# Oxidative stress in preeclampsia, more than enzymes

***El estrés oxidativo en la preeclampsia, más que las enzimas***

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## Introduction

Preeclampsia is a disease characterized by increased blood pressure (blood pressure (BP) > 140 mmHg), proteinuria (300 mg/L/day) and edema, that occurs regularly after the twentieth week of gestation. There are risk factors such as chronic hypertension, diabetes mellitus and obesity to the development of this disease. Preeclampsia is pregnancy-specific, affecting 2% to 7% of women, and is a leading cause of perinatal and maternal morbidity and mortality. Preeclampsia has been associated with growth retardation, low birth weight and premature birth, mothers may have placental abruption, renal failure, cerebral hemorrhage, disseminated intravascular coagulation and circulatory collapse.[1-3]

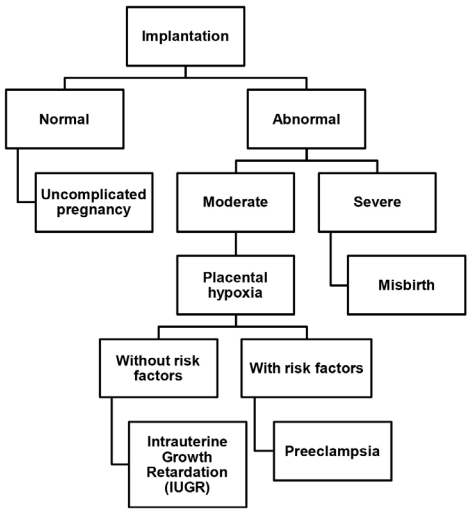
### Clinical features

Clinically, two types of preeclampsia have been established: 1) mild preeclampsia: when the patient is 140 mm Hg < BP <160 mmHg, 300 mg/L < proteinuria < 2 g/L, or its equivalent strip, 2) severe preeclampsia: when the patient has BP > 160 mmHg at rest on two occasions with a difference between measures of 6 h and proteinuria > 2g/L/day, or 3(+) to 4(+) by dipstick of a random sample, oliguria, edema 2(+) and / or cerebral or visual disturbances, epigastric pain, pulmonary edema, cyanosis, impaired liver function and thrombocytopenia.

### Pathophysiology

The pathogenesis of preeclampsia is still not completely understood due to multiple factors surrounding the onset of the disease, although it has been considered the hypothesis that endothelial dysfunction is a vital point, because changes in the structure of maternal vascular endothelial function generates multisystem damage. In an initial attempt the pathogenesis of preeclampsia has been divided into two stages: alterations of placental perfusion and maternal syndrome. Alteration of placental perfusion is associated with a failure of trophoblast invasion by mechanical factors, biochemical and / or immunological. In case of preeclampsia the invasion is deficient which favors fibrin and thrombosis, triggering atherosclerosis by modifying the size of normal veins, followed by ischemia and poor placental perfusion. Moreover, the maternal syndrome is characterized by higher BP and impaired renal function, there is vasospasm decreasing perfusion to all organs by the presence of pressor agents and activation of platelets (Fig. 1).

**Figure 1. Placental implantation process**

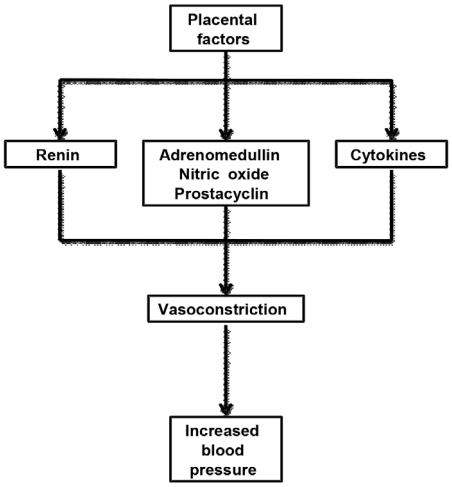



In women with preeclampsia it has been observed an increase in certain cytokines such as tumor necrosis factor alpha (TNF-a), an inducer of hypoxia-stimulated endothelial dysfunction. Cytokines activate neutrophils that generate oxidative insult. During placental development in women with preeclampsia there is a deficient signaling of

epidermal growth factor in combination with heparin (HB-EGF), responsible for trophoblast invasion, thus amending the trophoblast survival, differentiation and invasion, causing poor placental perfusion and hypertension. Another factor that influences the endothelial dysfunction is the alteration of production and disposal of nitric oxide (NO•) leading to increased peripheral resistance, BP and sensitivity to vasopressors.

It has also been shown that in the plasma of preeclamptic women the nuclear factor factor-kappa B (NF-κB) becomes active initiating a systemic inflammatory process by promoting the synthesis of cytokines (monocyte chemotactic protein (MCP), interleukin (IL)-6 and 8) and the expression of intracellular adhesion molecule-1 (ICAM-1), which maintains the systemic inflammatory process (Fig. 2). These same mechanisms are involved in the genesis of atherosclerosis, also seen in the spiral arteries of women with preeclampsia.

**Figure 2. Mechanisms involved in the development of hypertension of preeclampsia**




## Obesity and preeclampsia

Obesity is associated with chronic activation of inflammatory pathways in both adipocytes and in macrophages residing in or infiltrating the adipose tissue.[4] The possible ligation of Toll-like receptors on adipocytes or macrophages by dietary lipids could exploit the canonical inflammatory signaling pathway to activate NF-kB and produce inflammatory mediators.

## Oxidative stress

### 2.1. Free radicals

A free radical is a molecule or atom with one or more unpaired electrons in its outermost orbital, making it highly reactive and unstable, causing a chain reaction that damages cells and tissues.[5] Oxidative stress is defined as the biochemical imbalance by excessive production of free radicals that can not be controlled by the antioxidant systems. The damage occurs due to free radical generating covalent links among proteins, unsaturated fatty acids of biological membranes, carbohydrates and nucleic acids, inducing conditions such as diabetes, atherosclerosis, inflammation, ischemia / reperfusion, among others.[6;7]

The oxygen-derived free radicals superoxide anion ($O_2$-) and hydroxyl radical (OH•) are grouped under the term Reactive Oxigen Species (ROS) and stand out above others both by their abundance and their damage to biological systems. The hydrogen peroxide ($H_2O_2$) is not a free radical, but falls into the category of ROS because it is decomposed in the presence of transition metals (mainly Fe $2^+$) to produce OH•. Also, nitrogen is also capable of forming free radicals such as two oxides: the radical NO• and nitrogen dioxide radical ($NO_2$•), forming the so-called reactive nitrogen species (RNS). In turn, the radicals $O_2$• - and NO• react together to form peroxynitrite anion (ONOO-), among others.[7]

### 2.2. Nuclear factor Kappa-B (NF-κB)

Oxidative stress is a potent inducer of placental synthesis and release of proinflammatory factors. Most of these effects are mediated through the p38 mitogen-activated protein kinase (MAPK) and NF-κB pathways.[8;9] NF-κB signaling pathway is a complex network that regulates a cellular pathway involved in a myriad of physiological and pathological scenarios. There are at least two well-characterized signaling pathways leading to NF-κB activation, classical and alternative, and both rely on the catalytic activity of known IκB kinases (IKKs). The classical NF-κB signaling pathway is typically triggered by a vast number of proinflammatory cytokines, viruses, and bacteria, and hence, it leads to a coordinate inflammatory/immune response culminating in the expression of multiple cytokines, chemokines, adhesion molecules, and proinflammatory proteolytic enzymes. Alternative NF-κB signaling pathway is normally triggered by non-proinflammatory cytokines (e.g., lymphotoxin β (LTβ), B-cell activating factor (BAFF), and CD40 ligand (CD40L)) as well as some viruses (e.g., human T-cell leukemia virus (HTLV) and Epstein-Barr virus (EBV)).[10;11]

NF-κB signaling pathway is regulated by IKB family. Most stimuli activate NF-κB through IKB kinase-dependent (IKK-dependent) phosphorylation, polyubiquitination and subsequent proteasomal degradation of IKB proteins. The liberation of NF-κB allows translocation of heterodimer to the nucleus, where it can regulate the expression of specific genes typically involved in immune and inflammatory responses and in cell growth control.[12] Inducible genes that are known to be transactivated by NF-κB include, but are not limited to, IL-1β, IL-6, IL-8, TNF-a, interferon gamma (IFNγ), MCP-1, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1).[4]

By microarray studies it has been showed that in placenta of preeclamptic women there are increased expression of genes related to chemotaxis and the NF-κB pathway and decreased expression of genes related to antigen process-

ing and presentation, such as human leukocyte antigen B.[13] Although enhanced apoptosis is well known in placentas with preeclampsia, the role of transcription factor NF-κB in the process is still being debated. Increased trophoblastic apoptosis is at least partially induced by NF-κB.[14]

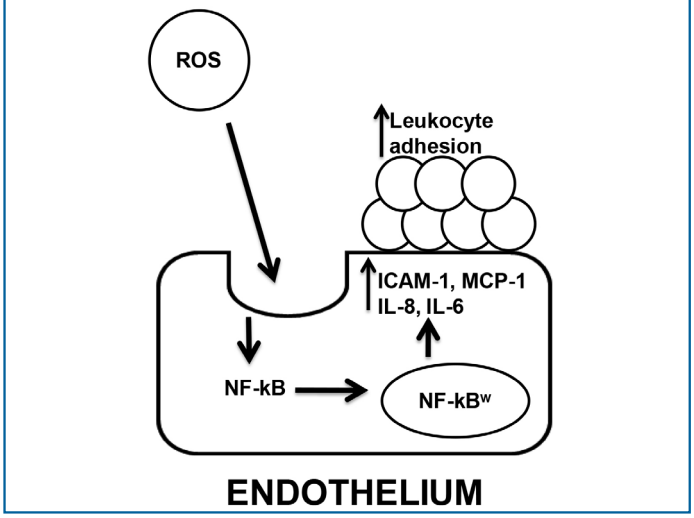
Glucocorticoid receptors can modulate NF-κB activity in the nucleus by regulating several key events including NF-κB DNA binding. Furthermore, glucocorticoids may serve as inhibitors of NF-κB activity via their ability to modulate the activity of proteins involved in the MAPK pathways including c-jun N-terminal kinase (JNK), p38, and MAP kinase phosphatase-1 (MKP-1), all of which can crosstalk and modulate NF-κB activity, and thus, inflammatory responses. [15-17]

Administration of vitamins C and E blocks activation of the p38 and stress-activated protein kinase MAPK and NF-κB pathways. Vitamin administration or p38 pathway inhibition also reduced COX-2 expression, TNF-a and IL-1β secretion, and the levels of apoptosis.

## Preeclampsia, oxidative stress and antioxidant systems

Pregnancy increases oxidative stress, a condition that can be aggravated with preeclampsia, because free radicals are detrimental to the integrity of the endothelium, causing maternal vascular dysfunction. According to this knowledge, preeclampsia is a condition where there is an imbalance between the endogenous antioxidant system and free radicals, mostly ROS. These species are caused by: mitochondrial aerobic metabolism, activation of NADPH oxidase, xanthine oxidase (XO), cytochrome P450 and lipid peroxidation process (Fig. 3).[18]

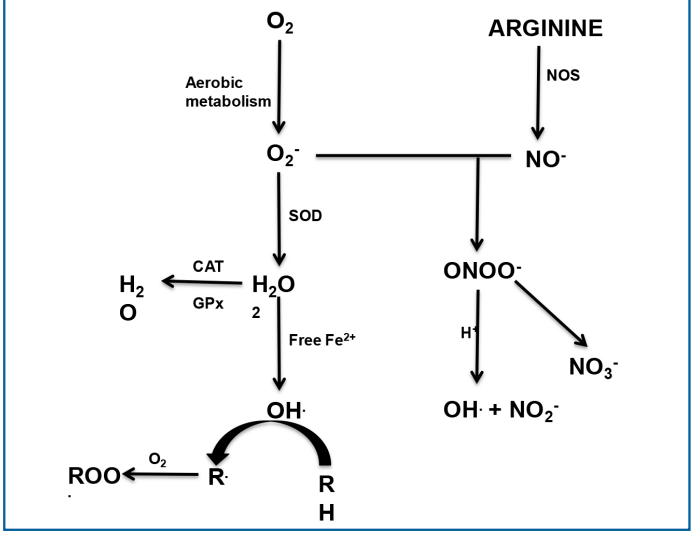
**Figure 3. Mechanisms that trigger the increase in leukocyte adhesion in preeclampsia**




ICAM-1: intracellular adhesion molecule-1, IL-6: interleukin-6, IL-8: interleukin-8, MCP-1: monocyte chemotactic protein-1, NF-κB: nuclear factor Kappa-B, ROS: reactive oxigen species

To stop the action of oxidizing agents or free radicals, the body has endogenous antioxidant mechanisms which are responsible for the degradation of those products, these may be enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)) and nonenzymatic (vitamin E, C, carotene, glutathione) (Fig. 4).[7;19] Under physiological conditions, these defense mechanisms maintain a low concentration of ROS in the cell and its activity is regulated, hence the balance between ROS production and antioxidant defenses determines the degree of oxidative stress.

**Figure 4. Intracellular formation of reactive oxigen species**




CAT: catalase, GPx: glutathione peroxidase, NOS: nitric oxide synthase, $NO_2$•: nitrogen dioxide radical, $NO_2$•: nitrogen trioxide radical, OH•: hydroxyl radical, ONOO-: peroxynitrite anion, SOD: superoxide dismutase

## Implications of oxidative stress

The ROS play an important role in the pathogenesis of premature delivery, fetal growth restriction, preeclampsia, eclampsia, hemorrhage and cerebral edema, cortical blindness, retinal edema and blindness, renal tubular necrosis, pulmonary edema and laryngeal edema, HELLP syndrome and hepatic rupture, placental infarction and placental abruption, maternal infections and maternal malnutrition.[20]

## SOD

SOD is the first antioxidant and is responsible for breaking two molecules of $O_2$- to $H_2O_2$, which in turn CAT of peroxisomes makes molecular $O_2$ and water. The SOD contains metal ions with redox activity in its catalytic center and there are three isoforms: 1) CuZn-SOD, cytosolic, 2) Mn-SOD, specifically in the mitochondria and 3) extracellular-SOD, secreted by many cells, including placental cells.[21] Deficiency in SOD activity in the placenta may increase the production of $O_2$- and the generation of lipid, protein and DNA oxidation. The SOD and NO• compete for $O_2$-, SOD deficiency causes increased $O_2$- and the interactions of the NO•, reducing vasodilation and resulting in the generation of ONOO- which subsequently leads to tyrosine nitrosylation protein. It also starts a chain reaction which affects polyunsaturated fatty acids due to the loss of $H^+$, forming hydroperoxides and other free radicals, causing damage to the endothelium. In addition, SOD modulates differentiation of trophoblast and its decrease leads to a greater sincitialisation that leads to apoptosis and increases the volume of trophoblast.[22]

**GPx**

The glutathione system comprises four major components, three enzymes: GPx, GR and glutathione-S-transferase (GST) and a non-enzymatic, reduced glutathione (GSH). There are five different types of GPx containing selenocysteine in its active site. GPx4, GPx1 and cytosolic enzymes are abundant in many tissues, GPx2 expressed in the gastrointestinal tract, GPx3 in the liver (form found primarily in the bloodstream) and GPx5, not selenium (Se) dependent, is specifically expressed in the epididymis. All of these isoenzymes reduce $H_2O_2$ and alkyl hydroperoxides, however, their specificity for the substrates can be different.[23]

Research has shown that the human placenta produces and secretes GPx3 and plasma levels increase during pregnancy, but other researchers report GPx levels unchanged or decreased in plasma and erythrocytes near the end of pregnancy. GPx levels in erythrocytes and plasma are highly sensitive to Se intake and this element is widely used for monitoring this enzyme. The decreased activity of GPx during preeclampsia contributes to oxidative stress and increased biological oxidation.[23] Reduced GPx could be associated with increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of preeclampsia.[24]

**Prevention and treatment of preeclampsia**

Clinical data (vasospasm, headache, blurred vision, epigastric pain, nausea and vomiting, diuresis), biophysical parameters (heart rate, blood pressure) and biochemical tests (proteinuria, blood count, platelet count, prothrombin time, partial thromboplastin time, urea, creatinine, uric acid, albumin, bilirubin, ALT, AST, LDH) allow the prediction or early detection preeclampsia, but have a low sensitivity and predictive value. Thus, preventive studies have focused on demographic, family, medical and obstetric complications associated with an increased risk of preeclampsia.[25;26]

Some methods have been developed to prevent and treat preeclampsia: a) dietary manipulations: low-salt diet, fish oil supplements, calcium supplements, b) magnesium sulfate: to prevent seizures and reduce the recurrence of seizures, c) cardiovascular drugs: diuretics, antihypertensive drugs, d) tocopherol (vitamin E) and ascorbic acid (vitamin C) and e) antithrombotics: low doses of aspirin (ASA), ASA / dipyridamole, ASA + heparin, ASA + ketanserin. Despite all these efforts, interruption of pregnancy is the definitive treatment.

## Conclusion

Preeclampsia is a hypertensive disorder of pregnancy in which enzymatic antioxidant defenses fail and tissues are injured. Antioxidant enzyme activity (SOD, GPx) is significantly decreased, while lipoperoxidation is increased in preeclamptic groups compared to normal pregnant women.[27] Beyond the enzymatic behavior, the NF-kB pathway unites the inflammatory and metabolic responses and, represents a common point for better understanding pregnancy and its predisposition to preeclampsia, also represents an alternative to develop new drugs to control this obstetric complication.

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