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Oxidative stress and apoptosis in preeclampsia

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ABSTRACT

We aimed to determine the oxidative stress and antioxidant status in preeclamptic placenta. Also, we investigated the apoptotic index of villous trophoblast and proliferation index of cytotrophoblasts. The study included 32 pregnant with preeclampsia and 31 normotensive healthy pregnant women. Malondialdehyde (MDA) and total antioxidant status (TAS) levels were measured in the placenta. For detection of apoptosis and proliferation in trophoblast, apoptosis protease activating factor 1 (APAF-1) and Ki-67 were used. Placental MDA levels in preeclamptic women were significantly higher than normal pregnancies ($p = 0.002$). There was no significant difference between the groups in the TAS levels of placenta ($p = 0.773$). Also, the apoptotic index in villous trophoblasts increased ($p < 0.001$), but proliferation index did not change in preeclampsia ($p = 0.850$). Increased oxidative stress and apoptosis in pathological placenta are not balanced by antioxidant systems and proliferation mechanisms.

1. Introduction

Preeclampsia is a common pregnancy specific disorder that is characterized by placental abnormalities and maternal vascular endothelial dysfunction (Roberts and Cooper, 2001). The pathophysiology of preeclampsia is still unclear, but reduced uteroplacental perfusion secondary to abnormal cytotrophoblast invasion of spiral arterioles is thought to lead to ischemia reperfusion injury to the placenta (Kaufmann et al., 2003).

Oxidative stress and free radicals are known to have important roles in the placental ischemia reperfusion injury. Placental ischemia reperfusion injury has contributed in generation of reactive oxygen species (ROS). The imbalance between the cellular generation of ROS and the capacity of antioxidants seems to play an important role in preeclampsia (Gupta et al., 2005; Açikgoz et al., 2006).

Proliferation and programmed cell death (apoptosis) are indispensible components of the trophoblast life cycle. There are aberrant cell turnover including an increased apoptosis in placental villous trophoblast of preeclamptic pregnancies (Allaire et al., 2000; Ishihara et al., 2002). Apoptotic pathway is stimulated from within the cell (intrinsic) or from an external signal (extrinsic). The activation of the intrinsic pathway changes mitochondrial membrane permeability, resulting in cytochrome *c* release into the cytosol. Cytochrome *c* is bound by apoptosis protease activating factor-1 (APAF-1) forming the apoptosome (Ott et al., 2002). There are no published studies that have investigated APAF-1 in patients with preeclampsia.

Numerous molecular markers of preeclampsia were investigated in the past; however, the significances of oxidative stress and apoptosis were not well documented. This study was designed to investigate the oxidative stress, total antioxidant status, apoptotic marker APAF-1 and proliferative marker Ki-67 antigen in the placenta of patients with preeclampsia.

2. Materials and methods

The study included 32 pregnant with preeclampsia and 31 normotensive healthy pregnant women. The study was approved by the Ethics Committee of Bulent Ecevit University. All participants were provided written informed consent. The samples were collected after obtaining informed consent from all the subjects.

Preeclampsia was defined according to the criteria of the American College of Obstetricians and Gynecologists (2002). Patients were excluded from the study if they had a history of diabetes, renal disease, hepatic disease, hypertension, cardiovascular illness, multiple pregnancies, HELLP syndrome (hemolysis, elevated liver function, low platelets), autoimmune diseases, or with a fetus having structural or genetic anomaly or infections. In

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the preeclampsia group, samples were collected when the patients first presented for evaluation and before initiation of any treatment. Thirty-one healthy pregnant women were recruited at a routine antenatal care visit or when they were admitted to the delivery room. Placental samples were taken from the maternal side of the placenta and immediately frozen at −80 °C until biochemical analysis. Tissue samples were minced and homogenized using a glass Teflon homogenizer (IKA, Staufen, Germany) as described previously by Ozturk et al. (2011). The lysates were centrifuged at 9000 × g at 4 °C for 30 min and the supernatants were immediately analyzed.

2.1. Malondialdehyde assay

MDA levels were assayed with a commercial kit (Bioxytech, CA, USA) that is based on the colorimetric method using Shimadzu UV 1601 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). MDA reacts with *N*-methyl-2-phenylindole to form an intensely colored carbocyanine dye with a maximum absorption at 586 nm. The results were calculated from standard curve. The intraassay CV was <%4.

2.2. Total antioxidative status assay

The TAS was analyzed using an ImAnOx (TAS) Kit (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The determination of the antioxidative capacity was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H_2O_2). The antioxidants in the sample eliminate a certain amount of H_2O_2. The residual H_2O_2 was determined by an enzymatic reaction involving the conversion of tetramethylbenzidine to a colorimetric product. Following addition of a stop solution to the samples, absorbance was measured at 450 nm using an ELISA reader (Poweam Medical, Nanjing, China).

2.3. Immunohistochemical staining

Samples from placental tissues were fixed in 10% buffered formalin solution, embedded in paraffin and cut into 5 μm sections. The sections were deparaffinized in xylene, and then were dehydrated. 3% hydrogen peroxide was used for blocking endogenous peroxidase activity. Antigen retrieval was carried out by incubation in citrate buffer (pH 6.0) for 10 min in a pressure cooker. Immunohistochemical staining was performed according to the standard streptavidin–biotin peroxidase complex method. The sections were exposed to the primary antibodies to Ki-67 (Biocare Medical, CA, USA, rabbit monoclonal anti-human IgG, clone: SP6, 1:100 dilution) and apoptosis protease activating factor 1 (APAF-1) (Lab Vision, Fremont, CA, USA, mouse monoclonal anti-human IgG, 1:100 dilution) for 60 min at room temperature. Then incubated with labeled secondary antibodies (biotinylated goat anti-Mouse IgG, anti-Rabbit IgG, Lab Vision, CA, USA) and peroxidase-conjugated streptavidin, then visualized by employing diaminobenzidine as the chromogen. Intense brown staining of the cytoplasm was considered as a positive apoptotic cell. A sample of colorectal carcinoma was used as a positive control for APAF-1 expression. Apoptotic cells were counted in villous trophoblasts, including cytotrophoblasts, syncytiotrophoblasts and syncytial knots. Apoptotic activity was expressed as the apoptotic index, i.e. the percentage of APAF-1 positive cells out of the total number of cells in the villous tissue counted (Prusac et al., 2011). Brown nuclear staining was regarded positive for the expression of Ki-67 antigen. A sample of melanoma was used as a positive control for Ki-67 expression. Ki-67 staining was determined solely for cytotrophoblasts, since there was no positive staining in syncytiotrophoblasts and trophoblasts of syncytial knots. It was expressed as proliferation index, i.e. percentage of positive cells in cytotrophoblasts out of the total number of cells in the cytotrophoblasts counted (Prusac et al., 2011).

2.4. Statistical analysis

All data were analyzed using SPSS 13 (SPSS, Inc., Chicago, IL) software and presented as mean ± standard deviation (SD). Differences of the parameters between the preeclampsia and control groups were evaluated by Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

Demographic data of the study population are summarized in Table 1. The mean blood pressure and maternal age of preeclampsia was statistically higher than that of the normal pregnancies. Gestational age and infant birth weight of the control group was statistically higher than those of the preeclampsia group. There was no statistically significant difference between the two groups with regard to parity.

The biochemical characteristics of the control and preeclamptic placentas are shown in Table 2. MDA levels of the placenta were evaluated as significantly higher in the preeclamptic women compared to the normal pregnancies. There was no significant difference between the two groups in the TAS levels of placenta.

Histopathological photographs of preeclamptic and normal placenta are shown in Figs. 1–3. Mean proliferation index in cytotrophoblast was 4.16 ± 1.26 in normal pregnancies and 4.12 ± 1.33 in preeclampsia ($p = 0.850$) (Fig. 4). Apoptotic index in villous trophoblasts increased in preeclampsia (2.03 ± 0.78) when compared with normal pregnancies (1.28 ± 0.52) ($p < 0.001$) (Fig. 5).

4. Discussion

There is increasing evidence that oxidative stress in uteroplacental tissues plays a pivotal role in the development of preeclampsia (Jauniaux et al., 2006). Free radicals cause oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues. Free radical induced oxidation of polyunsaturated fatty acids in cells, results in the formation of lipid peroxidation products such as MDA which is used as a biomarker of lipid peroxidation (Lykkesfeldt, 2007). Several studies have shown an increased lipid peroxides in placenta and maternal circulation of preeclamptic pregnancies (Poranen et al., 1996; Walsh et al., 2000; Gulmezoglu et al., 1996). In agreement with these studies, the results of our study indicated significantly high MDA levels of the placenta in preeclamptic compared to normotensive women.

The capacity of antioxidants to prevent oxidative damage may play a crucial role in preeclampsia. For this purpose we analyzed TAS levels in placenta. Mert et al. demonstrated that preeclampsia had elevated levels of total oxidant status and TAS in plasma when compared with healthy pregnant women (Mert et al., 2012). Ozturk et al. reported that the TAS level of the placenta was evaluated as significantly lower in the preeclamptic women compared to the normotensive women (Ozturk et al., 2011). We found no significant difference between the two groups in the TAS levels of placenta. In the light of previous studies and the recent study, we considered that lower antioxidant defense is probably secondary to enhanced depletion. These data provide convincing evidence that oxidative stress abnormally increases in the placentas of preeclamptic women.

The apoptosis rate is thought to have increased in preeclampsia as a result of hypoxia reperfusion injury and oxidative stress (Hung