

# Investigation of antioxidant enzyme levels in maternal and fetal erythrocytes in pregnant with preeclampsia\*

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## ÖZET

**Amaç:** Serbest radikaller tarafından oluşturulan lipid peroksidasyonun, preeklampside olası patolojik faktörlerden biri olduğu ileri sürülmektedir. Bu çalışmada; preeklampsili hastalarda gebeliğin üçüncü trimesterinde, maternal ve fetal dolaşımdaki eritrositlerin antioksidan enzim aktivitelerini ölçmede amaçlanmıştır. **Gereç ve Yöntem:** İlk hamileliğinin üçüncü trimesterinde olan 30'u preeklampsili, 31'i normal tansiyona sahip gebe kadınlardan alınan maternal ve umbilikal ven kanlarında plazma ve eritrosit içi glutatyon Peroksidaz (GSH-Px), Katalaz (CAT) ve Superoksit Dismutaz (SOD) aktiviteleri incelendi. **Sonuçlar:** Kontrol grubu ile preeklampsili hasta grubu arasında; yaşları ve gebelik haftası açısından bir fark bulunmamaktaydı ( $p>0.05$ ). Preeklampsili hastaların maternal dolaşımında; eritrosit içi GSH-Px ve SOD düzeylerinin kontrol grubuna göre daha yüksek olduğu (sırasıyla  $1857\pm13.2$ 'e karşı  $1387\pm123.8$  U/gHb ve  $2593.2\pm330.7$ 'ye karşı  $2041\pm200.3$  U/gHb;  $p<0.01$ ), CAT düzeyinin ise daha düşük olduğu ( $71.2\pm18.1$ 'e karşı  $137.3\pm27.1$  K/gHb;  $p<0.01$ ) tespit edilmiştir. Umbilikal dolaşımında, eritrosit içi SOD düzeyinin, preeklampsili hastalarda, kontrol grubuna oranla daha yüksek olduğu ( $18.18.5\pm151.5$ 'e karşı  $1535.8\pm169.2$  U/gHb;  $p<0.01$ ), buna karşılık CAT ve GSH-Px düzeyleri açısından aralarında bir fark bulunmadığı gözlenmiştir (sırasıyla  $84.8\pm14.3$ 'e karşı  $97.1\pm31.8$  K/gHb ve  $1207.5\pm117.5$ 'e karşı  $1211.4\pm107.7$  U/gHb;  $p>0.05$ ). İstatistiki incelemelerde bağımsız t testi kullanılmış ve  $p<0.05$  değeri anlamlı olarak kabul edilmiştir. **Tartışma:** Bu çalışmadan elde edilen veriler, preeklampsili gebelerde artan oksidatif stresin, hem maternal hemde fetal dolaşımda; reaktif oksijen türlerine karşı koruyucu bir savunma mekanizmasının gelişmesi ile sonuçlandığını göstermektedir. CAT ise preeklampsisi vakalarında gelişen bu koruyucu etkiye katkıda bulunmamaktadır.

**Anahtar Kelimeler:** Preeklampsisi, eritrosit, antioksidan enzimler.

## SUMMARY

**Investigation of antioxidant enzyme levels in maternal and fetal erythrocytes in pregnant with preeclampsia**

**Aim:** Free radical induced lipid peroxidation has been suggested as a possible pathogenic factor of preeclampsia. In this study, we were aimed to measure antioxidant enzyme activity in erythrocytes in maternal and fetal circulation in preeclamptic patients in the third trimester. **Material and metod:** Maternal and umbilical venous blood were obtained from thirty preeclamptic and thirty-one normotensif women with singleton pregnancy in the third trimester. The activities of glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) were determined in plasma and erythrocytes. **Results:** Patients ages and gestational weeks were not different in both groups ( $p>0.05$ ). In maternal erythrocytes of patients with preeclampsia, whilst GSH-Px and SOD levels were significantly higher than control ( $1857\pm13.2$  vs  $1387\pm123.8$  U/gHb and  $2593.2\pm330.7$  vs  $2041\pm200.3$  U/gHb;  $p<0.01$ , respectively), CAT levels were significantly lower ( $71.2\pm18.1$  vs  $137.3\pm27.1$  K/gHb;  $p<0.01$ ). Although, SOD levels in umbilical erythrocytes of patients with preeclampsia were significantly higher than control ( $18.18.5\pm151.5$  vs  $1535.8\pm169.2$  U/gHb;  $p<0.01$ ), CAT and GSH-Px levels were not different ( $84.8\pm14.3$  vs  $97.1\pm31.8$  K/gHb and  $1207.5\pm117.5$  vs  $1211.4\pm107.7$  U/gHb;  $p>0.05$ , respectively). Independent t test was used for statistical analysis.  $p<0.05$  was accepted as significant. **Conclusion:** The results demonstrate that increase in oxidative stress in preeclampsia results in development of defence mechanism both in maternal and fetal circulation to protect against reactive oxygen species. CAT has no impact on this protective effect in preeclampsia.

**Key Words:** Preeclampsia, erythrocyte, antioxidant enzymes.

Preeclampsia is associated with increased vascular reactivity and vasoconstriction with endothelial damage and dysfunction underlying the pathologic fe-

atures of this disorder. It is diagnosed primarily by the onset of hypertension and proteinuria in the latter half of gestation. Other manifestations of preeclampsia

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include reduced perfusion to organ and platelet activation (1). However, the etiology and pathophysiology of preeclampsia are poorly understood.

In recent years, much interest has been focused on the biological significance of (pygen Free Radicals (OFRs). Oxygen free radicals include superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $\cdot OH$ ). Superoxide anion is catalyzed to  $H_2O_2$  by SOD present in the biological system.  $H_2O_2$  is then catalytically reduced in the  $H_2O$  by CAT and GSH-Px. Thus, all OFRs are detoxified by these antioxidant enzymes.

In the normal physiologic, OFRs generation occur at low levels in tissues and also there is a balance between OFRs production and antioxidant enzyme activities. But, in several pathological situations such as diabetes, ischemia-reperfusion, and sepsis, OFRs concentration was increased as a result of an imbalance between the generation and inactivation of these oxygen free radical species (2). The overproduction of OFRs may cause detrimental effects on the cells. The most important detrimental effects of the OFRs are to lead to lipid peroxidation and formation of toxic reactive products, which may be involved in endothelial damage and dysfunction (3-5). OFRs-induced lipid peroxides have been shown to be increased in patients with preeclampsia because of overproduction of OFRs and/or insufficient antioxidant enzyme activities (6-7).

Red blood cells (RBCs) are particularly sensitive to OFRs and, like other cells of aerobic organism, RBCs are supplied with antioxidant defence mechanisms in order to be protected from the toxic actions of OFRs. The important defence mechanism in erythrocytes is the antioxidant enzymes such as SOD, CAT and GSH-Px (3).

In this study, it was aimed to investigate the levels of plasma and erythrocyte SOD, CAT and GSH-Px activities in maternal and fetal circulation of pre-eclamptic and normal pregnant in the third trimester.

#### **MATERIALS AND METHODS**

We included 30 women with preeclampsia (Pre-eclamptic group,  $27 \pm 5$  yr) in this study. None of the women studied had evidence of any active or potential infective process, such as urinary tract infection or chorioamnionitis. Fasting blood samples were withdrawn into evacuated heparinized tubes

from the antecubital vein. Maternal venous blood samples were taken at the same gestational age in both groups. Umbilical venous blood was drawn at the cord root immediately after delivery of the placenta. After centrifugation (3000 rpm,  $4^\circ C$ , 10 min) the obtained plasma was frozen at  $-50^\circ C$  until assay. The erythrocytes were subsequently washed twice with 2 volumes of 0.9 % sodium chloride solution. Following this, the erythrocytes were hemolysed with a twofold volumes of cold distilled water. After centrifugation (5000 rpm,  $4^\circ C$ , 10 min) the supernatant was frozen at  $-50^\circ C$  until assay. The plasma and supernatants were used to measure antioxidant enzymes activities.

SOD activity in plasma and supernatant was measured according to the method of Sun et al. (8) by determining the reduction of nitro blue tetrazolium (NBT) by super oxide anion produced with xanthine and xanthine oxidase. One unit of SOD was defined as the amount of protein or hemoglobin that inhibits the rate of NBT reduction by 50 %. Results were defined as units per gram protein or hemoglobin (U/g protein or g Hb).

CAT activity in supernatant was determined according to the method of Aebi (9) by monitoring the initial rate of disappearance of hydrogen peroxide (Initial concentration 10 mmol) at 240 nm in a spectrophotometer. Results were reported as constant rate per second per gram hemoglobin (K/g Hb).

GSH-Px activity in plasma and supernatant was measured according to Paglia and Valentine (10) by monitoring the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. Enzyme units were defined as the number of micromoles of NADPH oxidised per minute. Results were defined as units per gram protein or hemoglobin (U/g protein or g Hb).

Independent t test was used in statistical analysis of results with 2-tailed  $p < 0.05$  considered statistically significant. Data were reported as mean  $\pm$  standard deviation (SD) with 95 % confidence intervals.

#### **RESULTS**

The levels of plasma and erythrocyte SOD, CAT and GSH-Px activities are shown in table 1.

GSH-Px and SOD activities were significantly higher ( $1857 \pm 131.2$  vs  $1387 \pm 123.8$  U/g Hb and  $2593.2 \pm 330.7$  vs  $2041 \pm 200.3$  U/g Hb,  $p < 0.01$  res-

**Table 1.** Plasma and erythrocyte SOD, CAT and GSH-Px levels in preeclamptic and control groups.

Groups	SOD (U/g protein or Hb)	CAT (K/g Hb)	GSH-Px (U/g protein or Hb)
<b>Preeclamptic group (n=30)</b>			
Plasma (Maternal)	83.6±33.2		245.6±17.8*
Plasma (Fetal)	76.8±23.5*		150.4±19.1
Erythrocyte (Maternal)	2593.2±330.7*	71.2±18.1*	1857±131.2*
Erythrocyte (Fetal)	1818.5±151.5*	84.8±14.3	1207.5±117.5
<b>Control group (n=31)</b>			
Plasma (Maternal)	72.6±21.3		179.1±15.7
Plasma (Fetal)	51.1±14.9		149.8±11.8
Erythrocyte (Maternal)	2041.0±200.3	137.3±27.1	1387.9±123
Erythrocyte (Fetal)	1535.8±169.2	97.1±31.8	1211.4±103.7

\* Statistically significant (p<0.05) compared with control group.

pectively), whereas CAT was significantly lower (71.2±18.1 vs 137.3±27.1 K/g Hb, p<0.01) in erythrocyte of preeclamptic patients compared with control group. In umbilical erythrocytes, SOD was significantly higher (1818.5±151.5 vs 1535.8±169.2 U/g Hb, p<0.01), whereas CAT and GSH-Px were not different (84.8±14.3 vs 97.1±31.8 K/gHb and 1207.5±117.5 vs 1211±103.7 U/g Hb, p>0.05; respectively).

Maternal plasma GSH-Px levels were found to be higher in preeclamptic patients compared with control group (245.6±17.8 vs 179.1±15.7 U/g protein, p<0.05), whereas SOD was not different (83.6±33.2 vs 72.6±21.3 U/g protein, p>0.05). In preeclamptic group, umbilical plasma SOD levles was significantly higher (76.8±23.5 vs 51.1±14.9 U/g protein, p<0.05) than in control group, but GSH-Px was similar in both groups (150.4±19.1 vs 149.8±11.8 U/g protein, p>0.05).

## DISCUSSION

The etiology of preeclampsia still remains obscure.

However, oxidative stress, increased Oxygen Free Radicals (OFRs) production, has been implicated as a pathophysiological feature of women with preeclampsia (11). The effect of oxidative stress on vascular endothelial function is still not well defined. But, endothelial cell disfunction is the final common pathway in the pathophysiology of preeclampsia (12). It is known that endothelial cells (EC) are exposed to OFRs from both intracellular sources and products in the circulation.

The intracellular sources of OFRs are xanthine-xanthine oxidase system, activated polymorphonuclear leukocytes such as neutrophil, autooxidation of catecholamines, and arachidonic acid metabolism. The activity of xanthine oxidase, produces a large quantities of OFRs source in preeclampsia is neutrophils. Neutrophils are activated during prolonged period of reduced perfusion in preeclampsia (12, 13). In respons to this activation, neutrophils produce a large quantities of superoxide anion and hydrogen peroxide that can be diffuse ea-

sily into blood stream and surrounding tissues (14). OFRs, which is generated during reduced perfusion or by activated neutrophils, are known as a mediator of tissue injury.

Essentially, the toxic effects of OFRs, occur via lipid peroxidation. Because, increased OFRs can directly and indirectly initiate and propagate the process of lipid peroxidation (LPO) following by endothelial dysfunction which is play an important role in the pathogenesis of preeclampsia (5). LPO cause cell membrane damage, increased platelet aggregation, stimulation of mitogen and growth factor, inactivation of some enzymes and activation of proteases such as elastase (15, 17). Another important action of LPO is that they cause enhanced adhesion and activation of neutrophils to generate superoxide anion and hydrogen peroxide (12).

In the literature, there are several studies that show increased OFRs and lipid peroxidation in placenta and circulation of preeclamptic patients (6, 7, 15, 18). Poranen et al. (19) reported that lipid peroxidation was increased and the activity of antioxidant enzymes; SOD and Glucose 6-phosphate-dehydrogenase were decreased in preeclamptic placenta. Walsh and Wang (20) showed that lipid peroxides levels were significantly higher, and GSH-Px activity was significantly lower in preeclamptic placentas. In accordance with the study by Turoğlu et al (21), lipid peroxidation was found to be increased, while GSH-Px, SOD and Glutathion S-transferase activities were decreased in plasma of preeclamptic women.

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