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Original Article

How the nitric oxide and antioxidant enzymes work in the placental tissues of pregnant women with COVİD19 disease?

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A B S T R A C T

Objective: The aim of this study to examine the oxidative stress parameters in placental tissues and blood plasmas of pregnant women.
Materials and Methods: The biochemical parameters were examined in 37 pregnant women with Covid disease, 37 pregnant women who had covid, and 37 healthy pregnant women. The amounts of lipid peroxidation (LPO), glutathione (GSH), nitric oxide (NOx) and catalase (CAT), superoxide dismutase (SOD) enzyme activities were measured in each tissue and blood plasma.
Results: The LPO levels increased in the pregnant woman who has had Covid19 compared with healthy pregnant. Likewise, the LPO levels increased in pregnant women who were negative at the time of delivery. While the amount of GSH was high in healthy placental tissues, it was found to be quite low in Covid19 positive tissues. While the enzyme activities of SOD and NOx are quite high in placental tissues of healthy pregnant women, they are significantly low in Covid positive and negative pregnant tissues. While the CAT enzymes activities, another antioxidant enzyme, was at a very low level in healthy placental tissues compared to SOD enzyme, it was significantly increased in positive pregnant placental tissues.
Conclusion: The antioxidant enzyme activities and amounts show significant differences between groups in placental tissues. The changes in enzyme rates in placental tissues are proportional to blood plasma results.
Keywords: pregnant; Covid19; placenta tissue; blood plasma

Introduction

Coronavirus disease 19 (COVID-19) is a viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The impact of COVID-19 on our lives started in 2019 and was declared a worldwide pandemic [1]. In a short time, it affected the whole world population and the scientific world started researches on this subject. CoVs are enveloped, positive-stranded RNA viruses with a crown-like appearance under the electron microscope [2]. Genomic characterization suggests that alpha CoV and beta CoVs in bats and rodents are likely gene-induced. Members of this group of viruses cause respiratory, enteric, hepatic and neurological diseases in different animal species including camels, cattle, cats and bats. To date, seven CoVs (HCoV) that can infect humans have been identified. It is estimated that 2% of the population It has been stated that there are healthy carriers of a CoV and that these viruses are responsible for approximately 5-10% of acute respiratory tract infections [3]. While all humanity is affected by this pandemic, especially pregnant women are in the section where special attention is paid to the disease [4]. Many adaptive physiological changes are during pregnancy observed, especially in the hematological, cardiovascular and respiratory systems. [5-7]. In addition, it has been reported that obstetric complications such as miscarriage, preterm delivery, prenatal rupture of membranes, preeclampsia (PE),

abnormal fetal heart rate patterns and fetal distress are increased in pregnant women with COVID-19 [8]. Increased systemic inflammatory response, hypercoagulability, and decreased arterial oxygen saturation are associated with poor obstetric outcomes.
The placenta is a highly specialized endocrine organ that plays an important role in the adaptation to pregnancy and the normal growth and development of the fetus [9]. It is also the main unit of two-way communication and exchange between mother and fetus [10]. The function of the placenta; transferring the nutrient molecules, ions and gases necessary for the continuation of pregnancy and normal fetal development from the mother to the fetus, ensuring that the waste products formed as a result of the metabolism of the fetus pass into the maternal circulation [11]. It also plays an important role in the synthesis of hormones, peptides and steroids, which are crucial for a successful pregnancy [12].
The human placenta contains multiple enzyme systems responsible for drug oxidation, reduction, hydrolysis and conjugation. In particular, the antioxidant mechanism of these enzyme systems is used in many studies to express oxidation and reduction. In recent studies, it has been shown that reactive oxygen species (ROS) play a role in the pathogenesis and complications of many diseases. Therefore, thanks to these enzyme systems, foreign chemicals that pass through the placenta can be modified [13].

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At the same time, damage to the placenta is indicated by these enzyme systems (LPO, amount of GSH, SOD, CAT, MPx). In the current study, oxidative stress levels in pregnant women with and without Covid19 disease will be determined in the placental tissue and blood plasma, and the enzyme systems will be examined about the damage that occurs.

Material and methods

Research Design

The current study is a randomized control design. The pregnant women included in the sample group were selected according to the improbable sampling method. The sample size was determined as 111 individuals for three different groups in the G power analysis program, according to the t test, at a confidence interval of 0.80 according to the 0.5 effect value. The number of samples was determined as 37 people in each group. Inclusion criteria for the study; Pregnant women between the ages of 18-35, who can communicate in Turkish, who do not have a chronic disease, and who are willing to participate in the research. The pregnant women who do not meet these criteria are not included.

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

The tissue and blood samples: The samples were taken from pregnant women who admitted to Giresun University Gynecology and Pediatrics Training and Research Hospital, Department of Obstetrics and Gynecology outpatient clinic. Groups; The patient group diagnosed with Covid19 and delivered in this way (n=37) was determined as the group with negative findings at the time of delivery with the diagnosis of Covid19 (n=37) and the healthy pregnant women (n=37). The tissue and blood 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. The blood samples were taken into tubes containing anticoagulant, centrifuged and the plasma part was separated. The biochemical measurements were made in the plasma in the upper phase of the blood with EDTA.

Biochemical investigation of placental tissues and blood plasma

Total GSH determination: The amount of GSH was measured in the appropriate method [14]. With minor changes. After the stomach tissues were homogenized, the amount of enzyme in the supernatants was determined. The results of the GSH levels in the liver and stomach tissue were expressed as nmol/g tissue.

LPO determination: The level of LPO was determined by estimating malonyldialdehyde (MDA) using the thiobarbituric acid test [15]. After the stomach tissues were weighed and homogenized, they were treated with solutions suitable for the method. The results were determined by measuring the absorbance of the mixtures as nanomol MDA per gram tissue (nmol/g tissue).

CAT activity: Decomposition of H_2O_2 in presence of CAT was at 240 nm [16].

CAT activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25◦C and pH 7.8. Results were expressed as mmol/min/mg tissue.

SOD activity: SOD activity was measured according to the principle of superoxide radical formation of xanthine [17]. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction.

NOx measurement: The NOx levels in the samples were determined by the spectrophotometric method described by Miranda et al. [18].

Statistical analyses

The statistical calculations were made using an appropriate statistical program. The conformity of the data to the normal distribution was determined by the Kolmogrov Smirnov test. The data determined to be normally distributed were analyzed with the one-way ANOVA of variance test. Significance level was accepted as $p < 0.05$.

Experimental Procedure

The pregnant placental tissues and blood plasmas to be tested were obtained from Giresun Gynecology and Pediatrics Training and Research Hospital. The experiment was carried out with the permission of Ordu University clinical research ethics committee (12.07.2021/172). The institutional permission for the study was obtained from Giresun University Gynecology and Pediatrics Training and Research Hospital with the number E-87982892-929.

Results

The population of the study consists of 111 pregnant women in total (n=111). The 37 of them are Covid positive pregnant women, the 37 of them are Covid negative pregnant women and the 37 of them are healthy pregnant women. The oxidative stress parameters were determined in both blood plasma and placental tissue in these three groups. The oxidative stress in placental tissues and blood plasma samples was expressed through antioxidant enzyme systems. The enzyme activities are measured to show the formation of oxidative stress in the placental tissues and blood plasma of pregnant women with Covid19 disease. The enzyme activities in placental tissues are shown in the table 1 and the results of blood plasma are shown in the table 2. Table 1 shows that the LPO levels increased in the pregnant woman who has had Covid19 compared with healthy pregnant. Likewise, the LPO levels increased in pregnant women who were negative at the time of delivery. While the amount of glutathione was high in healthy placental tissues, it was found to be quite low in Covid19 positive tissues (Table 1).

While the enzyme activities of SOD and NOx are quite high in placental tissues of healthy pregnant women, they are significantly low in Covid positive and negative pregnant tissues.

Table 1: The oxidative stress parameters, the levels of GSH and LPO and the enzymes of CAT, SOD and iNOx in placental tissues taken from pregnant women with Covid 19 disease

Treatments	N	Amount of LPO *	Amount of GSH *	CAT Activity #	SOD Activity #	NOx Activity *
The healthy pregnant	37	24.65±0.2a	15.1±0.12c	80.4±0.22b	104±0.2c	17.5±0.14a
The pregnant woman who had covid 19 (covid negative)	37	34.8±0.1b	7.08±0.19b	61.4±0.16a	33.2±0.2a	24.5±0.16b
The pregnant with covid 19 (covid positive)	37	61.65±0.2c	5.46±0.17a	123.4±0.22c	61±0.1b	56.5±0.18c

*Means in the same column by the same letter are not significantly different to the Duncan test p<0.05). Mean damage index ± SE of thirty-seven placental tissues in each group. N: The number of placentas. * nmol/g tissue, #(mmol/min/mg tissue)*

While the CAT enzymes activities, another antioxidant enzyme, was at a very low level in healthy placental tissues compared to SOD enzyme, it was significantly increased in positive pregnant placental tissues. The antioxidant enzyme activities and amounts show significant differences between groups in placental tissues. The changes in enzyme rates in placental tissues are proportional to blood plasma results (Table 2).

Discussion

COVID-19 is an infection caused by viruses that cause Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). It causes severe acute respiratory tract infections and progresses as mild and severe symptoms [19, 20]. Due to the very rapid spread of the virus, almost the entire population of the country and the world has been infected. In fact, a serious public health emergency has reached the lethal level in communities where healthcare providers are underserved [21].

Therefore, it resulted in serious damages in terms of economic, psychological, education and health by causing restrictions on all continents in mass. This virus, which affects every part of the society, has brought especially children and pregnant women into consideration. These groups, which would be greatly affected by the use of drugs, had to fight the virus only with their antioxidant systems. Especially in pregnant women, the use of drugs is not foreseen in many societies because they do not know how it will affect the fetus. A hypoxia intolerant picture develops with diaphragmatic elevation, increased oxygen consumption and edema in the respiratory tract mucosa during pregnancy. In a flu epidemic in 1918, mortality rates were reported as 2-6% in the whole population and 37% in pregnant women. [22]. In another study, information about the clinical features of COVID-19 infection in pregnant women, pregnancy outcomes and the potential for vertical transmission are given. The emergency preventive measures were taken in nine pregnant women who were diagnosed with COVID-19 and developed pneumonia. The newborn nasopharyngeal swab samples, amniotic fluid, cord blood, breast milk samples were collected and severe acute respiratory syndrome (SARS-CoV-2) was detected in 2 newborns. As a result, it was stated that vertical transmission is possible and should be evaluated in detail [23]. The major source of oxidative stress in pregnancy is the placenta [24]. The placenta is a structure rich in polyunsaturated fatty acids, so it is the main source of lipid peroxides secreted into the maternal circulation. In normal pregnancy, placental lipid production is kept under control by placental antioxidants. Major antioxidant enzymes such as SOD, CAT, glutathione-S-transferase (GST), glutathione reductase (GR) and non-enzymatic antioxidants such as GSH are present in the placenta [25]. The production of free oxygen radicals in healthy pregnancy decreases with the increase of antioxidant defense systems.

It was determined in some studies that LPO increased significantly in the maternal cycle during this period when metabolic needs and oxygen requirements of tissues increased. In the present study, antioxidant enzyme systems were evaluated and determined in placental tissue and in blood plasma in terms of parallelism. A total of 111 pregnant placental tissues and blood plasma were evaluated in the study. The groups will be 37 people; It is set to be covid 19 positive (positive at birth), covid 19 negative (negative at birth) and healthy pregnant women.The free radicals interact with cell components such as proteins, lipids and nucleic acids and cause significant structural and functional disorders in cells. LPO is a chemical event initiated by free radicals and involves the oxidation of unsaturated fatty acids in the membrane structure. Once this event starts, it proceeds in the form of autocatalytic chain reactions. Tissue damage due to LPO can be determined by measuring LPO degradation products. The one of these products is malondialdehyde (MDA). An increase in MDA level in tissues is an indication of the onset of oxidative stress. In our findings, the LPO level in the placental tissues and plasmas of pregnant women with covid 19 was significantly higher than in healthy pregnant women. The results consistent with these data are also seen in placenta tissue studies conducted in parallel with the current study.

Nitric oxide, synthesized from L-arginine by a family of enzymes known as NO synthases (NOSs), is an important messenger molecule that plays a critical role in a wide variety of physiologic functions including neuronal transmission, vascular relaxation, immune modulation, and cytotoxicity [26].

Nitric oxide and its by-products (RNM) from antioxidant enzyme systems inhibit mitochondrial respiration; It maintains the existing balance by stimulating or inhibiting cell death. While the production of NO in physiological concentration is necessary for the maintenance of functions; moderate and high levels trigger oxidative stress [27]. NO is a molecule responsible for oxidative damage [28,29]. In many studies, it has been determined that oxidative stress is increased in various tissues. In the current study, it was determined that NOx activity proportionally increased in the placenta and blood plasma of pregnant women with covid 19 compared to healthy pregnant women.

At the same time, it was determined that it showed moderate activity in covid negative pregnant women. The another intracellular enzyme system in the fight against free radicals is superoxide dismutase (SOD) and catalase (CAT). SOD is an important enzyme that neutralizes superoxides. The CAT enzyme is the enzyme that converts H_2O_2, which is also considered a radical, into water [30]. H_2O_2 cannot be considered a strong radical on its own.

Table 2: The oxidative stress parametes, the levels of GSH and LPO and the enzymes of CAT, SOD and iNOx in blood plasma taken from pregnant women with Covid 19 disease

Treatments	N	Amount of LPO	Amount of GSH	CAT Activity	SOD Activity	NOx Activity
The healthy pregnant	37	32.4±0.17a	4.5±0.18c	14.8±0.3a	4.2±0.17c	124.6±0.17c
The pregnant woman who has had covid 19 (covid negative)	37	62.5±0.17b	2.4±0.01b	25.9±0.2b	1.5±0.02b	62.5±0.16b
The pregnant with covid 19 (covid positive)	37	113.2±0.12c	1.5±0.02a	50.2±0.2c	0.6±0.02a	53.6±0.15a

*Means in the same column by the same letter are not significantly different to the Duncan test p<0.05). Mean damage index ± SE of thirty-seven placental tissues in each group. N: The number of placentas. * nmol/g tissue, #(mmol/min/mg tissue)*

Because it does not contain unpaired electrons [31,32]. However, H_2O_2 is considered a reactive oxygen species and plays an important role as a radical source. In the presence of transition metals such as H_2O_2, $Fe+2$ and $Cu+2$, it can react with superoxide to form the hydroxyl radical, which is a very reactive radical [33,34].

Therefore, tissues have antioxidative mechanisms to regulate the amount of H_2O_2. It is natural that there is an increase in the activity of the CAT enzyme in order to reduce the amount of H_2O_2 that is excessive in tissue damage and traumatic situations. Therefore, a high amount of CAT activity was detected in the tissues of pregnant women with covid 19. Likewise, the H_2O_2 produced by CAT is tried to be neutralized by electron transfer thanks to GSH in organisms and tissues. The decrease in the level of GSH in the tissue is actually an expression of the acceleration of the transformation depending on the electron transfer in the environment. Therefore, the decrease in GSH level in pregnant tissues with covid indicates that it is trying to reduce and fight H_2O_2 in the environment. Likewise, the level of GSH in blood plasmas decreased in line with the tissues.

As a result, it was determined that the oxidative stress parameters between the groups were significantly different and covid 19 disease caused quite a damage to the mother and fetus. At the same time, considering the apgar score evaluation, it was determined that the weight of the fetus was slightly lower compared to the control group. It was also determined that some positive pregnant women had babies with excessive dyspnea, tachypnea and meconium during delivery. Considering these data, we can say that this virus may cause some discomforts in both pregnant women and fetus.

Disclosure

Authors have no potential conflicts of interest to disclose.

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