ROLE OF OXIDATIVE STRESS IN PREECLAMPSIA AND NORMOTENSIVE PREGNANCIES

D. Kostadinova-Slavova*

Department of Obstetrics and Gynecology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT
Preeclampsia is described as a systemic illness associated with pregnancy. It is characterized by symptoms such as edema, proteinuria (the presence of excess proteins in the urine), hypertension (high blood pressure), and dysfunction. Recent research has extensively investigated the role of oxidative stress in the pathophysiology of preeclampsia. Oxidative stress refers to an imbalance between the production of reactive oxygen species and the body's ability to detoxify them or repair the resulting damage. Despite the research focus on oxidative stress, there is currently no consensus among researchers regarding the mechanisms that lead to the occurrence of preeclampsia in expectant mothers. The present review aims to advance the understanding of the underlying causes of preeclampsia by addressing the main biomarkers of oxidative stress.

In summary, preeclampsia is a complex condition associated with pregnancy, and its pathophysiology has been the subject of extensive research, particularly in connection to oxidative stress. However, the lack of agreement among researchers indicates that the exact mechanisms leading to preeclampsia in expectant mothers are still not fully understood and may vary across different studies or perspectives.

Key words: preeclampsia; oxidative stress; antioxidant system; peroxidation

INTRODUCTION
A pregnancy complication known as preeclampsia is characterized by significant disruption of vital organ and system functions. Multiple organ failure, edema, arterial hypertension, and proteinuria (>0.3 g/l in daily urine) are among the signs and symptoms of preeclampsia. During the second half of pregnancy, preeclampsia develops (1). Preeclampsia (PE) is the term for issues that arise at any stage of pregnancy and come before eclampsia, excluding the period of time prior to the onset of seizures (2). This categorization divides preeclampsia into two groups: moderate and severe (or mild and severe), with eclampsia coming next (3). The diagnosis of the condition followed by an attack is important because it requires immediate intensive treatment, more often delivered by the most gentle method depending on the obstetric situation. In recent decades, ideas about the heterogeneous nature of this syndrome have expanded (4). Preeclampsia can occur before 34 weeks (early onset) and after 34 weeks (late onset), during labor or in the postpartum period. It has been shown that early and late PE may have different pathophysiology (5). Early PE, unlike late PE, is usually accompanied by ischemic disturbances in the placenta and fetal growth retardation. Late-onset PE is associated with low-grade chronic inflammation, higher body mass index, and insulin resistance (6).

Risk factors for the onset of PE include advanced age, obesity, diabetes mellitus, and preexisting arterial hypertension. The pathogenesis of preeclampsia is diverse and not fully understood (7). The main links in pathogenesis are generalized vasoconstriction, hypovolemia, impaired rheological properties of blood, thrombo endothelial dysfunction, and development of disseminated intravascular coagulation syndrome. The immunogenetic factor and the immunological conflict between the mother and the fetus (hyperreaction of the
mother’s body to the fetoplacental complex) are important for developing PE (8). In turn, immune disorders disrupt the functional state of the central nervous system, metabolic processes, and hormonal status. A leading, initiating, and supporting role in the PE development belongs to the placenta. Vascular disorders initially occur in the placenta, and generalized vascular damage occurs in the kidneys, liver, lungs, and brain. Pathomorphologists consider the presence of signs of a delay in the second wave of cytotrophoblast vascular invasion (16–18 weeks of gestation) as a reliable sign of previous PE (9).

**ETIOLOGY AND PATHOGENESIS**

Preeclampsia pathogenesis is complicated by the lack of clear diagnostic criteria for the disease and its subtypes. The studied patients had established PE, and there were no blood samples taken from women before the clinical manifestation of the complication (5). This fact makes it difficult to define clear relationships between specific mediators, mechanisms, and clinical manifestations. Experimental animal models are unable to provide definitive insight into the pathogenesis of preeclampsia due to their limited applicability to the human form of the disease. Several theoretical mechanisms for the development of preeclampsia have been proposed to reconcile fetoplacental abnormalities and the clinical manifestations of pregnancy (10). Hysterectomy during cesarean section in women with preeclampsia showed that both the depth of invasion and the numerical density of interstitial trophoblasts were significantly reduced. Basic research and clinical data indicate that the maladaptation and inadequate uterine artery cytotrophoblast invasion and remodeling characteristic of preeclampsia result from intrinsic factors acting in combination with extrinsic uterine factors (11). These extrinsic factors acting around the uterine arteries include impaired decidual remodelling, impaired uterine killer cell function, and deficient expression of adhesion molecules by the endothelium. These factors may interact in a cascading manner, with the cells being the predominant decidual lymphoid cells (11).

During early pregnancy, they accumulate as a dense infiltrate around invading cytotrophoblast cells and participate in spiral artery remodelling by producing cytokines that are involved in angiogenesis and vascular stability, such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and angiopoietin (12). Preeclampsia is a disease of placentation caused by reduced activity or half-life of NO, with secondary low tissue concentrations of l-Arg (L-arginine) resulting from overexpression of arginase (11-13). In response to hypoxia, which results from abnormal placentation and placental hypoperfusion, the placenta releases a variety of proinflammatory factors into the maternal circulation, including sFlt1 (soluble receptor tyrosine kinase 1) and free radicals, which initiate endothelial dysfunction and subsequent multisystem organ and tissue damage. Histologically, the consequence of poor placentation is the appearance of obstructive lesions at the distal ends of the spiral arteries, acute atherosis characterized by fibrinoid necrosis, and the accumulation of lipid-laden macrophages or foam cells (13).

Mesenchymal stem cells (MSK) play an important role in the pathology of preeclampsia. MikroRNK-136 expression was expressed in decidua-derived MSK from women with PE compared with women with a normal pregnancy. Moreover, MikroRNK-136 significantly increased apoptosis and suppressed the proliferation of mesenchymal stem cells, which could be inhibited by trophoblastic invasion. This suggests that microRNA-136 expressed in decidua, which is elevated in PE, is a potential causative factor for PE (14).

Placental development is influenced by oxygen \((O_2)\) levels. At low oxygen levels, cytotrophoblasts proliferate in vitro, and hypoxia-inducible factor-1 (HIF-1α) activates several genes involved in the cellular response to oxygen starvation. Hypoxia promotes in vitro differentiation of trophoblast stem cells into spongiotrophoblasts and HIF1-α is required for placentation. The environment in the developing embryo is “physiological” hypoxia, which activates HIF1-α and leads to proper development of the placenta, cardiovascular system, and hematopoiesis (15). Thus, \(O_2\) acts not only as a terminal electron acceptor in mitochondrial oxidative phosphorylation but also as a signal responsible for transcription factor activation. Therefore, the formation of oxygen gradients in developing embryos activates the expression of hypoxic genes in a dose-dependent manner (16). Also, TGF-β3

KOSTADINOVA-SLAVOVA D.
mRNA expression correlated with HIF-1α protein levels. TGF-β3 levels are increased in hypoxia, and HIF-1α plays a dominant role in regulating TGF-β3 promoter activity. Therefore, hypoxia-induced TGF-β3 expression is due to increased HIF-1α activity on the TGF-β3 promoter (15-17).

ENDOTHELIAL DYSFUNCTION

In preeclampsia, vascular reactivity changes, and the coagulation cascade is activated as a result of changes in the structure and function of the vascular endothelium of pregnant women, which are manifested by high blood pressure (hypertension), protein in the urine (proteinuria), and/or swelling of the hands, feet, and/or person (18). Endothelial changes represent an altered state of endothelial cell differentiation in response to a sublethal injury or cytokine stimulation. In pregnant women with preeclampsia, pathological changes occur as a result of glomerular endothelins the cells enlarge due to the increased lipid content and sometimes clog the capillary lumen (19, 20). Many researchers assign a special role to neutrophils and platelets traditionally, polymorphonuclear neutrophils (PMNs) have been considered short-lived, highly differentiated granulocytic leukocytes, characterized by the presence of a segmented nucleus and distinct arrays of cytoplasmic granules (20, 21). In addition to their main well-known function—participation in the fight against pathogens of various diseases—they are involved in the development of systemic inflammation in preeclampsia, systemic lupus erythematosus, antiphospholipid syndrome, and migrating into the walls of blood vessels and tissues (22). Neutrophil migration in tissues includes the following stages: binding, rolling, adhesion, crawling, and transmigration. This process is initiated by stimulation of the endothelium by other activated leukocytes or bacterial antigens (22, 23). The activated endothelium expresses high levels of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P- and E-selectins on its surface. Neutrophil recruitment is mainly driven by the binding of P-selectin glycoprotein ligand 1 (PSGL1), ESL1, CD44, and L-selectin (24, 25).

Neutrophil adhesion can be facilitated by the activation of proinflammatory cytokines, chemoattractants, or growth factors. In addition, the adhesion of neutrophils to the endothelium ensures the interaction of chemokines with the heparan sulfates of the endothelial cells (26-28). Neutrophils express high levels of integrins CD11a-CD18 (LFA1/lymphocyte function-associated antigen 1) and CD11b-CD18 (MAC1/macrophage-1 antigen), which bind to endothelial cell surface molecules such as intracellular adhesion molecules 1 and 2 (ICAM1 and ICAM-2) (29). Neutrophil transmigration requires integrins and cell adhesion molecules (CAMS) such as ICAM1, ICAM2, and VCAM1, as well as platelet endothelial cell adhesion molecule 1 (PECAM1, also called CD31), CD99, junctional adhesion molecules (JAMS), epithelial cell adhesion molecules (ECAM), and other endothelial cell molecules (30, 31). Transmigration occurs between paracellular or across transcellular endothelial cells, and to cross membranes, neutrophils release specific proteases such as matrix metalloproteinases (MMPs) and serine proteases (29-31). These enzymes affect neutrophil migration by degrading elastin and collagen molecules and increasing vascular permeability. Proteins are under hormonal regulation during pregnancy, and neutrophils can attract other neutrophils through the expression of interleukin-17 (IL-17), which induces the release of chemokines and cytokines such as interleukin-6 (IL-6) and macrophage inflammatory protein-2 (MIP-2) from other cells that recruit neutrophils (32).

Leukocytes examined in the decidua tissue of matched blood samples showed a significant increase in CD45+ and CD15+ neutrophils migrating into the decidua between 6 and 20 gestation weeks (33). High levels of CD66b expression further characterize these types of polymorphonuclear neutrophils, and CD66b, also called carcinoembryonic antigen-associated cell adhesion molecule 8 (CEACAM8), is expressed specifically on neutrophils and eosinophils and plays important role in adhesion and activation (33, 34). In addition to helping to form thrombi, platelets have a significant impact on different body cells, including blood cells. Along with neutrophils, different cross-linking agents can activate (prime) platelets. This results in the immune response and the emergence of different inflammatory reactions being amplified by the interaction of these two types of peripheral blood cells (35). Platelets express adhesion molecules (E-adhesin), which allows neutrophils to interact with platelets and vice versa, thereby stimulating neutrophil activity (35, 36). These complexes enter vascular endothelial cells and cause intracellular
disruption of calcium metabolism in ion channels (37).

The calcium accumulation in the cytoplasm causes vascular smooth muscle cells to contract more forcefully and for a longer period of time (38). This process also disrupts the endothelial cells' ability to produce NO (nitric oxide), which could be the cause of arterial hypertension in preeclampsia (27). A normal pregnancy involves changes to the cardiovascular system, such as a reduction in vascular tone and reactivity. Patients with preeclampsia who are pregnant show higher vessel reactivity. Resistance arteries also have a decreased capacity for relaxation because there is a decrease in the production of endothelial vasodilator nitric oxide (36-39).

ROLE OF THE PLACENTA IN PREECLAMPSIA

Clinical data and lesions of preeclampsia within days of pregnancy termination indicate that the placenta is a major source of factors contributing to endothelial cell dysfunction in preeclampsic pregnancies (40). Inadequate trophoblastic invasion and unsuccessful uterine artery remodelling are the causes of preeclampsia. In normal pregnancy, the uterine spiral arteries that supply the intervening space of the placenta increase significantly in diameter and become refractory to vasomotor agents (41). This involves the replacement of the endothelium by invading trophoblast cells adopting an endothelial cell adhesion molecule phenotype and the replacement of the internal elastic lamina and smooth muscle by trophoblast and fibrinoid matrix. This transformation is complete by 20 weeks of gestation (42). The venous distension accounts for the increased blood supply to the intervillous space required to meet the demands of the rapidly growing fetoplacental unit during the later stages of pregnancy (43).

Preeclamptic placentas show abnormal expression of integrin molecules that regulate cell-cell and cell-matrix interactions. As a result, trophoblastic invasion is inhibited, and spiral artery remodelling is often limited to the decidual parts so that the myometrial segments do not expand and remain contractile (44). Defective spiral artery remodelling in preeclampsia (and IUGR) likely results in decreased uteroplacental perfusion and foci of placental hypoxia or ischemia. Placental infarcts occur with increased frequency in preeclampsia, consistent with focal ischemia (45). Placental hypoxia or ischemia can lead to the release of products into the maternal circulation, which then initiates the pathophysiological changes in preeclampsia (41, 45, 46).

OXIDATIVE STRESS

Oxidative stress (OS) is defined as “an imbalance between oxidants and antioxidants leading to disruption of redox balance and control and/or molecular damage (47).” During pregnancy, the body's oxidative imbalance negatively affects the development of the fetus and causes various complications, depending on the stage of development. Lipid peroxidation and OS are the main factors responsible for the free radical generation from a poorly perfused placenta, leading to the adhesion of platelets and leukocytes to the vascular endothelium, causing vasoconstriction and increased peripheral vascular resistance (48). In addition, placental vasoconstriction leads to a decrease in uteroplacental circulation, which causes an additional release of inflammatory cytokines and antiangiogenic factors and is involved in the formation of a vicious circle of worsening oxidative stress and the development of vascular endothelial dysfunction. Pregnancy can increase oxidative stress in the body due to increased energy needs and metabolic processes associated with fetal development, but this effect is not absolute and may vary from woman to woman (49).

THE PROOXIDANT AND ANTIOXIDANT SYSTEM IN PREGNANCY

Pregnancy is associated with biochemical changes in which the energy needs and absorption of oxygen by the mother's body increase (50). As a result, OS and ROS/reactive nitrogen species (RNS) production are increasing. OS pathogenesis involves ROS, the most common of which are superoxide (O2•−), hydrogen peroxide (H2O2), and hydroxyl radical (•OH). Under physiological conditions, 95% of the oxygen consumed by the cells is used for mitochondria regeneration, and the remaining 5% is converted into ROS as a result of enzymatic peroxidation (51). During pregnancy, ROS triggers the initiation of a prostaglandin cascade, resulting in preterm labor, cervical dilation, and vasoconstriction leading to preeclampsia. A parallel process is known as nitrosative stress (NS), which is...
Nitric oxide plays a major role in blood pressure regulation and blood vessel relaxation. During pregnancy, NO is produced in the placenta and regulates blood flow to the uterus and fetus. ROS high levels further damage DNA in the cells and interfere with NO, thereby disrupting normal vasodilation and leading to increased formation of the highly reactive ONOO⁻. Peroxynitrite and high levels of NO damage cells and tissues, including the endothelial cells lining the inside of blood vessels, and disrupt the normal function of the placenta (54). As a result of various mechanisms, the increased oxidative stress during pregnancy can be a consequence of: 1) increased formation of free radicals, formed by the oxidation of carbohydrates and proteins, from the autoxidation of fatty acids in triglycerides and phospholipids; 2) exhaustion of the activity of the antioxidant system in the body; 3) disorders of mitochondrial oxidative enzymes and the exchange of prostaglandins and leukotrienes; 4) disorders in glutathione metabolism; 5) influence of gene polymorphism (54).

Malondialdehyde (MDA) is used as an OS marker, i.e. to assess lipid peroxidation. Lipid peroxidation represents oxidative damage to lipids and the increased formation of lipid peroxides, the end product of which is MDA (55). Extremely elevated levels of oxidative stress and lipid peroxidation are observed in pregnant women with pregnancy-induced hypertension. Malondialdehyde values correlated positively with blood pressure values, and the highest MDA values were observed in pregnant women with high blood pressure values (56). This gives us the right to assume that MDA can be used as a marker of the severity of preeclampsia, which will determine the tactics for further management of such patients after receiving the results of in-depth research. ROS production and byproducts of aerobic energy metabolism are maintained at a physiological level of activity by antioxidant substances (57). The imbalance between antioxidants and ROS during oxidative stress is characterized by altered mitochondrial function, decreased protein activity, nucleic acid damage, and the induction of apoptosis (50-52).

Excessive ROS production induces the autophagy process. In early pregnancy, induction of autophagy preserves trophoblast function in the low-oxygen, nutrient-rich placental environment. Inappropriate regulation of the autophagy axis by ROS leads to abnormal autophagy activity and contributes to the development of preeclampsia and intrauterine growth restriction (58). The role of oxidative stress in the development of PE is being actively studied. The obtained results show that it plays a significant role in the development of preeclampsia because it causes damage to the vascular endothelium and, accordingly, vascularization of the placenta, in addition to triggering an immune response in the body. It is also known that in preeclampsia there is a disturbance in the NO production system (59).

The body's antioxidant defense system prevents, eliminates, and restores the effects of the reactions of ROS, RNS, NO, and biological molecules to protect against them (60). According to their mechanism of action, antioxidants are divided into enzymatic: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), ceruloplasmin, heme proteins, thioredoxin (TRX) and paraoxonase (PON1) and non-enzymatic: glutathione (GSH), vitamin E, vitamin C, albumin, bilirubin, uric acid, creatinine, cysteine, carotenoids, flavonoids, coenzyme Q (reduced), metal ion binding proteins (ferritin, metallothionein), and blood plasma proteins (transferrin, ceruloplasmin, albumin) (61). Depletion of the antioxidant defense system during pregnancy increases the level of ROS, RNS, NO and, under certain conditions, can damage fetal tissues and organs, including the lungs, brain, and retina, and thus endanger life or affect the quality of the life of the baby and the mother (60).

Adequate nutrition for a woman in the stage before conception, during pregnancy, and breastfeeding improves the results for the mother and the child, and reduces the frequency of obstetric and neonatal complications. Insufficient intake of essential vitamins and...
trace elements can lead to biological competition with the development of severe consequences for both the mother and the fetus (62). Modern antioxidant therapy is represented by various drugs (α-tocopherol, vitamin C, selenium, α-lipoic acid, etc.) that are used to prevent pregnancy complications, especially in groups at high risk of obstetric complications. Antioxidants are represented by a large group of vitamins (vitamins C, E, A, etc.), flavonoids, and trace elements. The trace elements - iron (Fe), zinc (Zn), copper (Cu), selenium (Se), cobalt (Co), chromium (Cr), molybdenum (Mo), and iodine (I) are vital (essential), in the absence or deficiency in the body of the pregnant woman, disorders occur in the processes of growth and development of the fetus (63).

In a study conducted with pregnant women (from 16 to 22 weeks) at high risk of developing preeclampsia according to Doppler measurements, a decrease in the development of preeclampsia was reported. The women were divided into two groups, one receiving treatment with antioxidants (1000 mg vitamin C and 400 IU vitamin E) and the other a placebo. In the antioxidant group, only 8% of women developed preeclampsia compared to 17% of women on placebo therapy (64). Wibowo et al. (65) reported positive results for preeclampsia in pregnant women with low oxidant status and supplemental antioxidant intake. In the study, the group of pregnant women taking antioxidants had a three-fold lower incidence of preeclampsia compared to the placebo group (p<0.05). In a prospective study by Klemmensen et al., (66), it was reported that the additional intake of vitamins C and E by pregnant women significantly reduced the incidence of preeclampsia. In addition, several studies report that intake of antioxidants can prevent the development of severe forms of preeclampsia/eclampsia/HELLP syndrome, and severe forms of preeclampsia leading to preterm birth (66).

**CONCLUSION**

Pregnancy involves many important biochemical changes, including increased production of hormones (gonadotropin (hCG), estrogens, and progesterone), as well as increased blood supply to the uterus and fetus. These changes are important for the maintenance of pregnancy and the development of a healthy fetus. Preeclampsia is one of the most serious complications of pregnancy, so early detection improves outcomes. There is currently no reliable screening test to predict PE development. Combining biomarkers studied by different methods will likely lead to better predictive performance.

**ACKNOWLEDGEMENTS**

This study was funded by scientific project №5/2023 of Medical faculty, Trakia University, Stara Zagora, Bulgaria.

**REFERENCES**

KOSTADINOVA-SLAVOVA D.


KOSTADINOVA-SLAVOVA D.


46. Jung, E., Romero, R., Yeo, L., Gomez-Lopez, N., Chaemsathong, P., Jaovisidha,


53. Phoswa, W., Khaliq, O. The role of oxidative stress in hypertensive disorders of pregnancy (preeclampsia, gestational hypertension) and metabolic disorder of pregnancy (gestational diabetes mellitus). Oxidative medicine and cellular longevity, 1-10, 2021.


64. Kumar, N., Das, V., Agarwal, A., Pandey, A., Agrawal, S., Singh, A. Pilot Interventional Study comparing fetomaternal outcomes of 150 mg versus 75 mg aspirin starting between 11 and 14 weeks

