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

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ABSTRACT

Objective: The present study aims to establish the oxidant/antioxidant status in serum samples from pregnant women above the threshold for Down syndrome risk according to the quadruple test.

Methods: Thirty maternal serum samples that were above 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 have been matched with control group consisting of 30 pregnant women whose DS risk were below threshold. Malondialdehyde (MDA) level, glutathione peroxidase (GSH-Px) and non-enzymatic superoxide radical scavenger activities (NSSA) were detected in all serum samples.

Results: It was found that NSSA was significantly decreased in the study group as compared to the control group (P=0.006). MDA 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

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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). DS 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 loss of chromosomal balance is considered to be 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, increased in 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).

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 threshold for DS risk at term according to the quadruple test. It was also aimed to determine the differences in oxidant/antioxidant status between low and high risk pregnancies for DS.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Ankara University Faculty of Medicine (Decision date & number: December 26, 2011 & 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 vas calculated *a priori* with the G*Power v.3.1.3 statistics program (12). Two tailed α error probability value of 0.05 and assumption of 0.8 effect size with 0.8 power (1-β error probability), sample size was calculated as 27 samples in each group with a total of 54 samples. The sample size was increased approximately 10 percent 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 the others and the pregnant women who were smokers, diabetics using insulin, having pregnancy by *in vitro* fertilization and risk found for trisomy 18 and neural tube defect above the threshold were excluded from the study. A total of 764 pregnant women were enrolled

during this period and 143 of them were excluded according to the mentioned criteria. DS risk at term above 1/270 according to 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), other 30 pregnant women whose age, body weight and gestational week matched to 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 serum. After separation of the serum they were divided into 3 different tubes, one for the routine quadruple test, and the other 2 tubes were stored at -80 $^{\circ}$C (for oxidant/antioxidant assays and repeat of routine if needed). For the quadruple test AFP, total hCG, uE_3 and inhibin A levels were measured in the serum by Beckman Coulter DxI 800 Immunoassay autoanalyzer (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. MDA level (μmol/L) was measured by the thiobarbituric acid reactive substances (TBARS) method (15). GSH-Px 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. NSSA (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 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, 9 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 15^{th} gestational week, 10 in 16^{th} gestational week, 6 in 17^{th} gestational week, 8 in 18^{th} gestational week and 2 in19^{th} gestational week. Comparison of demographic data between the groups is presented in the table 1. As shown in the table 1, there were no statistically significant differences in age, body weight and gestational week between the groups.

Table 1: Age, body weight and gestational week values of the pregnant women in 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

Median DS risk ratios of the study and control group were 1 in 113 and 1 in 17500 respectively (P<0.001). There were no statistically significant differences between the groups for MDA and GSH-Px. NSSA values of the study group were lower than that of the control group (2.224 vs. 4.786 respectively, P=0.006, table 2).

Table 2: DS risks of groups and oxidant/antioxidant parameters measured in serum samples obtained from the pregnant women in study and control groups [Median (lowest and highest values)].

Parameters	**Control group (n=30)**	**Study group (n=30)**	**Mann-Whitney U test**
DS risk[#]	1/17500 (1/50000-1/375)	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

The distribution of NSSA values is also presented in box plot graphic in the figure 1.

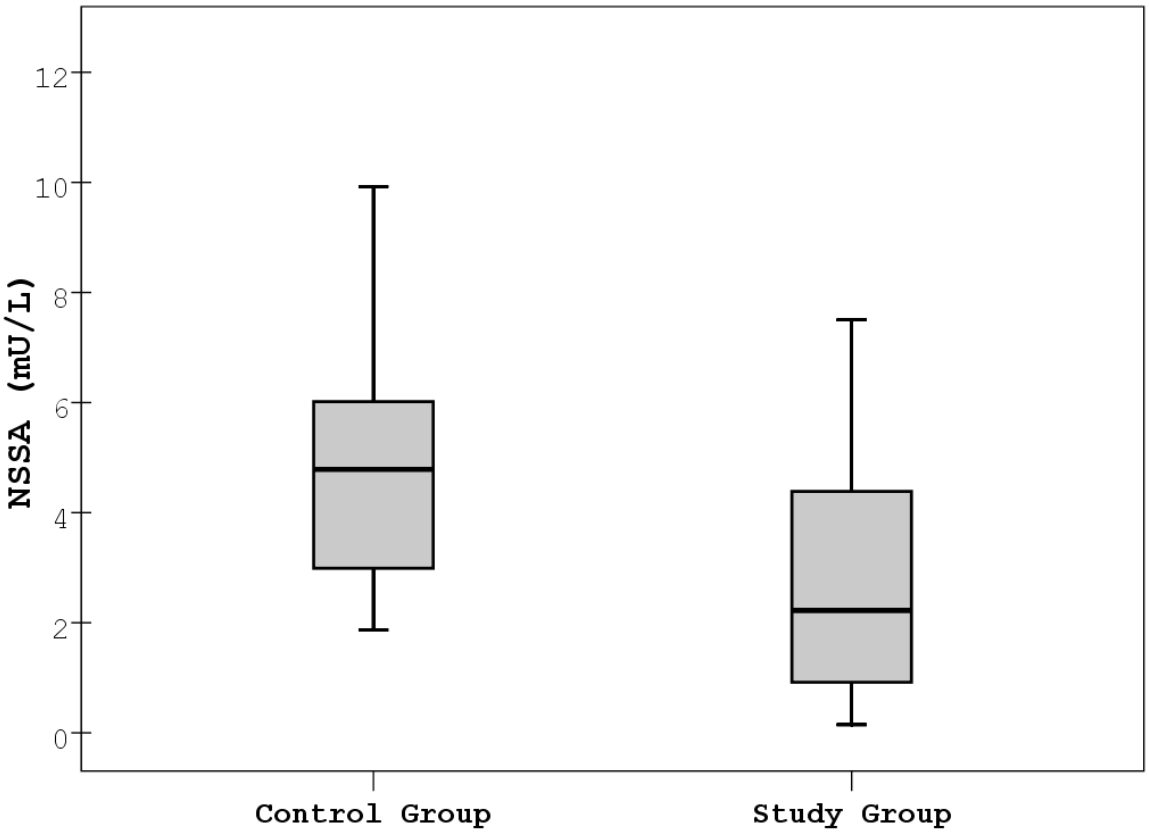

Figure: Distribution of NSSA values among the groups.

Correlation analyses between DS risk, test parameters and demographic data in the study and control groups are given in the table 3. MDA 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 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*	

Grey: Study group; White: Control group; * Statistically significant according to Spearman test; ns: non-significant

DISCUSSION

DS is the most common genetic cause of mental retardation in humans. It occurs one in approximately every 700 live births and DS incidence is higher at the time of conception. Over the last decades several prenatal screening programs 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 (IUGR), 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 maternal serum SOD level might be added to the panel to improve the sensitivity of the prenatal screening program (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-folds 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 comparatively 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 glutathione 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 literature 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 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 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*. recommending to give mothers antioxidant supplements around the time of conception and that of Czeizel *et al*. advising antioxidant supplementation during first trimester (23, 25).

Another important finding of our study is that there was 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 according 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 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 as compared to that of the controls. But, between the high and low risk groups there was no significant change in MDA level which is an oxidant stress marker. However, MDA level increased as gestational age increased during second trimester in the high and low risk groups. In the light of these findings, we think that it may be useful to supplement all pregnant women with antioxidant vitamins and/or foods rich in antioxidants around the time of conception possibly to prevent bearing a DS fetus.

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