Avrupa Bilim ve Teknoloji Dergisi
Sayı 38, S. 94-98, Ağustos 2022

Araştırma Makalesi

www.ejosat.com ISSN:2148-2683

European Journal of Science and Technology
No. 38, pp. 94-98, August 2022

Research Article

Sağlıklı ve preeklamptik plasental dokularda oksidatif stres parametrelerinin ve nitrik oksitin değerlendirilmesi

Ozlem Aydin Berktas[1], Sadettin Oguzhan Tutar[2]

[1]Faculty of Healthy Science, Department of Nursing, Giresun University, 28100, Giresun, Turkey. 0000-0002-7235-4890, ozlem.berktas@giresun.edu.tr

[2]Faculty of Medicine, Department of Obstetrics and Gynecology, Giresun University, 28100, Giresun, Turkey. 0000-0003-0208-6680, oguzhantutar48@gmail.com

(İlk Geliş Tarihi 24 Mart 2022 ve Kabul Tarihi 15 Haziran 2022)

(**DOI:** 10.31590/ejosat.1092209)

ATIF/REFERENCE: Berktas, A. O., Tutar, S. O. (2022). The evaluation of oxidative stress parameters and nitric oxide in healthy and preeclamptic placental tissues. *Avrupa Bilim ve Teknoloji Dergisi*, (38), 94-98.

Öz

Bu çalışmada preeklamptik gebelerde oksidatif hasar parametreleri araştırıldı ve sağlıklı dokularla karşılaştırıldı. 53 preeklamptik ve 53 sağlıklı gebe plasentada antioksidan enzimler belirlendi. Plasenta dokusunda serbest oksijen radikallerinin hasarı, antioksidan enzim sistemleri (lipid peroksidasyon (LPO), glutatyon (GSH) ve katalaz (CAT) miktarları, miyeloperoksidaz (MPx) enzim aktiviteleri ve nitrik oksit düzeyleri ölçülerek belirlendi. Preeklampsi geçirmiş gebede LPO düzeyleri sağlıklı gebelere göre yükselmiştir. Sağlıklı plasenta dokularında GSH miktarı yüksek iken, preeklampsi dokularında oldukça düşük bulundu. Preeklampsili kadınların plasental dokularında CAT, MPx enzim aktiviteleri ve NO düzeyleri oldukça yüksek iken, sağlıklı dokularda düşük bulunmuştur. Plasental dokularda antioksidan enzim aktiviteleri ve miktarları gruplar arasında önemli farklılıklar göstermektedir. Bu veriler doğrultusunda oksidatif stres parametrelerinin doku düzeyindeki hasarı oldukça iyi gösterdiğini söyleyebiliriz. Sonuç olarak elde edilen veriler plasenta dokusunda oksidatif hasarı göstermekte ve antioksidan mekanizmanın çalıştığını göstermektedir.

Anahtar Kelimeler: Preeklampsi, Oksidatif hasar, Reaktif oksijen türleri, Antioksidan enzimler.

The evaluation of oxidative stress parameters and nitric oxide in healthy and preeclamptic placental tissues

Abstract

In the present study, oxidative damage parameters in preeclamptic pregnant women were investigated and compared with healthy tissues. Antioxidant enzymes were determined in 53 preeclamptic and 53 healthy pregnant placentas. The damage of free oxygen radicals in the placental tissue was determined by measuring by antioxidant enzyme systems [The amounts of lipid peroxidation (LPO), glutathione (GSH) and catalase (CAT), myeloperoxidase (MPx) enzyme activities and nitric oxide levels (NO)]. The LPO levels increased in the pregnant woman who has had preeclampsia compared with healthy pregnant. While the amount of GSH was high in healthy placental tissues, it was found to be quite low preeclampsia tissues. While CAT, MPx enzyme activities and levels of NO were quite high in the placental tissues of women with preeclampsia, they were found to be low in healthy tissues. The antioxidant enzyme activities and amounts show significant differences between groups in placental tissues. In line with these data, we can say that oxidative stress parameters show the damage at the tissue level quite well. As a result, the data obtained show oxidative damage in the placental tissue and indicate that the antioxidant mechanism works.

Keywords: Preeclampsia, Oxidative damage; Reactive oxygen species; Antioxidant enzymes.

1. Introduction

Preeclampsia is the most complex multisystem disorder among all pregnancy complications associated with hypertension and proteinuria that occur during pregnancy. Since it is a system disorder, the cause has not been determined exactly (Burton and Jauniaux, 2015). In particular, studies are on enzyme systems, and their effects on free radicals and oxidative stress are now very popular. Preeclampsia is characterized as an oxidative damage that develops due to the increase of free oxygen radicals and the decrease of antioxidant sources. Some studies suggest that placental oxidative stress may play a role in the etiopathogenesis of preeclampsia (Hauth et al., 2000). In cases where reactive oxygen species increase, the body's defense system is defeated and oxidative stress occurs. ROS (reactive oxygen species) are molecules containing unpaired electrons in their outer orbitals (hydroxyl radical, superoxide anion radical, and nitric oxide radical). In addition to these radicals, there are reactive oxygen species such as hydrogen peroxide and peroxynitrite, but also structures containing unpaired electrons. They attack phospholipid structures, especially by acting on the cell membrane. They react with polyunsaturated fatty acids, forming lipid peroxides. Thus, it has been widely cited as a promoter of lipid peroxidation in relation to preeclampsia (Hung et al., 2002). The decreased uteroplacental perfusion results in ischemic damage to the placenta. may be a catalyst for increased oxygen radical-based lipid peroxidation to trigger oxidative stress in the placenta.

Placenta; It is a specialized organ that coordinates adaptation during pregnancy and manages normal growth and development (Burton and Jauniaux, 2015). The placenta, which provides continuous two-way exchange between mother and baby, provides ion transport and the passage of waste products into circulation (Lanoix et al., 2008; Myatt, 2006). It plays a role in the synthesis of all molecules necessary for a healthy pregnancy and ensures the continuation of many reactions thanks to its multiple enzyme systems. Thanks to these enzyme systems, foreign chemicals that pass through the placenta can be modified (Myatt, 2006; Syme et al., 2004). Therefore, in the present study, antioxidant enzyme systems were determined in preeclamptic placentas to help elucidate the oxidative stress mechanism, which is thought to have important roles in the pathogenesis of preeclampsia.

2. Materials and Methods

2.1. Experimental Procedure

The preeclamptic pregnant placental tissues were obtained from Giresun Gynecology and Pediatrics Training and Research Hospital. The experiment was carried out with the permission of Gumushane University clinical research ethics committee (09.06.2021/4). The institutional permission for the study was obtained from Giresun University Gynecology and Pediatrics Training and Research Hospital with the number E-87982892-929.

2.1.1. Chemicals

All chemicals used in the experiment were obtained from Sigma-Aldrich.

2.1.2. The tissue samples

Who applied to Giresun University Gynecology and Pediatrics Training and Research Hospital, Department of Obstetrics and Gynecology outpatient clinic, filled out the informed consent form, and volunteered two groups, healthy and preeclamptic were formed from pregnant women. In the first of these experimental groups, there were 53 preeclamptic pregnant women and in the other 53 healthy pregnant women. The tissue samples taken from these groups at the time of birth were stored at -80oC and then the biochemical parameters were measured. The placental tissues were ground with liquid nitrogen and homogenized with appropriate homogenates (different for each parameter). The homogenates were centrifuged for each enzyme using a refrigerated centrifuge as specified in the literature. The clear supernatant was also used for enzyme activities such as CAT (catalase), SOD (superoxide dismutase), NOx (nitric oxide) enzymes activities and the levels of GSH (glutathione) and LPO (lipid peroxidase).

2.1.3. Biochemical investigation of placental tissues

Total glutathione (GSH) determination: GSH levels in placental tissues were measured in accordance with the literature (Sedlak and Lindsay, 1968).

Lipid peroxidation (LPO) determination: LPO levels were determined using thiobarbituric acid based on the measurement principle of the variability of the MDA level in the medium (Ohishi and Yagi, 1979).

Catalase (CAT) activity: Catalase activity was measured according to the appropriate literature. The results were obtained by measuring at 240 nm in the homogenate obtained by adding appropriate buffers (Aebi, 1984).

Myeloperoxidase (MPx) activity: The supernatants prepared according to the principle will be used as the enzyme source in the measurement of MPx activity. The absorbance will be recorded by measuring at 460 nm wavelength at 30 second intervals and for 5 minutes (Priebat et al., 1982).

NOx (Nitric oxide) levels: The NOx levels in the samples were determined by the spectrophotometric method described by Miranda et al., 2001 (Miranda et al., 2001).

2.1.4. Statistical analyses

The number of samples calculated according to the G power analysis test was calculated in the appropriate SPSS program. Statistical differences were expressed and significance levels independent sample t test results at $p< 0.05$ were considered significant.

3. Results

In the current study, 106 pregnant placentas were used. The sample number was adjusted to include 53 preeclamptic pregnant and 53 healthy pregnant women. The oxidative stress in placenta tissues obtained from pregnant women was expressed by the parameters of antioxidant enzyme systems. The enzyme activities in placental tissues are shown in the Table 1.

Table 1: The oxidative stress parameters, the levels of GSH and LPO; the enzyme activities of CAT, MPx and NOx in placental tissues taken from pregnant women with preeclampsia.

Means in the same column by the same letter are not significantly different to the Duncan test $p<0.05$). Mean damage

Treatment	N	Amount of LPO (nmol/g tissue)	Amount of GSH (nmol/mg tissue)	CAT activity (mmol/min/mg tissue)
The preeclamptic pregnant placentas	53	21.4±0.2	17.4±0.18	80.53±0.2
The healthy pregnant placentas	53	15.6±0.1	24.5±0.14	69.49±0.1

index ± SE of fifty-three placental tissues in each group.

As seen in Table 1, LPO level, which is an indicator of tissue damage, was found to be quite high in pregnant women with preeclampsia compared to healthy tissue. Parallel to LPO, CAT and NOx enzyme activities in tissues are also quite high in preeclamptic pregnant tissues. While GSH level was significantly high in healthy pregnant placental tissues, it was found to be significantly low in preeclamptic pregnant women. Likewise, MPx crushing activity, which is an important indicator of neutrophil infiltration, was found to be as low as GSH in tissues (Fig. 1).

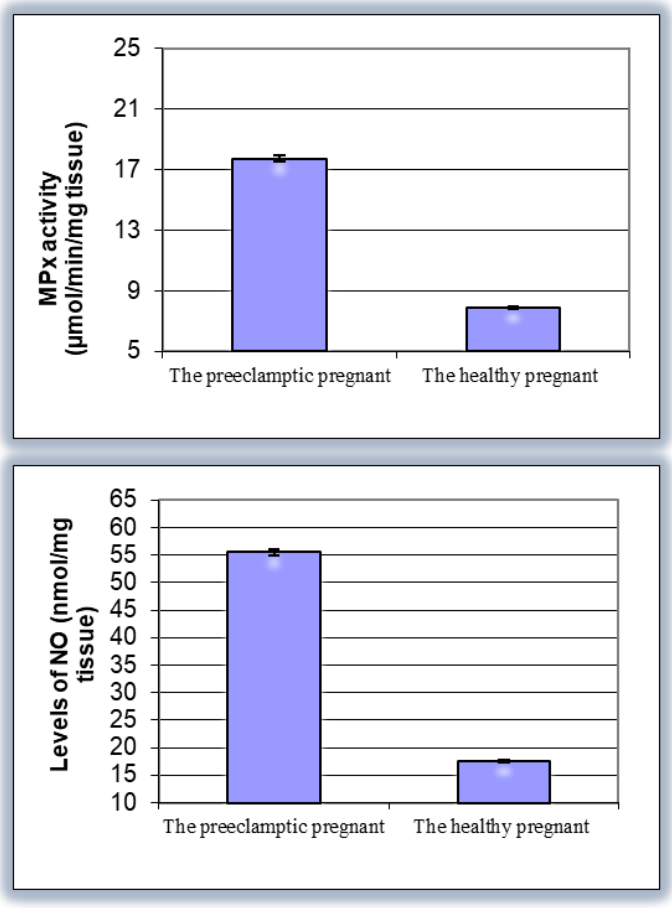

Fig. 1. The oxidative stress parameters, the levels of NO and the enzyme activities of MPx in placental tissues. Means in the same column by the same letter are not significantly different to the One-way ANOVA ($p<0.05$).

4. Discussion

Preeclampsia is a multisystemic syndrome that occurs after the 20th week of pregnancy and is characterized by hypertension and proteinuria (Young et al., 2010).

More than sixty thousand maternal deaths worldwide are caused by preeclampsia every year, especially in developing countries. Preeclampsia, which is a pregnancy-specific disease, disappears after delivery. It is important to identify which patients are at risk for the development of preeclampsia in order to identify cases that may benefit from follow-up and treatment, but the pathogenesis of preeclampsia is still not fully understood. According to some studies, it is stated that preeclampsia is caused by changes in placental microcirculation (Young et al., 2010).

The placenta, which provides the bilateral relationship between mother and fetus during pregnancy, is a specialized endocrine organ. The passage of nutrients, ions and gases necessary for normal fetal development, and the incorporation of waste materials into the circulation are provided here. In order to have a healthy pregnancy period, the synthesis of molecules such as hormones, proteins and steroids takes place in the placenta (Lanoix et al., 2008; Syme et al., 2004). In particular, many enzymes responsible for drug oxidation, hydrolysis, reduction and conjugation are found in the placenta. Thanks to these enzyme systems, the functioning of the chemical substances coming to the placenta is directed. Mostly, these enzymes modify the compounds formed by the oxygen molecule that aerobic organisms are constantly exposed to. In all organisms, there is a balance between enzyme systems and free radicals, and when this balance is disturbed, free oxygen radicals appear. The free radical reactions cause oxidation of lipids, proteins and polysaccharides and DNA damage, which exerts significant toxic biological effects (Saugstad, 1996). It is very natural that the production of oxygen radicals increases during the pregnancy period when tissue oxygen demand increases (Gitto et al., 2009; Chamy et al., 2006). During this period, the main source of oxidative stress is the placenta. Because placental lipid peroxides pass into the maternal circulation (Saugstad, 1996). But; In normal pregnancy, lipid peroxides in the placenta are under control by antioxidant enzymes (Walsh, 1997). There are high levels of antioxidant enzymes such as SOD, CAT, GPx, MPx and glutathione reductase in the placenta (Walsh, 1997; Adiga et al., 2007). However, the production of lipid peroxides is quite high in the placental tissue, especially in preeclamptic pregnant women. Accordingly, it was determined in some studies that malondialdehyde (MDA) increased and enzymatic and non-enzymatic antioxidants decreased (Walsh, 1997; Adiga et al., 2007). In addition, it was determined that the activity of nitric oxide synthase, a secondary enzyme in both placenta and plasma, increased (Lowe, 2000; Bowen et al., 2001; Moncada, 1992).

In the current study, pregnant women were divided into two groups (preeclamptic and healthy) and, oxidative damage in placental tissues compared. Primarily, the level of lipid peroxidation was investigated in preeclamptic pregnant tissues. MDA level, which is an indicator of lipid peroxidation, was found to be significantly higher than in healthy pregnant women (Saugstad, 1996). It is natural that there is an increase in free radical production according to the increasing oxygen demand during pregnancy. In particular, they seek help from enzymatic

and non-enzymatic enzyme systems in order to minimize the damage during the oxidative stress process. Since LPO present in the placenta during normal pregnancy needs more defense than the stress state of the environment, it tries to quickly block the reactive oxygen system. Therefore, it tries to eliminate the damage of the environment by secreting more MDA. The reason why LPO level is higher than healthy pregnant placentas is that it stimulates the antioxidant system to quickly eliminate stress. In many studies, results were obtained that are compatible with our findings (Saugstad, 1996; Walsh, 1997; Adiga et al., 2007).

Against the increasing reactive oxygen species in the tissues, the amount of GSH takes action to neutralize the radicals. Meanwhile, GSH is oxidized by donating electrons to the unpaired electrons of the radicals and turns into oxidized glutathione (GSSG). This structure is converted to GSH by the GR enzyme. Therefore, if radical production is high in the environment, the amount of oxidized GSH will also be high, and the amount is expected to be low compared to healthy pregnant placentas. Consistent with the literature, the amount of GSH in preeclamptic tissues was also found to be significantly low (Lowe, 2000; Bowen et al., 2001).

Again, the CAT enzyme, which is found in a significant amount in the tissues, eliminates the effect of H2O2, which has a weak character. Normally not a strong radical, H2O2 can be a very strong source in the presence of transition metals such as Fe+2 and Cu+2. For this reason, during the production of reactive oxygen, the CAT enzyme eliminates the damage by converting the H2O2 formed in the environment into water and molecular oxygen. Therefore, the catalase enzyme, which is present at certain levels in healthy tissues, shows more activity in case of stress and tries to reduce the amount of H2O2. In line with this information, it is expected that CAT activity is higher in preeclamptic tissues compared to healthy tissues (Saugstad, 1996; Walsh, 1997; Adiga et al., 2007). Another enzyme important in the management of oxidative stress is MPx. In case of tissue damage, this enzyme releases neutrophils to the area and creates hypochlorous acid (HOCl) from H2O2 and chloride ions. HOCl increases the production of hydroxyl radicals. Hydroxyl radicals, as it is known, are very important oxygen radicals and cause LPO by showing dangerous and harmful effects on biomolecules. According to the data obtained, while MPx activity is quite low in healthy pregnant women, the activity is very high in preeclamptic tissues. Our findings are consistent with many studies.

Nitric oxide, synthesized from L-arginine by a family of enzymes known as NO synthases (NOS), is an important messenger molecule that plays a critical role in a wide variety of physiological functions, including neuronal transmission, vascular relaxation, immune modulation, and cytotoxicity (Moncada, 1992). Nitric oxide and its by-products (RNM) from antioxidant enzyme systems inhibit mitochondrial respiration; It maintains the current balance by stimulating or inhibiting cell death. While NO production at physiological concentration is necessary for the continuation of functions; moderate and high levels trigger oxidative stress (Tresguerres et al., 2008). NO is a molecule responsible for oxidative damage and many studies have shown increased oxidative stress in various tissues. In the present study, it was determined that NOx activity increased proportionally in the placental tissues of preeclamptic pregnant women compared to healthy tissues (Lowe, 2000; Bowen et al., 2001; Furchgott and Zawadzki, 1980; Palmer et al., 1987). In line with all these data, it can be said that preeclamptic pregnant women are exposed to oxidative stress compared to healthy pregnant women. We believe that the repair of this condition, the cause of which is still unknown, can be started by preventing free radical damage.

Compliance with Ethical Standards

Ethical Approval: The experiment was carried out with the permission of Gumushane Univ. Clinical Research Ethics Committee (09.06.2021/4).

Financial support: The financial support of this study was provided by the researchers themselves.

Conflict to Interest: All authors declare that there are no conflicts of interest.

Authors' Contributions: The collection of placental tissues provided by Dr. O. T. The biochemical analysis of tissue samples and measurement of parameters were carried out by Dr. O. B.

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