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RESEARCH ARTICLE

PATHOPHYSIOLOGY AND BIOMARKERS OF PRE-ECLAMPSIA

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ABSTRACT

Preeclampsia is a pregnancy specific syndrome, characterized by high blood pressure and proteinuria after 20 weeks of gestation in previously normotensive women.
Preeclampsia accounts over 60,000 maternal deaths annually worldwide.
In the pathophysiology of preeclampsia is involved abnormal uterine vascular changes, placental ischaemia, increased oxidative stress, release of antiangiogenic proteins into maternal plasma, excessive maternal inflammatory, endothelial injury, generalized endothelial dysfunction, atherosis, , hipercoagulative state, multiorganic manifestation, genetic implication.
There were identified and studied maternal serum markers for detection of preeclampsia. Those markers that prove to be the most effective in prediction of preeclampsia could enter in clinical practice.

INTRODUCTION

Preeclampsia is a pregnancy related disease characterized by hypertension: 140/90mm Hg or greater taken twice a day more than 4 hours apart after 20 weeks of gestation (except molar pregnancies) and proteinuria: 0.3 g or greater after a 24-hour urine sample in previously normotensive women [ACOG, 2002].

Preeclampsia (PE) affects 2-8% of all pregnancies [Cnossen JS, 2009], accounting over 60,000 maternal death annually worldwide [Duhig KE, 2015] and about 1.8% perinatal deaths [Ghulmiyah L, 2012].

Although older and recent research, the exact cause of preeclampsia remains unknown. PE is a two stage disorder [Cunningham FG, 2014, Redman CW, 2014]: stage I is caused by defective placentation, faulty endovascular trophoblastic remodeling of spiral arterioles which produces placental hypoxia; stage II, "maternal preeclampsia" is linked to preexisting maternal conditions such as: obesity [Mbah AK, 2010], diabetes, cardiovascular and renal diseases, immunological disorders or hereditary influences, more susceptible to developing endothelial cell activation or inflammation.

Defective implantation

In normal pregnancy, between 12 and 20 weeks of gestation, spiral arterioles invasion by extravillous cytotrophoblast occurs within decidua basalis and even into the inner one-third of the myometrium. This invasion induces spiral arteries enlargement, removing these vessels from the vasopressors control. It is an adaptative phenomenon for uteroplacental blood flow, increasing necessary for maternal-fetal exchanges [Cunningham FG, 2014, Brosens IA, 1972, Churchill D, 1999, Huisman MA, 2010].

Stallmach,1999 *et al*. believe that in PE there is an abnormal immunological conflict between trophoblastic cells and maternal immune defense cells; in decidua increases the number of maternal natural killer cells CD56 and lymphocytes $CD8^{+}$T followed by lack or faulty cytotrophoblastic invasion in uteroplacental arteries or their atherosis. Decidual natural killer cells (dNK) are found in direct contact with trophoblasts and stromal cells; they produce proangiogenic factors: VEGF (Vascular Endothelial Growth Factor) and PlGF (Placental Growth Factor) which promote vascular growth in decidua. Decidual NK also produce cytokines and chemokines and their cytotoxic potential is blocked during normal pregnancies.

Uteroplacental flow

It was noticed that Na^{+} injected into intervillous space has been removed 2 or 3 times faster in normotensive pregnant women compared with preeclamptic women in which a reduced uteroplacental blood flow was present [Berceanu Sabina, 1998].

Several studies [Churchill D, 1999, Rohini S Deshpande, 2007, Tobinaga CM, 2014] have shown in women which will develop PE an appearance of a depression of systolic wave, a

"notch", in 22-24 weeks of gestation or even earlier in 12-16 weeks, when the ultrasound exploration is done with vaginal transducer. Using a three-dimensional power Doppler histogram, Pimenta,2013 *et al*. described the placental vascularity index which was decreased in women with any pregnancy-associated hypertensive disorders.

Endothelial lesions

Using electron microscope in PE was found: endothelial damage, areas where the endothelium was wholly lacking, penetration of plasma constituents in the vascular wall, myointimal cells proliferation, invasion of the vessels wall by foamy macrophages, changes referred to as acute atherosis [Cunningham FG,2014]. Consecutively, is troubled the balance between vasoconstrictor factors, especially thromboxane and endothelin which are elevated in PE, and the vasodilators: prostacyclin and nitric oxide. The effects of these changes include a reduction in uteroplacental flow and placental hypoxia. Maternal vascular diseases like chronic hypertension, autoimmune diseases with hypertension and multiple pregnancies with increased trophoblastic tissue mass predispose to PE, possible by reduction of uteroplacental blood flow. These ischemic lesions may be followed by the release of placental factors into maternal circulation which in turn produces extensive endothelial damage, characteristics of PE and E (eclampsia).

In preeclamptic patients, was found cytokines, sign of inflammation: IL-1β [Adams KM,2003], IL-6 [Adams KM, 2003, Roland Linda, 2010, Udenze Ifeoma, 2015, Mihu D, 2014, Lau SY,2013], IL-8 [Adams KM,2003], IL-10 [Lau SY,2013], IL-16 [Adams KM, 2003, Yang Gu, 2008], IL-18 [Adams KM, 2003, Roland Linda,2010], TNFα [Adams KM, 2003, Udenze I, 2015, Mihu D,2014, Lau SY, 2013, Sibai BM,2009], and CRP (C-reactive protein) [Udenze I, 2015] that increase in placenta as well as in peripheral blood. These cytokines have been proposed as predictive markers of PE.

Even in the first trimester of pregnancy an abnormal immunological or inflammatory response generate an increase of PTX3 (pentraxin) which belongs to the same family as CRP. PTX3 levels in women who subsequently develop PE are already elevated in the first trimester. To identify pregnant women who will develop PE, Cetin I 2009 *et al*. propose to determine the levels of PTX3 in the first trimester of pregnancy.

Baschat AA, 2009 *et al*. had determinated by ELISA method PTX3 blood levels in 11-14 weeks of gestation. The authors didn't found a statistically significant relationship between serum PTX3, maternal demographics and placental Doppler studies in the first trimester of pregnancy in women who subsequently develop PE.

Ozler A,2012 *et al*. studied in PE and HELLP syndrome serum levels of neopterin (NEO), an important indicator of cellular immunity secreted by activated monocytes, TNFα and IL-6. The authors found serum levels of NEO well correlated with severity of PE. but no significant differences in the serum levels of IL-6 and TNFα between the groups (uncomplicated pregnancy, mild PE, severe PE, HELLP syndrome)

Endothelial dysfunction

The human endothelium, which weighs 1.5 kg, is considered the largest autocrine, paracrine and endocrine organ. This organ regulates vascular tone, platelet activation, monocyte adhesion, thrombogenesis, inflammation, lipid metabolism and vessels growth and remodeling [Espinoza J, 2010]. In PE, endothelial injury is demonstrated by the increased levels of substances released by endothelial cells that comprise: fibronectin, laminin, tissue plasminogen activator, plasminogen inhibitor-1 and von Willebrand factor. Damaged endothelial surfaces will increase platelet adherence and clot formation; it releases platelet-derived growth factors, vasoconstricting agents and procoagulants, which accelerates the coagulation [Churchill D, 1999]. The resulting vasospasm and microthrombi will cause additional endothelial cell damage which also will further reduce uteroplacental blood flow in maternal organs (kidney, liver, lung, brain) giving rise to a vicious circle. The increased production of vasoconstrictors (endothelin, thromboxane, angiotensin, serotonin) and a reduced synthesis of vasodilators (prostacyclin, prostaglandin E_2, nitric oxide) associated with an enhanced response to vasopressor agents (angiotensin II, noradrenaline, PGF2α, thromboxane) will increase the vasospasm and consecutively the blood pressure. Circulating levels of endothelin, a potent vasoconstrictor produced by damaged endothelial cells, are increased in PE [Espinoza J, 2010, Veillon EW, 2009, Frey HA, 2013, Stjernquist M, 1998].

The severity of PE consecutively to endothelial damage can be identified by increasing uric acid, serum creatinine and creatinine clearance levels.

In PE also increase plasma atrial natriuretic peptide levels [Adam B, 1998].

Nitric Oxide and preeclampsia

Nitric oxide (NO) plays an important role in maintaining feto-placental blood flow and in lowering the resistance in the peripheral maternal precapillary arterioles. NO is synthesized from L-arginine by endothelial cells under the influence of the nitric oxide synthase (NOS) enzyme and it is calcium-dependent. In endothelial cells after stimulation by stress factors, bradykinin, serotonin, NO diffuses into the smooth muscle cells of the vascular walls, where it activates guanylate cyclase which increases cGPT (cyclic guanosine monophosphate) from GPT; cGPT closes calcium channels, it decreases intracellular calcium and subsequently produces, smooth muscle relaxation in vessels. NO is inactivated by reactive oxygen species (ROS) which increase in PE oxidative stress and this inactivation induce maternal and fetoplacental arterial circulation dysfunction. NO is a vasodilator and platelets antiaggregant.

Experimentally blocking NOS in pregnant rat produce a similar syndrome to PE [Costantine MM,2009].

Angiogenic and antiangiogenic proteins

Placental vasculogenesis is dependent on many factors involved in angiogenesis and its limitation [Akbar SA. 2009, Audibert F, 2010, Massuyama H, 2010, Goel A, 2013, Troisi Rebecca 2008]. VEGF and angiopoietins family are the main angiogenic factors [Goel A,2013]. Angiogenesis limitation is controlled by several antiangiogenic factors in relationship with the induced "dialog" between human chorionic gonadotropin (hCG) and extravillous trophoblast. Another angiogenic factor is PlGF [Troisi Rebecca, 2008]. An antiangiogenic factor involved in the development of PE is soluble Fms-like tyrosine kinase 1 (sFlt-1) which is a variant

of Flt-1 receptor for PlGF and VEGF. Elevated maternal serum levels of sFlt-1 decrease and inactivate plasma concentrations of free PlGF and VEGF and this leads to endothelial dysfunction [Sibai BM, 2008]. Another antiangiogenic factor is s-endoglin (sEng) also called CD105, which is a coreceptor of TGFβ (Transforming Growth Factor β) family [Baumann MU, 2008]. The increase of antiangiogenic factors like sFlt-1 and sEng occurs long time before the onset of clinical symptoms of PE and can be used in early detection of severe PE (24-32 weeks of gestation) and of late PE (over 35 weeks of gestation) [Baumann MU, 2008]. In several studies about PE was investigated either singular determination of these factors [Duhig KE, 2015, Massuyama H, 2010] either the ratio between them and angiogenic factors [Troisi Rebecca 2008, Karumanchi A 2014, Haggerty CL, 2012, Vatten LJ, 2012, Verlohren S, 2010].

Wathen K-A, 2009 *et al*. have studied maternal serum endostatin an anti-angiogenic factor produced by endothelial cells. The authors concluded that maternal serum endostatin concentrations were elevated in 16-20 weeks of gestation women and was associated with subsequent PE.

Vatten JL, 2012 *et al*., Shokry, 2010 *et al*., Sunderji S,2010 *et al*. concluded that low PlGF and elevated sFlt-1 levels in maternal circulation are associated with subsequent development of PE.

Espinoza J, 2010 *et al*. communicated that an excess of antiangiogenic factors are involved in PE, placental abruption, HELLP syndrome, Mirror syndrome. Some authors found that in PE angiogenic imbalances was associated to parvovirus and cytomegalovirus infections [Xie F,2010], twin-to-twin transfusion syndrome, unexplained fetal death, fetal growth restriction. It seems that angiogenic imbalance is not necessary or sufficient for the development of PE because not all women with pregnancy complications have increased values of antiangiogenic factors. In addition, angiogenic imbalances are also found in other diseases and complications of pregnancy than PE. These imbalances can have a genetic predisposition or other factors may determine PE. Mirror syndrome or Balantine's syndrome consists in associating a fetal hydrops with placentomegaly and severe maternal edema but the association with PE is only in 50% of cases. Elevated antiangiogenic factors were found in partial or complete mole with early-onset preeclampsia before 20 weeks of gestation. The authors consider that chronic uteroplacental ischemia limits the nutrients for fetus who may signal the placental release of antiangiogenic factors that increase the maternal blood pressure in attempt to compensate for limited blood flow in placenta and fetal tissues.

Massuyama H, 2010 *et al*. have studied the profiles of circulating angiogenic factors and adipocytokines in order to differentiate pregnant women who will have early-onset PE (24-32 weeks of gestation) compared to late onset PE (over 35 weeks of gestation). The sFlt-1/PlGF ratio in early-onset PE was significantly higher than that in late-onset PE. It was associated with a significant increase of leptin in both subgroups of PE.

Buhimschi IA, 2010*et al*. concluded that the determination of urinary sEng can't predict the type of PE (early or late onset) and its severity. The determinations of sFlt-1 and sFlt-1/sEng ratio are superior in diagnosis, date of appearance of PE, its severity and evolution.

For prediction a prognosis in the current pregnancy, Salomon LJ,2003 *et al*. and Sibai MB,2008 *et al*. had determined serum inhibin A, PlGF, sFlt-1, elevated in severe PE, and PlGF/sFlt-1 ratio in pregnant women between 12-19 and 24-28 weeks of gestation which had had a previous history of PE or chronic hypertension. The levels of these factors had no value in predicting PE during current pregnancy.

Byers BD, 2009 *et al*., studied a mouse obese model treated with sFlt-1; the offspring of obese mice had also obesity, vascular dysfunction and preeclampsia-like syndrome.

Buhimschi IA, 2008 *et al*. have determined the proteomic profiling of urine and have identified fragments of SERPINA1 (Serine peptidase inhibitor) and albumin as biomarkers of PE before the appearance of clinical symptoms.

Roberts MJ, 2008 analyzed Buhimschi proteomix in the diagnosis and pathophysiology of PE and he believed that this new method, proteomix would identify PE with severe evolution and maternal life-threatening (eclampsia, abruption placentae, HELLP syndrome, renal failure) which can have different urine proteic markers.

Wortelboer EJ, 2010 *et al*. had investigated the predictive value of maternal serum pregnancy-associated plasma protein A (PAPP-A), free β subunit of human chorionic gonadotrophin (fβhCG), placental protein 13 (PP13), PlGF, desintegrin a and metalloproteinase 12 (ADAM12) for the first trimester identification of early-onset PE. The authors had demonstrated that PP13, PlGF and PAPP-A values were lower in women who had developed early-onset PE whereas ADAM12 and βhCG did not differ in control women and in those with PE. In PE complicated by SGA (small-for-gestational-age) fetuses, all markers, except f βhCG had lower values.

Audibert F, 2010 *et al*., conducted a screening for PE and HELLP diagnosis using first trimester maternal serum markers like: PAPP-A, free βhCG, inhibin-A, PP13, PlGF, desintegrin a, MMP12 (ADAM12) associated with uterine artery Doppler examination in nulliparous women at 11-13 weeks of gestation. A combined screening model with clinical characteristics, PAPP-A, inhibin –A, PlGF could detect 75% of early-onset PE at a 10% false-positive rate.

Romero R *et al*. 2008 determined PP13 in maternal plasma concentrations and found low levels in early-onset and severe late-onset PE. In early onset PE the sensitivity was 100% and the specificity of 85%.

Ajayi F *et al*. 2008 found in PE elevated expression of serine protease HtrA1 involved in trophoblast cell migration and invasion.

Lipid peroxidation and fatty acids metabolism in preeclampsia

Pregnancy is accompanied by hyperlipidemia induced by pregnancy hormones, especially Human Placental Lactogen [Wiznitzer A, 2009]. During pregnancy increase triglycerides [Wiznitzer A, 2009], free fatty acids, cholesterol [Wiznitzer A, 2009], LDL-C (low-density lipoprotein cholesterol), HDL-c (high-density lipoprotein cholesterol), VLDL (very low-density lipoprotein cholesterol), lipoproteins (a), apolipoproteins A-1 (Apo A-1), and B (Apo B) which are involved in the etiology of atherosclerosis.

In a multivariate analysis, Wiznitzer A *et al*. 2009 found that the triglycerides levels but not HDL levels were associated with pregnancy complications (PE, diabetes mellitus).

Oxidative stress

Recent studies have confirmed a common pathogenic mechanism in PE, pulmonary cancer, lung metastases from breast cancer, myocardial infarction and graft rejection in heart transplant named oxidative stress which consists in the presence of active oxygen substances in excess comparatively to buffer capacity of antioxidants [Roberts MJ, 2004].

The common *antioxidants* include: vitamin C, E, β-carotene, glutathione [Mickhael MS, 1994]. In PE, in the presence of hyperlipidemia due to vasospasm, is formed by electron transfer mechanisms, reactive oxygen species that lead to synthesis of peroxides from toxic lipid and protein oxidation.

Markers of oxidative stress include a variety of metabolic byproducts as thiol oxidized, lipid peroxides and isoprostanes. Lipid peroxidation of polyunsaturated fatty acids cause the release of volatile compounds generically called alkanes and their methylated products. Till now have been isolated 107 such compounds involved in oxidative stress and demonstrable in respiratory air [Moretti M, 2004]. The determination of these oxidative stress markers could allow the diagnosis of PE before the appeareance of clinical signs of it.

Lipid peroxides are unstable toxic compounds that can affect endothelial cell functions by various mechanisms present in PE, particularly increased capillary endothelial permeability and platelet aggregation [Vatten LJ, 2012, Moretti M, 2004].

In PE, prostacyclin synthase and cyclooxygenase enzymes are inhibited by high levels of lipid peroxides with decreased release of endothelial prostacyclin and increasing ratio Thromboxane/Prostacyclin. Lipid peroxides would increase calcium penetration into vascular smooth muscle cells and potentiate vasoconstriction. Oxidized LDL increases the adhesion of neutrophils to the endothelium followed by the release of IL-1β from mononuclear cells. LDL also lowers synthesis of prostacyclin, increases production of endothelin, activates nitric oxide synthesis and increases platelet activation of PAF-1 (Platelet Aggregation Factor 1).

There were investigated more peroxides which could be oxidative stress markers such as malonyldialdehyde (MDA), lipoproteins a, apolipoproteins Apo A and Apo B. MDA found constant increased in PE could be a marker test for severe PE [Var A, 2003]

Catarino C *et al*. 2008, studied in PE fetal lipoproteins changes in relationship with maternal lipoproteins. In PE women were found significant increased values of total cholesterol, LDLc, HDLc, TG, apoA1 and apoB. In PE, in fetal umbilical cord blood were found decreased values of HDLc and apoA1 and significant increased values of TG, LDLc/HDLc. It was found a positive relation between triglycerides (TG) and proteinuria.

Baker AM *et al*. 2009 had determined lipid profiles in 50 women at 15-20 weeks of gestation. Pregnant women with mild PE had higher TG levels and a higher total cholesterol and HDL ratio than controls. Women with severe PE had lower levels of LDL than controls and a less atherogenic lipid profile than controls. The authors consider that midgestation dyslipidemia is associated with mild but not severe PE. This findings suggests a different pathophysiology between mild and severe PE.

Reactive oxygen species may be involved in endothelial cell dysfunction and PE. Several studies have shown that lipid peroxidation products such as malonyldialdehyde and F2-isoprostanes are elevated in the plasma and placentas of women with PE.

The glutathione peroxidases (GPx) play a critical role in the control of lipid peroxidation especially in catalyzing of H_2O_2, lipids and other organic hydroperoxides Boutet M, 2009. Boutet M *et al*. 2009 have found an increase of HO-1 mRNA levels and of the glutathione peroxidases (GPx-1, GPx-4) which can be exploited as first trimester markers to identify pregnant women who will develop PE.

Genetic predisposition

In PE was identified a gene mutation T235 of angiotensinogen. The HLA-G (Human Leukocyte Antigen G) polymorphism gene was also correlated to PE [Churchill D, 1999]. Triche EW *et al*. [2014] identified from 22 million records in PubMed, 729 articles about 535 genes associated with PE.

CONCLUSION

The primary event in the development of PE appears to be in relationship with abnormal trophoblastic invasion of uteroplacental vessels and their lack of enlargement followed by keeping reactivity to vasoconstrictors. The cause of this impaired implantation and their sequence were not well defined but is believed to be followed by placental hypoxia which subsequently leads to the release of factors in maternal circulation. The nature of these factors is not clarified but it is believed that they produce a generalized vascular endothelial dysfunction constant present in pregnant women with PE. The roles of reduced production of prostacyclin and nitric oxide, increased production of thromboxane, endothelin, increased sensitivity to angiotensin II appear to be secondary. Recent studies on the involvement of the fatty acids and lipoproteins metabolism may require clarifications.

The vasospasm followed by a low perfusion of various tissues and organs underlies the pathophysiology of PE. Vasoconstriction produces resistance to blood flow and consequently increase blood pressure. In severe PE, the vasospasm is associated with vascular endothelial damage followed by loss of vascular endothelial tightness. Local tissue hypoxia and vasospasm leads to hemorrhage, necrosis and other organic disorders. There are some data that suggests that PE would be associated with an abnormal production of vasoconstrictor prostaglandins like thromboxane and with decreased synthesis or increased inactivation of prostacyclin, nitric oxide and other vasodilators or their associations.

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