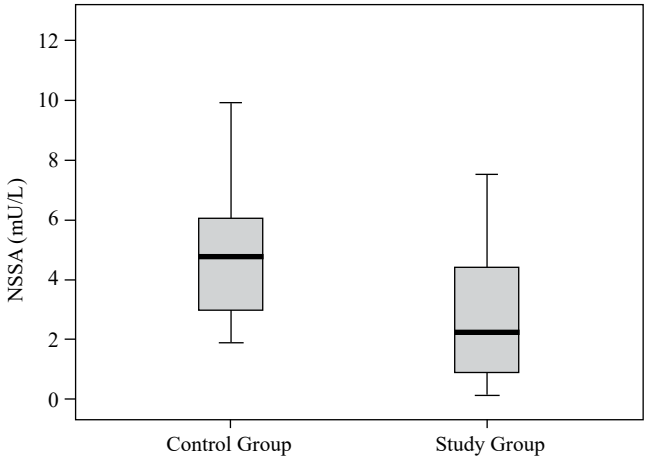

Figure: Distribution of NSSA values among the groups. NSSA = non-enzymatic superoxide radical scavenger activity.

Table 3: Crosstab of correlation analyses between DS risk, test parameters and demographic data in the study and control groups

		Study group						
		Age	Body weight	Gestation week	MDA	GSH-Px	NSSA	DS risk
Control Group	Age		$r = 0.55$; $p = 0.003$*	ns	ns	ns	ns	ns
	Body weight	ns		ns	$r = 0.47$; $p = 0.017$*	ns	ns	ns
	Gestation week	ns	ns		$r = 0.37$; $p = 0.042$*	ns	ns	ns
	MDA	ns	ns	$r = 0.71$; $p < 0.001$*		ns	ns	ns
	GSH-Px	ns	ns	ns	ns		ns	ns
	NSSA	ns	ns	ns	ns	ns		ns
	DS risk	$r = 0.40$; $p = 0.03$*	ns	ns	ns	ns	ns	

*Statistically significant according to Spearman test; ns: non-significant; MDA = malondialdehyde; NSSA = non-enzymatic superoxide radical scavenger activity; GSH-Px = glutathione peroxidas; DS = Down syndrome.

DISCUSSION

Down syndrome is the most common genetic cause of mental retardation in humans. It occurs 1 in approximately every 700 live births and DS incidence is higher at the time of conception. Over the past decades, several prenatal screening programmes, including the quadruple test, have been developed to detect pregnancies carrying DS-affected fetus (1, 7, 18, 19). Various clinical conditions, including intrauterine growth retardation, preeclampsia, gestational hypertension and gestational diabetes, have been found to be associated with oxidative stress during pregnancy (20–22). To the best of our knowledge, there is no study yet in the literature investigating the possible relation between oxidant stress and DS risk given by the prenatal screening tests. For this reason, we aimed in this study to establish oxidant/antioxidant status in serum samples from pregnant women above threshold for DS risk at term, according to the quadruple test, and to determine the differences in oxidant/antioxidant status between low- and high-risk pregnancies for DS.

Ognibene *et al* investigated SOD enzyme activity in serum samples from DS-affected pregnancies. The authors found that SOD enzyme activity increased significantly in DS- affected pregnancies as compared to unaffected controls. They concluded that the maternal serum SOD level might be added to the panel to improve the sensitivity of the prenatal screening programme (5). Perrone *et al* investigated isoprostan (IP) concentrations in amniotic fluids obtained from pregnant women with advanced maternal age. They compared IP levels in normal pregnancies and DS-affected pregnancies and found that IP concentrations increased nine-fold in amniotic fluids from DS-affected pregnancies as compared to the controls (4). In another study, Meguid *et al* investigated TBARS, vitamins B_{12}, C and E, homocysteine and folic acid in DS children and mothers bearing DS children compared with matched healthy controls. The authors found that mothers who had DS children had higher levels of TBARS and homocysteine and lower levels of folic acid and vitamin B_{12}, C and E as compared to the controls. They also found that DS children had lower levels of homocysteine, folic acid and vitamin B_{12}, C and E, as compared to healthy children. The authors concluded that abnormal folic acid and homocysteine metabolism is a maternal risk factor for DS children, and these mothers had an imbalance in their oxidant/antioxidant status (23). Sulthana *et al* found that reduced GSH in erythrocytes and total antioxidant status in plasma significantly decreased in children with DS, as compared to the healthy controls. They concluded that DS children have increased levels of oxidant stress and recommended antioxidant supplementations to decrease the morbidity in DS children (24).

In another study, Czeizel *et al* investigated the possible association between the use of nutritional supplements during the first gestational month and the origin of DS. They observed a significant preventive effect of folic acid and iron in pharmacological doses against DS. They also reported that the use of antioxidant vitamins was rare in the first month of pregnancy. The authors concluded that if the association between some genetic mutations in folic acid metabolism and DS incidence is assumed to be correct, the periconceptional use of large-dose folic acid and/or iron, together with antioxidants, might provide an opportunity for the primary prevention of DS (25).

In the context of this knowledge, how can our results be evaluated? First of all, the present study is the first one investigating the association between maternal oxidant/antioxidant status and DS risk given by the prenatal screening tests. The most striking finding of the present study is that NSSA decreased significantly in the pregnant women with high risk for bearing DS fetus, as compared to the controls. The decreased NSSA might indicate that the pregnant women with high risk were exposed to more oxidant stress and that, consequently, their antioxidant capacity was reduced. No data could be obtained in our study for the patho-physiological mechanism(s) and/or about non-enzymatic antioxidant(s) that are responsible for the decrease in NSSA. It has been shown previously that increased oxidant stress in pregnancy is associated with several clinical conditions (20–22). However, it is not well known what decreased antioxidant capacity in pregnancy is indicated principally. Although decreased NSSA in the high-risk group suggested an increase in oxidative stress, the observation that no difference was found in MDA levels between the groups indicated that oxidant stress did not occur severely enough to observe advanced lipid peroxidation reactions in serum. Additionally, there were no statistically significant differences in GSH-Px activities between the groups.

The decrease in NSSA in the high-risk group indicates that there is a reduction in non-enzymatic antioxidant capacity, and this finding is consistent with that of Meguid *et al* (23). Since NSSA shows non-enzymatic antioxidant capacity, we may suggest replacing it by giving pregnant women dietary supplements like antioxidant vitamins and/or foods rich in antioxidants. Our suggestion is in agreement with that of Meguid *et al* who

recommend giving mothers antioxidant supplements around the time of conception and that of Czeizel *et al* who advise antioxidant supplementation during the first trimester (23, 25).

Another important finding of our study is that there was a significant positive correlation between MDA levels and gestational week in both study and control groups (Table 3). This finding indicated that oxidant stress gradually increased in maternal serum during the second trimester. It is reported that pregnant women have significantly increased MDA levels as compared to non-pregnant women (26). We could not compare MDA levels with non-pregnant women because we did not have a non-pregnant control group, which is a weakness in the design of this study. Additionally, significant positive correlation between MDA levels and body weight in the high-risk group was observed. This finding is consistent with that of Prazny *et al* who suggest that obesity may contribute, by unknown mechanism(s), to oxidant stress expressed by MDA (27).

One of the weak points of the present study was being restricted to few oxidant and antioxidant parameters which make it difficult to evaluate the oxidant/antioxidant status completely. The scope of the study may be enriched by studying oxidation markers like 8-hydroxy-2′-deoxyguanosine, 8-isoprostane, nitrotyrosine and antioxidant markers like thioredoxin. Another shortcoming was the lack of knowledge of the nutritional status of the women during pregnancy. We did not have any data about food consumption and if there was any vitamin and/or antioxidant supplementation in progress. It can be suggested that larger and more comprehensive studies may be designed to overcome these weaknesses.

In conclusion, non-enzymatic antioxidant capacity in pregnant women with a high risk of bearing a DS-affected fetus decreased, compared to that of the controls. However, between the high- and low-risk groups, there was no significant change in the MDA level, which is an oxidant stress marker. However, the MDA level increased as gestational age increased during the second trimester in the high- and low-risk groups. In light of these findings, we think that it may be instructive to supplement all pregnant women with antioxidant vitamins and/or foods rich in antioxidants around the time of conception, to prevent the possibility of bearing a DS fetus.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Durak for his valuable suggestions during the analysis of oxidant/antioxidant status. The authors acknowledge the Obstetrics Outpatient Clinics of Ankara University Faculty of Medicine for filling out the prenatal screening information and order form. None of the authors have conflicts to disclose.

REFERENCES

1. Megarbane A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethore MO et al. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet Med 2009; **11:** 611–6.
2. Perluigi M, di Domenico F, Fiorini A, Cocciolo A, Giorgi A, Foppoli C et al. Oxidative stress occurs early in Down syndrome pregnancy: a redox proteomics analysis of amniotic fluid. Proteomics Clin Appl 2011; **5:** 167–78.
3. Zana M, Janka Z, Kalman J. Oxidative stress: a bridge between Down's syndrome and Alzheimer's disease. Neurobiol Aging 2007; 28: 648–76.
4. Perrone S, Longini M, Bellieni CV, Centini G, Kenanidis A, De Marco L et al. Early oxidative stress in amniotic fluid of pregnancies with Down syndrome. Clin Biochem 2007; **40:** 177–80.
5. Ognibene A, Ciuti R, Tozzi P, Messeri G. Maternal serum superoxide dismutase (SOD): a possible marker for screening Down syndrome affected pregnancies. Prenat Diagn 1999; **19:** 1058–60.
6. Jovanovic SV, Clements D, MacLeod K. Biomarkers of oxidative stress are significantly elevated in Down syndrome. Free Radic Biol Med 1998; **25:** 1044–8.
7. Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. Lancet 2003; **361:** 835–56.
8. Halliwell B, Gutteridge JMC, eds. Free radicals in biology and medicine. 3rd ed. Oxford, New York: Oxford University Press; 1999.
9. Jackson MJ. An overview of methods for assessment of free radical activity in biology. Proc Nutr Soc 1999; **58:** 1001–6.
10. McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000; **108:** 652–9.
11. Durak I, Canbolat O, Kacmaz M, Ozgen G, Ozturk HS. Antioxidant interferences in superoxide dismutase activity methods using superoxide radical as substrate. Clin Chem Lab Med 1998; **36:** 407–8.
12. Faul F (ed): G*Power. Version 3.1.3 ed. Germany: Universtadt Kiel; 2011.
13. Beckman Coulter Access Family of Immunoassay Systems Assay Inserts [CD-ROM]; 2006.
14. Benetech Prenatal Risk Assessment. Toronto, Canada: Benetech Clinical Software Solutions; 2008.
15. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch Biochem Biophys 1962; **98:** 253–61.
16. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; **70:** 158–69.
17. SPSS 15.0 for Windows. 15.0 ed. Chicago: SPSS Inc.; 2006.
18. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 2003; **7:** 1–77.
19. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984; **1:** 926–9.
20. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta 2009; **30:** S43–8.
21. Rogers MS, Wang CC, Tam WH, Li CY, Chu KO, Chu CY. Oxidative stress in midpregnancy as a predictor of gestational hypertension and pre-eclampsia. BJOG 2006; **113:** 1053–9.
22. Lappas M, Hiden U, Desoye G, Froehlich J, Hauguel-de Mouzon S, Jawerbaum A. The role of oxidative stress in the pathophysiology of gestational diabetes mellitus. Antioxid Redox Signal 2011; **15:** 3061–100.

23. Meguid NA, Dardir AA, El-Sayed EM, Ahmed HH, Hashish AF, Ezzat A. Homocysteine and oxidative stress in Egyptian children with Down syndrome. Clin Biochem 2010; **43:** 963–7.
24. Sulthana SM, Kumar SN, Sridhar MG, Bhat BV, Rao KR. Levels of non enzymatic antioxidants in Down syndrome. Indian J Pediatr 2012; **79:** 1473–6.
25. Czeizel AE, Puho E. Maternal use of nutritional supplements during the first month of pregnancy and decreased risk of Down's syndrome: case-control study. Nutrition 2005; **21:** 698–704.
26. Gohil JT, Patel PK, Gupta P. Evaluation of oxidative stress and antioxidant defence in subjects of preeclampsia. J Obstet Gynaecol India 2011; **6:** 638–40.
27. Prazny M, Skrha J, Hilgertova J. Plasma malondialdehyde and obesity: is there a relationship? Clin Chem Lab Med 1999; **37:** 1129–30.