

## **Plasma antioxidant enzyme levels in smoker and non-smoker pregnant women and their correlation with uterine arterial Doppler parameters.**

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### **Abstract**

**Introduction:** Cigarettes contain many free radicals and cause oxidative stress in the body. Its contents decrease blood fluidity and blood stream by affecting the vascular endothelium; further, they directly or indirectly impair placental perfusion.

**Material and method:** Catalase (CAT), malondialdehyde (MDA) and total sulfhydryl (SH) levels were analysed in venous blood specimens obtained from 49 smoker and 35 non-smoker pregnant women (age, 18-35 y) who were in the second trimester. Uterine arterial Doppler ultrasonography was performed to evaluate uterine perfusion. Pulsatile index (PI), resistance index (RI) and systole/diastole ratio (S/D) was measured. The smoker group was further subdivided into two groups: 1. those who smoked for <5 y and 2. Those who smoked for >5 y. Obtained data were analysed using the SPSS 11 statistical programme.

**Results:** No significant differences were observed between the smoker and non-smoker groups in terms of demographic parameters and right-left uterine arterial Doppler parameters. The mean S/D ratio was significantly higher in the smoker group (P=0.01). The mean PI, mean RI and plasma MDA, CAT and total SH levels were similar in both groups. Although not statistically significant, the mean PI, RI and S/D ratio values of the smoker pregnant women who smoked for  $\geq 5$  y was found to be higher than of those who smoked for <5 y.

**Conclusion:** There is no correlation between plasma antioxidant enzyme levels and uterine artery Doppler parameters; however, the duration of smoking may affect uterine perfusion.

**Keywords:** Cigarette, Oxidative stress, Uterine artery, Pregnancy.

*Accepted on November 8, 2018*

### **Introduction**

Oxidative stress (OS) is caused by the imbalance between pro-oxidants and antioxidants [1,2]. Smoking is an external source of free radicals as a factor of oxidative stress. One puff of cigarette smoke generates 1014 and 1015 free radical molecules from the tar and gas phases, respectively [3]. High levels of free radical uptake initiate directly and indirectly lipid peroxidation in the body and increase endothelial damage caused by lipid peroxidation in smokers [4].

Placental perfusion depends on blood flow from the ovarian and uterine arteries. Uteroplacental blood flow continuously increases throughout pregnancy [5]. It is unclear as to which factors induce uterine blood flow in the first two thirds of pregnancy. The increase in maternal placental blood flow is mainly attributed to vasodilatation, whereas that in foetal-placental blood flow is attributed to the continuous increase in placental vessels [6].

Increasing oxidative stress is the direct effect of cigarette smoking. The antioxidant defense system is the next effect of smoking [7]. It causes endothelial damage, leading to the development of atherosclerosis and increased blood pressure [8]. It causes malnutrition in tissues because it reduces blood fluidity [9].

Many authors have suggested that nicotine receptors play a role in pathologies caused by cigarette smoking, but the effect of OS is recently attracting attention. Evidence suggests that smoking causes maternal and fetal OS [10].

Our study aimed to evaluate antioxidant enzyme levels and uterine artery Doppler parameters in smoker and non-smoker pregnant women and determine whether there is any correlation between these parameters.

## Material and Methods

Totally, 49 smoker and 35 non-smoker pregnant women (age, 18-35 y) who applied to our centre between October 2004 and December 2005 and were in the second trimester were included in the study. Women aged between 18-35 y, women with an at least 2 y gap between pregnancies and women who did not smoke during their previous and present pregnancies were included in the non-smoker group; women who smoked before their pregnancy and continued to smoke at least thrice a day during their present pregnancy were included in the smoker group. Patients with endocrine disease who were diagnosed before pregnancy, patients with systemic disease that may affect uteroplacental blood flow and antioxidant enzyme levels, and chronic drug users were not included in the study. Age, last menstrual period, gestational week, gravidae, parity, resume and family history of each pregnant woman were reviewed and recorded. Smoking time and the number of cigarettes smoked per day were included in the patient information form. All pregnant women were examined, and examination reports according to their gestational weeks were requested.

The smoker pregnant women were further subdivided into two groups: 1. those who smoked for <5 y and 2. Those smoked for  $\geq 5$  y.

### Collection and storage of plasma samples

5 ml of peripheral venous blood samples from pregnant women from the EDTA tube were taken with 10 minutes centrifugation at 3000 rpm 4°C (1000 NF Core R Turkey). Obtained plasmas were stored at -80°C. When the number of suitable samples is reached enough, it is dissolved in the room heat collectively.

### Measurement of plasma MDA levels

Yagi's method was modified and used to measure the plasma MDA levels [11]. MDA concentrations obtained from the graph plotted using the standards were calculated in nmol/ml.

### Measurement of total SH levels

The method reported by Sedlak et al. was modified and used [12]. The results obtained from the graph plotted using the standards were calculated in mmol/L.

### Measurement of plasma catalase levels

Reagents (27 mM H<sub>2</sub>O<sub>2</sub>) were prepared in phosphate buffer (50 mM, pH 7). Samples were analysed using a spectrophotometer (Shimatzu UV-1601) at 240 nm with the reagent warmed to 37°C. Absorbance values were obtained at 0 and 2 min. Decreasing H<sub>2</sub>O<sub>2</sub> absorbance values were calculated (in U/ml) from the molar absorptivity of H<sub>2</sub>O<sub>2</sub>.

### Evaluation of uterine artery Doppler in patients

To evaluate uterine perfusion in the pregnant women, the uterine artery blood flow was evaluated by Doppler

ultrasonography, and PI, RI and S/D ratio was recorded. Using a 3.5 MHz convex probe with General Electric's Brand 'Logiq 9' (Milwaukee, USA) model Doppler ultrasonography device with harmonic features by the Radiology Department, the blood flow waveforms at the region where both uterine arteries cross the external iliac arteries were evaluated by the same specialist, and PI, RI and S/D ratio were recorded.

### Statistical analysis

Statistical analyses were performed using the SPSS 11 statistical programme. Plasma enzyme values and uterine artery Doppler parameters of smoker and non-smoker pregnant women were compared using the Student's t-test. The right and left uterine artery Doppler ultrasonography results of the study and control group were compared using the paired t-test. Pearson correlation analysis was applied for correlating data for the smoker pregnant group.  $p < 0.05$  was considered statistically significant.

## Results

49 smokers and 35 non-smokers were included in the study. The demographic characteristics of the study and control group are shown in Table 1. There was no significant difference between the smokers 'and non-smokers' mean age, gravidity, parity and gestational weeks (GW).

**Table 1.** Distribution of study and control group according to demographic parameters.

	Smokers group (N=49) (Mean $\pm$ SD)	Non-smokers group (N=35) (Mean $\pm$ SD)	P value
Age	26.5 $\pm$ 4.2	26.6 $\pm$ 4.5	0.92
Gravidity	2.1 $\pm$ 0.9	2.4 $\pm$ 1.5	0.22
Parity	0.9 $\pm$ 0.7	1.1 $\pm$ 1.1	0.27
GW	18.0 $\pm$ 2.5	17.1 $\pm$ 2.7	0.12

The mean S/D ratio in smoking pregnant group was significantly higher than the non-smoking pregnant group ( $P=0.016$ ) (Table 2). Mean PI and mean RI values were similar in smokers and non-smokers.

**Table 2.** Mean uterine artery Doppler parameters of study and control group and plasma enzyme levels of the study and control group.

	Smokers group (N=49) (Mean $\pm$ SD)	Non-smokers group (N=35) (Mean $\pm$ SD)	P value
Mean PI	1.05 $\pm$ 0.45	1.16 $\pm$ 0.54	0.319
Mean RI	0.60 $\pm$ 0.15	0.64 $\pm$ 0.24	0.300
Mean S/D	2.52 $\pm$ 0.64	2.19 $\pm$ 0.53*	0.016
MDA (nmol/L)	5.98 $\pm$ 7.67	5.76 $\pm$ 3.84	0.87
T-SH (mmol/L)	0.55 $\pm$ 0.69	0.43 $\pm$ 0.04	0.23

PI: Pulsate Index; RI: Resistance Index; S/D: Systole/Diastolic Ratio; \* $P < 0.05$ : statistically significant.

## Plasma antioxidant enzyme levels in smoker and non-smoker pregnant women and their correlation with uterine arterial Doppler parameters

As seen in Table 2, there was no significant difference in plasma MDA levels between the study and control groups.

25 of the 49 women who smoked were found to be smoking less than 5 y, 24 of them were using 5 y or more of smoking cigarettes (Table 3).

**Table 3.** Average uterine artery Doppler parameters according to smoking durations and Plasma MDA, SOD and catalase enzyme levels (Mean  $\pm$  SD).

Duration smoking	of <5 y	>5 y	P value
	N=25	N=24	
Mean PI	1.11 $\pm$ 0.56	0.98 $\pm$ 0.29	0.72
Mean RI	0.59 $\pm$ 0.18	0.61 $\pm$ 0.11	0.76
Mean S/D	2.49 $\pm$ 0.59	2.55 $\pm$ 0.70	0.76
MDA	6.37 $\pm$ 10.34	5.59 $\pm$ 3.29	0.72
T-SH	0.69 $\pm$ 0.95	0.41 $\pm$ 0.06	0.15
CAT	441.44 $\pm$ 196.71	536.83 $\pm$ 195.72	0.09

**Table 4.** Correlation between plasma antioxidant enzyme levels and uterine artery Doppler parameters in smokers and non-smokers (*r* (p)). *r*: correlation coefficient.

	MDA	T-SH	CAT
	<i>r</i> (p) value	<i>r</i> (p) value	<i>r</i> (p) value
Smokers group PI	0.16 (p=0.27)	0.20 (p=0.15)	0.22 (p=0.12)
Non-smokers group PI	0.10 (p=0.54)	0.15 (p=0.36)	0.03 (p=0.84)
Smokers-group RI	0.03 (p=0.78)	0.06 (p=0.68)	0.04 (p=0.75)
Non-smokers group RI	0.14 (p=0.40)	0.0191 (p=0.26)	0.037 (p=0.82)
Smokers group S/D	0.21 (p=0.13)	0.12 (p=0.56)	0.16 (p=0.26)
Non-smokers group S/D	0.11 (p=0.51)	0.08 (p=0.48)	0.22 (p=0.19)

No significant correlation was found between plasma antioxidant enzyme levels and uterine artery PI, uterine artery RI, uterine artery S/D ratio (Table 4) in smokers and non-smokers.

## Discussion

The antioxidant enzyme concentration and activity in human placenta are increasing in proportion to gestational age [13]. In the study conducted in 2016, it has been shown that smoking during pregnancy negatively affects the oxidant/antioxidant balance [14]. Studies on the toxicity of cigarette smoke are mostly focused on oxidative stress [15,16]. In a study conducted by Zhou et al., smoking has been shown to reduce SOD, CAT and glutathione peroxidase (GSH-Px) enzyme activity [17].

The oxidative stress increases and the antioxidant defense system works more and more. The increase in total serum lipid

is accompanied by an increase in MDA [18].

The effect of cigarette on placental perfusion remains unclear [19]. In patients with pathologic uterine perfusion, oxidative stress markers may be used in addition to clinical Doppler studies. Plasma antioxidant capacities of 25 pregnancies between 18-23 w with pathologic uterine perfusion were found to be lower than those of normal uterine perfusion ( $p < 0.05$ ) [20]. In this study, the mean S/D ratio in the smoker pregnant group was significantly higher than the non-smoker pregnant group ( $P = 0.01$ ). Mean PI and mean RI values were similar in smokers and non-smokers.

2459 non-smokers and 248 smokers were included in the 20<sup>th</sup> gestational week, the umbilical artery RI and the mean uterine artery RI were higher in smokers [21]. In another study in which smokers and pregnant smokers were evaluated, significant changes were observed in maternal circulation parameters associated with nicotine, and it was understood that uterine blood flow values did not change [22]. In our study, there was no correlation between antioxidant enzyme levels and MDA level, which is an oxidation indicator, and uterine artery Doppler parameters.

Albuquerque et al. [23], it has been shown that the uterine artery RI value may be related to the duration of smoking and the amount of cigarettes. In the research conducted in 2009; smokers; it was found that serum nitric oxide concentrations in maternal and infant mothers who smoked during pregnancy were associated with the number of cigarettes consumed per day when separated by two groups of smokers who smoked 5 per day for 2 years before conception and continued to drink during pregnancy [24]. In a study of third trimester pregnancies smoking was not shown to be directly related to the hemodynamics of the uterine artery [25].

Although not statistically significant, the mean PI, RI and S/D ratio values of those who smoked for >5 y were found to be higher than of those who smoked for <5 y. The plasma MDA, SOD and CAT enzyme levels of those who smoked for <5 y and >5 y were found to be similar.

## Conclusion

There is no correlation between plasma antioxidant enzyme levels and uterine artery Doppler parameters; however, the duration of smoking may affect uterine perfusion.

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