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ESTUDO DE MICROPARTÍCULAS NA PRÉ-ECLÂMPSIA GRAVE

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ESTUDO DE MICROPARTÍCULAS NA PRÉ-ECLÂMPSIA GRAVE

Diss

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LISTA DE ABREVIATURAS

APC – *allophycocyanin*, aloficocianina

BMI – *body mass index,* índice de massa corporal

CD – *cluster of differentiation,* cluster de diferenciação

CID – coagulação intravascular disseminada

COEP – Comitê de Ética e Pesquisa

COX-2 – *cyclooxygenase-2,* ciclooxigenase-2

CXCR4 – *CXC chemokine receptor type 4,* receptor de quimiocina CXC do tipo 4

Cy5 – *cyanine 5,* cianina 5

DBP – *diastolic blood pressure*, pressão sanguinea diastólica

FITC – *fluorescein isothiocyanate,* isotiocianato de fluoresceína

FSC – *foward scatter,* dispersão frontal

GA – *gestational age*, idade gestacional

GLA – *gama-carboxyglutamic acid*, ácido gama-carboxiglutâmico

HELLP – *hemolysis, elevated liver enzymes and low platelet*, hemólise, enzimas hepáticas elevadas e plaquetas baixas

I-CAM 1 – *intercellular adhesion molecule* 1*,* molécula de adesão intercelular 1

IgG1 – *immunoglobulin G1*, imunoglobulina G1

IgM – *immunoglobulin M*, imunoglobulina M

INFγ – *interferon γ*

iNOS – *inducible nitric oxide synthase*, óxido nítrico sintase induzível

IL – *interleukin*, interleucina

mmHg – milímetro de mercúrio

MP – *microparticle*, micropartícula

mRNA – *messenger ribonucleic acid,* ácido ribonucléico mensageiro

NDOG2 – *trophoblast monoclonal antibody (clone NDOG2),* anticorpo monoclonal anti-trofoblasto (clone NDOG2)

NF-κB - *nuclear factor kappa B,* fator nuclear kappa B

NO – *nitric oxide*, óxido nítrico

PBS – *phosphate buffered saline*, tampão fosfato salino

PE – *preeclampsia*, pré-eclâmpsia

PE – *phycoerythrin*, ficoetrina

PerCP – *peridinin chlorophyll protein*, proteína clorofila peridinina

PMP – *platelet microparticle,* micropartícula de plaqueta

PS – *phosphatidylserine*, fosfatidilserina

ROS – reactive oxygen species, espécies oxigênio reativas

SBP – *systolic blood pressure,* pressão sanguinea sistólica

STBM – *syncytiotrophoblast microparticles,* microparticula do sinciciotrofoblasto

SSC – *side scatter,* dispersão lateral

TF/FT – *tissue factor*, fator tissular

TNF-α – *tumor necrosis factor-alpha,* fator de necrose tumoral alfa

TTP – púrpura trombocitopênica trombótica

V-CAM 1 – *vascular cell adhesion molecule 1,* molécula de adesão a célula vascular 1

INTRODUÇÃO

A Pré-eclâmpsia (PE) é uma doença multisistêmica específica da gestação, que caracteriza-se clinicamente pelo aparecimento de hipertensão e proteinúria após a 20ª semana de gestação.

Por ser uma doença cuja única resolução baseia-se na interrupção da gestação, a PE é responsável por 10% a 15% de mortes maternas em todo mundo e é ainda importante causa de morte fetal devido à restrição ao crescimento intrauterino e prematuridade.

É importante classificar e diferenciar os casos de PE leve e grave. Segundo o *American College of Obstetricians and Gynecologists* (2002): a PE leve é caracterizada por hipertensão com pressão sistólica ≥140mmHg e diastólica ≥90mmHg em pelo menos duas medições separadas por intervalo de 4 horas; e proteinúria ≥300mg em urina de 24 horas ou ≥1+ pelo método de fita. A PE grave é caracterizada por hipertensão com pressão sistólica ≥160mmHg e diastólica ≥110mmHg em pelo menos duas medições separadas por um intervalo de 4 horas; e proteinúria ≥5g na urina de 24 horas ou ≥3+ pelo método de fita. A forma grave da PE pode evoluir para outras manifestações clínicas de risco, como a eclâmpsia, a Síndrome HELLP (*Hemolysis, elevated liver enzymes and low platelet*) e a coagulação intravascular disseminada (CID).

A gestação normal está associada a adaptações anatômicas e funcionais do sistema cardiovascular da gestante para acomodar as novas demandas fisiológicas, no entanto na PE esta adaptação é inadequada. Embora o conhecimento seja limitado, já foram identificados fatores de risco para o desenvolvimento da PE como: primiparidade, gestação múltipla, obesidade, PE prévia, fatores genéticos e comorbidades maternas. Várias hipóteses têm sido levantadas na tentativa de explicar a patogênese da PE, mas apesar da extensiva pesquisa, os mecanismos envolvidos nesta disfunção vascular ainda não são bem compreendidos. Recentemente, pesquisas têm reportado elevados níveis de micropartículas (MP) na PE e sugerido seu envolvimento nas manifestações clínicas associada a esta doença, em especial a hipertensão.

As MP são conhecidas como uma população heterogênea de pequenos fragmentos liberados da membrana das células durante ativação celular e apoptose. Muitos tipos celulares, como células endoteliais, plaquetas e leucócitos, liberam estas MP *in vitro*, mas vários estudos têm demonstrado a presença destes fragmentos *in vivo*. Sabe-se que as MP são liberadas durante o remodelamento da membrana plasmática. O súbito aumento dos níveis de cálcio citosólico muda o estado transmembrana, resultando em externalização de fosfatidilserina e ativação de enzimas citosólicas, levando à clivagem do citoesqueleto. Este fenômeno resulta em vesiculação da membrana e liberação das MP para o meio.

Embora estejam presentes no sangue periférico de indivíduos saudáveis, pesquisas revelam um aumento importante em certas condições patológicas. Estas condições incluem as doenças autoimunes, diabetes, câncer e doenças infecciosas. As MP são consideradas

potentes vetores de informação biológica e protagonistas na rede de comunicação celular, tais como indução de modificações endoteliais, angiogênese e diferenciação. As MP *in vivo* parecem estar envolvidas na regulação da coagulação e função vascular, pois estas atuam como potentes indutores pró-inflamatórios e modificadores da expressão gênica nas células endoteliais.

Sabe-se que os processos de coagulação e inflamação co-existem na PE. Desta forma, a principal motivação para o desenvolvimento deste trabalho foi elucidar a relação entre as MP e a PE grave, uma vez que, pelo nosso conhecimento, há poucos trabalhos envolvendo esta associação, tendo como limitante a menor variedade nos tipos de MP avaliadas e o tamanho amostral. Como até o momento nenhum marcador laboratorial mostrou-se efetivo no diagnóstico da doença, sendo hoje feito essencialmente pelas características clínicas e proteinúria apresentadas pela gestante, torna-se oportuno conhecer possíveis analitos biológicos que permitam diagnosticar ou acompanhar a evolução da PE.

Apesar de inúmeras pesquisas sobre essa condição, a etiologia da PE permanece por ser elucidada e não há como prever a ocorrência da mesma antes do aparecimento dos sintomas. Sendo assim, o presente estudo tem como objetivo avaliar a origem e o número de MP e associá-los ao desenvolvimento da PE grave.

Cumpre ainda ressaltar que este trabalho será apresentado com base nos artigos científicos elaborados e submetidos, sendo o primeiro capítulo referente ao artigo de revisão, e o segundo capítulo correspondente aos resultados obtidos neste estudo.

RESUMO

Objetivo: O presente estudo teve como objetivo avaliar micropartículas (MPs) a partir de fontes diferentes em gestantes com pré-eclâmpsia grave (PE), em comparação com gestantes normotensas e mulheres não gestantes.

Estudo: Este estudo de caso-controle avaliou 28 gestantes com PE grave, 30 gestantes normotensas e 29 mulheres não gestantes. MPs de neutrófilos, células endoteliais, monócitos, plaquetas, leucócitos, eritrócitos e sinciciotrofoblastos foram avaliados usando citometria de fluxo.

Resultados: Foi observado um aumento no total de MPs nas gestantes com PE grave, em comparação com gestantes normotensas e mulheres não gestantes (P = 0,004 e P = 0,001, respectivamente). MPs derivadas de eritrócitos estavam aumentadas nas gestantes com PE grave, comparativamente com gestantes normotensas (P = 0,002). Uma correlação positiva foi observada entre a contagem de plaquetas e do número de MPs derivados de plaquetas (P = 0,05). Uma correlação positiva também foi encontrada entre o número de MPs derivadas de células endoteliais e o número de MPs derivadas de plaquetas, leucócitos, neutrófilos e linfócitos (P <0,05).

Conclusão: a contagem de MP pode ser útil para o diagnóstico de PE grave, e as MPs derivadas de eritrócitos parece ser um bom marcador para PE grave. Além disso, MPs derivadas de células endoteliais estão associados com a inflamação e coagulação em PE grave.

Palavras-chave: coagulação, inflamação, micropartículas, pré-eclâmpsia

CAPÍTULO 1

Artigo de revisão intitulado: INTERACTION OF MICROPARTICLES AND PREECLAMPSIA

Abstract

Preeclampsia (PE) is a multi-system disorder, characterized by hypertension and proteinuria, occurring after the twentieth week of pregnancy. Despite intensive research, PE is still one of the leading causes of maternal mortality, and reliable screening tests or effective treatments of this disease have yet to be discovered. The most common procedure is to deliver the baby and the placenta, often prematurely, in the interest of providing the most appropriate conditions for the baby or the mother. Therefore, improving the overall understanding of the role of microparticles in PE may well be useful for new clinical diagnoses and therapeutic approaches.

Microparticles (MPs) are small vesicles released after cell activation or apoptosis, which contain membrane proteins that are characteristic of the original parent cell. MPs have been proven to play key roles in thrombosis, inflammation, and angiogenesis, as well as to mediate cell-cell communication by transferring mRNAs and microRNA from the cell of origin to target cells. It has been suggest that MPs, mainly placenta-derived syncytiotrophoblast microparticles (STBMs), may well play an important role in the pathogenesis of PE.

Keywords: Preeclampsia, microparticles, coagulation, inflammation, syncytiotrophoblast.

PREECLAMPSIA

Preeclampsia (PE) is a multi-system obstetric disorder, whose natural occurrence can only be found in primates and humans [1]. Two percent of women with PE will progress to eclampsia leading to convulsions and potential maternal and fetal death. PE is characterized either by a systolic blood pressure of ≥140mmHg or by a diastolic blood pressure of ≥90mmHg on two or more consecutive occasions, 4 hours apart; together with proteinuria (either ≥300mg protein/day or protinuria by dipstick urine >1+) occurring after the twentieth week of pregnancy in women who had presented no prior symptoms [2]. PE, as compared a normal pregnancy, is associated with increased intravascular coagulation [3, 4], fibrin deposition [5], and inflammatory response [6, 7].

Several hypotheses have been postulated in an attempt to explain the pathogenesis of PE, as described in Table 1 [1, 8, 9]. Although the PE etiology is still unknown, the theory most widely discussed emphasizes the abnormal placenta and describes the PE as a disorder that occurs in two stages. The first stage begins with the abnormal placentation and production of placental factors, such as proteins and cytoplasmic debris falling into the maternal circulation. The second, called the “mother stage”, is the multisystemic maternal syndrome of PE and depends not only on the action of these circulating factors, but also on the health of the pregnant woman, including diseases that affect the vascular system, including preexisting heart or renal diseases, metabolic diseases, genetic factors, and obesity [8, 9].

Table 1. Theories that explain the PE pathogenesis

• Placentation abnormalities (defects in the trophoblast and spiral arteries)
• Angiogenic factors
• Maladaptive cardiovascular and vasoconstriction
• Genetic predisposition
• Immunologic intolerance between maternal and fetal tissue
• Platelet activation
• Vascular endothelial damage or dysfunction

The placenta abnormalitie is caused by an insufficient trophoblast invasion by the spiral arteries that fail to remodel the vessels and remains as small-caliber vessels. This leads to a restriction of placental blood flow, turning the environment into a uteroplacental

hypoxia. The inadequate placentation results in reduced blood flow in the fetal-placental unit, which can lead to poor fetal growth [1, 10, 11].

Currently, PE has been considered as a syndrome, and not a disease, caused by isolated or combined alterations, whose vascular endothelial changes are recognized as a central process [12].

Despite intensive research, PE is still one of the leading causes of maternal mortality, and reliable screening tests or effective treatments of this disease have yet to be discovered. [12]. The most common procedure is to deliver the baby and the placenta, often prematurely, in the interest of providing the most appropriate conditions for the baby or the mother. [13].

MICROPARTICLES

Microparticles (MPs) were first described by Wolf in 1967 as a “dust” procoagulant formation around an activated platelet [14]. Today, MPs are known as a heterogeneous population of small fragments (0.05-1μm) released from the cell membrane during cell activation and apoptosis. Moreover, it is well established in the literature that all eukaryotic cells have the capacity to release MPs [15, 16].

The cell membrane is characterized by its distribution of phospholipids, with phosphatidylcholine and sphingomyelin on the outside, and phosphatidylethanolamine and phosphatidylserine (PS) on the inside. The initial step in the formation of MP is the remodeling of the membrane, with the formation of blebs within it. This step requires an increase in intracellular calcium levels, consequently resulting in the rearrangement and loss of the phospholipidic membrane’s asymmetry, coupled with the externalization of PS to the outer surface. Concomitant to the loss of membrane asymmetry, calcium-sensitive enzymes are activated and promote the cleavage of the filaments of the cytoskeleton leading to the formation of blebs on the membrane and the release of MPs [16, 17, 18].

MPs have commonly been considered inert cell debris, but numerous studies have shown their participation in the exchange of intercellular signals and biological information. There are two main mechanisms through which intercellular signaling can occur. First, the circulating MPs act as signs that affect the cellular properties and activate receptors on target cells, by presenting bioactive molecules attached to the membrane. Second, the MPs directly mediate signaling by transferring part of their contents to cell receptors, resulting in cell activation, phenotypic cellular modification, and the reprogramming function [15, 19]. In the

membrane, MPs also expose a variable spectrum of bioactive substances, receptors, and adhesion molecules [15]. MP membranes also carry chemokines, cytokines, enzymes, growth factors, and signaling proteins [19, 20].

MPs have been proven to play key roles in thrombosis, inflammation, and angiogenesis, as well as to mediate cell-cell communication by transferring mRNAs and microRNA from the cell of origin to target cells [15]. MPs are considered potent tools in the cellular communication network, such as the induction of endothelial changes, angiogenesis, and differentiation [15, 16]. The nature and physical characteristics of the MPs need to be better studied, since most studies assess only their amount, origin, and biological activity. Current knowledge about the formation of MPs derived from experiments with isolated or cultured cells shows that activation and apoptosis promote the release of MPs *in vitro*; however, the mechanism *in vivo* remains unknown [18].

MPs are rich in phospholipids and can be derived from endothelial cells, erythrocytes, platelets and leucocytes [17, 21]. Although they are present in the peripheral blood of healthy individuals, with platelet-derived MPs representing approximately 70% to 90% of all circulating MPs, a significant increase in certain pathological conditions could be observed. These conditions include autoimmune diseases, diabetes, cancer, and infectious diseases [14, 16].

Sheremata et al. [22] observed an increase of platelet-derived MPs in patients with multiple sclerosis, when compared to a normal control group. Tramonato et al. [23] found a significant increase in MPs derived from endothelial cells in the plasma of diabetic patients, as compared to non-diabetic individuals. Kalinkovich et al. [24] showed an increase of MPs expressing C-X-C chemokine receptor type 4 (CXCR4) in the blood and bone marrow of patients with acute myeloid leukemia. Goswami et al. [25] observed increased levels of MPs derived from the syncytiotrophoblast in pregnant women with PE, as compared to a group of normotensive pregnant women with fetal growth restriction. Campos et al. [26] found a significant increase in circulating MPs derived from platelets, erythrocytes and leukocytes in patients infected with *Plasmodium vivax*.

MP protein compositions determine the biological effects of MPs, which vary depending on the cell from which they originated and the type of stimulus involved in their formation. The phospholipid composition of MPs isolated from the synovial fluid of patients with rheumatoid arthritis differs in composition from those isolated from healthy individuals [16, 17]. MPs expose their membrane proteins in specific cells that originated them, which can in turn be used to study their exact origin [18]. The different pattern of expression of these proteins can be distinguished within a subpopulation of circulating MPs released after

apoptotic stimuli from those resulting from cell activation. For example, the comparison of the protein expression in MPs derived from microvascular endothelial cells revealed that the endothelial markers CD31 and CD62E are strongly expressed by MP when released from apoptotic cells; however, CD51 and CD54 are preferentially expressed in MP when released by cell activation [14]. Flow cytometry is the most widely used method to analyze MPs by employing antibodies to cell markers and specific binding of annexin V to phosphatidylserine [17, 18, 27].

MPs from different cell types have different *in vitro* effects on vascular and blood cells, and are commonly involved in regulating coagulation and vascular functions [20]. MPs act as potent pro-inflammatory mediators, beginning an array of signal transduction pathways and gene expression profiles in endothelial cells, thereby affecting their function. MPs derived from platelets stimulate the expression of cyclooxygenase-2 (COX-2) and prostacyclin [28], the production of cytokines in endothelial cells, and an increase in adhesion molecules on the endothelial surface, resulting in monocyte adhesion and platelet activation [29]. These can also directly activate and stimulate monocytes to produce cytokines and reactive oxygen species (ROS), resulting in an inflammatory response [30]. MPs derived from leukocytes also induce the increase of adhesion molecules on endothelial cells and initiate the production of interleukin 6 and 8 [31]. Those derived from endothelial cells can also activate neutrophils, resulting in endothelial adhesion [32].

Regarding the haemostatic function, The MPs present a high level of procoagulant activity, given that they contain anionic PS and express the tissue factor (TF). The PS facilitates the gathering of the components of the clotting cascade that contain gama-carboxyglutamic acid (GLA), such as factors VII (FVII), IX, X, and protrombin. PS-MPs are derived mostly from megakaryocytes and seem to express receptors for both collagen and the von Willebrand factor, as can be seen in the activated platelet [33].

TF is the main regulator of blood coagulation, since this is a receptor for FVII/VIIa. Circulating TF-MP may provide an alternative source of TF that would be recruited to the growing thrombus and reinitiate clotting [33]. The presence of PS may induce a conformational change in TF that increases its specific activity [34]. Some studies suggest that monocytes are likely to be the major source of TF-MPs in health and disease, while endothelial cells may release TF-MP in certain diseases [35, 36].

MPs are able to act on endothelial cells [37], as well as the regulation of vascular tonus, most notably by decreasing the production of nitric oxide (NO) [38]. The latter is a powerful vasodilator, an anti-platelet agent, and a major factor for endothelial cell survival [30]. MPs are also able to influence smooth muscle cells directly through the activation of the

transcription factor NF-κB, leading to the enhanced expression of cyclooxygenase-2 (COX-2) with a subsequent increase in prostacyclin productions, respectively, resulting in vasodilatation [38].

MICROPARTICLES AND PREECLAMPSIA

Normal pregnancy is associated with extensive changes in hemostasis and generalized maternal inflammatory response. The hypercoagulability and inflammation states are increased in PE. Detailed understanding of the links between the blood coagulation and inflammation are imperative to the elucidation of the etiology of PE [39, 40]. Since MPs are involved in both processes, to understand the role of MPs in PE can contribute to a better understanding of the etiopathogenesis of this disease.

Studies have shown that MPs are commonly increased in pregnancy, since this is a medical condition associated with the anatomical and functional adaptation of the vascular system of a mother to accommodate the new physiological demands. However, this increase is especially important in pregnant women with PE, which shows an extensive activation of endothelial cells, leukocytes, and the coagulation system [30, 41, 42].

Recently, several groups reported high levels of circulating MPs in plasma of pregnant women with PE and suggest their involvement in hypertension associated with the disease [42, 43]. Some studies have shown not only MPs derived from platelets, endothelium, and leukocytes, but also from MPs derived from syncytiotrophoblast [44].

Elevated concentrations of erythrocyte-derived MPs have appeared in PE, which are most likely due to hemolysis and haemoconcentrations, since these process are often associated with this syndrome [12]. Increased MPs from T cells, monocytes, and granulocytes were reported in PE, and the number of granulocyte-derived MPs correlates with elastase, a marker of granulocyte activation and secretion [12, 45, 46].

Gonzalez-Quintero et al. [47], in a study comparing the levels of MPs derived from endothelial cells (CD31+ / CD41+) in the plasma of pregnant women with PE, pregnant women with gestational hypertension, and a control group of healthy pregnant women, observed significantly increased levels in the first group as compared to the other two groups. This study also noted that these MPs expressed the adhesion molecule CD31. The theory of endothelial dysfunction in the pathogenesis of PE has gained importance with the identification of endothelial adhesion molecules, such as VCAM-1, ICAM-1, CD31, E-

selectin, and fibronectin in the plasma of pregnant women with PE [48, 49]. These adhesion molecules are expressed constitutively and regulate the trafficking of circulating inflammatory cells to sites of cellular damage [47].

Meziani et al. [41] found evidence that women with PE have increased levels of MPs derived from monocyte/lymphocyte and platelet (PMPs) when compared to normal pregnant women. Microparticles from preeclamptic, but not healthy pregnant women, induced an *ex vivo* vascular hyporeactivity toward serotonin in human omental arteries and mouse aortas. Hyporeactivity was associated with increased NO production and was reversed by an NO synthase inhibitor. In the presence of a COX-2 inhibitor, serotonin-mediated contraction was partially reduced in arteries treated with healthy microparticles but was abolished after treatment with MPs from preeclamptic women. MPs were associated with an up-regulation of inducible nitric oxide (iNOS) and COX-2 inflammatory proteins through the activation of the NF-kB transcription factor in the vascular wall. When PMPs and MPs from other sources were separated and tested for vascular reactivity, it was observed that only PMPs stimulated NO release, suggesting that its inflammatory properties would be associated with nitrosative and oxidative stress in the vascular wall and that the positive role of this type of MP would result in the sudden increase of blood pressure in the PE. However, it could be observed that MPs from other sources, most probably derived from leukocytes, induce the release of vasoconstrictor products and COX-2, especially the 8-isoprostane, whose increase has been observed in the placenta of preeclamptic women [42, 50].

Lok et al. [51] observed a positive correlation between the number of PMPs and the platelet count when compared preeclamptic and normotensive pregnant women during pregnancy and postpartum. In both groups, the PMPs represented the highest percentage of all MPs, but these were reduced in preeclamptic women as compared to normotensive pregnant. However, in postpartum, no difference could be observed among the groups as regards PMP. The reduction in platelet count often observed in PE should explain the lower levels of circulating PMPs in this group. However, the increase in the number of PMPs after birth is most likely due to elevation and normalization in the platelet count.

There is a contrast in the findings of different studies. Lok et al. [51] observed no increase in the total levels of MPs in pregnant women with PE when compared to normotensive pregnant women. However, other studies have reported high numbers of MPs, mainly syncytiotrophoblast membrane microparticles (STBMs), in pregnant women with PE when compared to normotensive ones [52]. This contrast may be related to variations in the characteristics of patients in each study, the study design, and/or the type of antibodies used to detect the MPs [51].

The syncytiotrophoblast membrane cell is an additional source of MPs during PE and their levels increase significantly during pregnancy, which is expected, since there is a gradual increase in the placental volume. Placental oxidative stress destabilizes the syncytiotrophoblast cells, resulting in an increased release of MPs containing oxidized lipids. These are surface membrane fragments shed from the outer layer of the placenta directly into the maternal blood. Other STBMs enter the maternal circulation via decidual veins, which should lead to maternal systemic effects [51, 53].

This type of MP has also been shown to cause endothelial dysfunction [16]. Due to oxidative stress in the intravillous space, the STBMs carrying TF accumulates in this area, starting the local effect on the placental hemostasis. Alternatively, the increased blood pressure, inflammation, and other pathological conditions should result in increased levels of maternal MPs that reach the intravillous placental space through maternal spiral arteries, affecting placental hemostasis [54, 55].

STBMs reach their highest level in the third trimester [12, 56]. Preeclamptic women in this period, as compared to normotensive pregnant, have increased STBMs, which is thought to directly reflect placental hypoxia and apoptosis [12, 56-60]. Indeed, hypoxia leads to excessive ROS generation in the placenta. In normal pregnancies ROS generation is low, and antioxidative pathways are able to inactivate endogenous ROS, thereby limiting placental damage. However, in PE these adaptive mechanisms are overwhelmed by an enhanced production of ROS, in turn leading to an apoptotic/necrotic cascade and STBM formation [61].

The presence of STBMs specifically promoted cell death and/or reduced the proliferation of endothelial cells, as well as activated superoxide production in neutrophils isolated from preeclamptic women [12, 56, 61]. Furthermore, in normotensive pregnant women, the walls of the uteroplacental arteries are invaded by trophoblasts. In PE, reduced trophoblast invasion is combined with an accumulation of apoptotic trophoblasts in the arteries walls, increasing the STMBs levels [62]. In PE, the networks of interstitial and endovascular trophoblast invasion are affected by maternal factors [63, 64]. The interstitial invasion is affected by the premature increase of oxygen in the placenta and a reduced proliferation, while the endovascular network is affected by macrophage-induced apoptosis of perivascular and intramural trophoblasts. Both events limit the number and extent of adaptation of spiral arteries, required for growth fetal [65].

Previous studies have shown no significant differences in the number of total circulating MPs of preeclamptic women, as compared to healthy pregnant women, although

higher levels of MPs derived from T lymphocytes, B lymphocytes, and granulocytes could be observed in pregnant women with PE, as compared to normotensive pregnant women [20, 66]. The increase in this particular subgroup of MPs in PE may well represent a possible mechanism for the development of vascular dysfunction and seems to reflect a modified status of immune system activation and higher inflammatory response [20]. Increased levels of MPs derived from lymphocytes may be due the release of activated lymphocytes in the maternal circulation, as these also tend to increase in the placental tissue during PE [67]. These MPs can cause direct or indirect endothelial injury by inducing new MPs by activating other cells, creating a vicious circle [20]. Studies have shown that, in PE, neutrophils are activated when they pass through the placenta, which would explain the increased levels of MPs derived from this cellular type [68]. The increased number of MPs derived from leukocytes observed in PE should reflect the activation of leukocytes, mainly monocytes and neutrophils, as it one of the core characteristics of this disease [69]. It could also observed that MPs derived from monocytes produce high levels of inflammatory cytokines [70].

CONCLUSION

The data reported in other studies in recent years has discussed the involvement of MPs in physiological and pathological conditions, mainly in inflammatory diseases.

Taking in account that the cellular origin, contents, and forms of MPs are variable, some may well represent target treatments of pathological states, in turn reducing their harmful effects linked to procoagulant and proinflammatory properties in the vessel wall and target organs, especially since the MPs are able to regulate the gene expression involved in inflammation and the regulation of oxidative stress caused by the vascular function.

It is important to note that the relationship between MP levels during a normal and during a complicated pregnancy is still not fully understood. Moreover, it is well-known that the number of MPs is variable in normotensive pregnancy, as compared to preeclamptic pregnancy. Thus, the question to be posed is whether or not MPs should be considered a future marker in the diagnosis of PE or a new therapeutic resource capable of reducing the endothelial dysfunction.

Other studies are warranted to answer this question, given that the elucidation of the mechanisms involved in the effects of MPs may well represent a highly effective contribution to additional intervention strategies concerning PE.

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REFERENCES

1) Trogstad L, Magnus P, Stoltenberg C: Pre-eclampsia: Risk factors and causal models. Best Pract Res Clin Obstet and Gynaecol 2011:25:329-342.

2) American College of Obstetricians and Gynecologists (ACOG): Practice bulletin: Diagnosis and management of preeclampsia and eclampsia. Obstet Gynecol 2002:99:159-167.

3) Schjetlein R, Abdelnoor M, Haugen G, Husby H, Sandset PM, Wosloff M: Hemostatic variables asindependent predictors for fetal growth retardation in preclampsia. Acta Obstet Gynecol Scand 1999: 78:191-197.

4) Higgins JR, Walshe JJ, Darling MR, Norris L, Bonnar J: Hemostasis in the uteroplacental and peripheral circulations in normotensive and preeclamptic pregnancies. Am J Obstet Gynecol 1998:179:520-526.

5) Weiner CP, Brandt J: Plasma antithrombin III activity: an aid in the diagnosis of preeclampsia-eclampsia. Lancet 1977: 2:1249-1252.

6) Rákóczi I, Tallián F, Bagdány S, Gáti I: Platelet life-span in normal pregnancy and pre-eclampsia as determined by a non-radioisotope technique. Thromb Res 1979:15:553-556.

7) Redman CW, Bonnar J, Beilen L: Early platelet consumption in preeclampsia. Br Med J 1978:1:467-469.

8) Turner JA: Diagnosis and management of pre-eclampsia: an update. Int J Womens Health 2010:30: 327-337.

9) Young BC, Levine RJ, Karumanchi SA: Pathogenesis of Preeclampsia. Annu Rev Pathol Mech Dis 2010:5:173-192.

10) Hawfield A, Freedman BI: Pre-eclampsia: the pivotal role of the placenta in its pathophysiology and markers for early detection. Ther Adv Cardiovasc Dis 2009:3:65-73.

11) Wang A, Rana S, Karumanchi SA: Preeclampsia: The Role of Angiogenic Factors in Its Pathogenesis. Physiology 2009:24:147-158.

12) Rodie VA, Freeman DJ, Sattar N, Greer IA: Pre-eclampsia and cardiovascular disease: metabolic syndrome of pregnancy? Atherosclerosis 2004:175:189- 202.

13) Coomarasamy A, Honest H, Papaionnou S, Gee H, Kahn KS: Aspirin for prevention of preeclampsia: a systemic review. Obstet Gynecol 2003:101:1319-1332.

14) Wolf P: The nature and significance of platelet products in human plasma. Br J Haematol 1967:13:269-288.

15) Mause SF, Weber C: Microparticles: Protagonists of a Novel Communication Network for Intercellular Information Exchange. Circ Res 2010:107:1047-1057.

16) Meziani F, Tesse A, Andriantsitohaina R: Microparticles are vectors of paradoxical information in vascular cells including the endothelium: role in health and diseases. Pharmacol Rep 2008:60:75-84.

17) Chironi GN, Boulanger CM, Simon A, George FD, Freyssinet JM, Tegui A: Endothelial microparticles in diseases. Cell Tissue Res 2009:335:143-151.

18) Boulanger CM, Amabile N, Tedgui A: Circulating Microparticles: A Potential Prognostic Marker for Atherosclerotic Vascular Disease. Hypertension 2006:48:180-186.

19) Tan KT, Lip GY: The potential role of platelet microparticles in atherosclerosis. Thromb Haemost 2005:94:488-492.

20) VanWijk M J, Nieuwland R, Boer K, Van der Post JAM, VanBavel E, Sturk A: Microparticle subpopulations are increased in preeclampsia: Possible involvement in vascular dysfunction? Am J Obstet Gynecol 2002:187:450-456.

21) Orozco AF, Jorgez CJ, Horne C, Marquez-Do A, Chapman MR, Rodgers JR, Bischoff FZ, Lewis DE: Membrane Protecded Apoptotic Trophoblast Microparticles Contain Nucleic Acids. Am J Pathol 2008:173:1595-1608.

22) Sheremata WA, Jy W, Horstman LL, Ahn YS, Alexander JS, Minagar A: Evidence of platelet activation in multiple sclerosis. J Neuroinflammation 2008:5:1-6.

23) Tramontano AF, Lyubarova R, Tsiakos J, Palaia T, DeLeon JR, Ragolia L: Circulating Endothelial Microparticles in Diabetes Mellitus. Mediators of Inflamm 2010:1-8.

24) Kalinkovich A, Tavor S, Avigdor A, Kahn J, Brill J, Petit I, Gonchberg P, Tesio M, Netzer N, Naparstek E, Hardan I, Nagler A, Resnick I, Tsimanis A, Lapidot T: Functional CXCR4-Expressing Microparticles and SDF-1 Correlate with Circulating Acute Myelogenous Leukemia Cells. Cancer Res 2006:66:11013-11020.

25) Goswami D, Tannetta DS, Magee L, Fuchisawa A, Redman CWG, Sargent IL, Von Dadelszen P: Excess Syncytiotrophoblast Microparticle Shedding is a Feature of Early-onset Pre-eclampsia, but not Normotensive Intrauterine Growth Restriction. Placenta 2006:27:56-61.

26) Campos FMF, Franklin BS, Teixeira-Carvalho A, Filho AL, de Paula SC, Fontes CJ, Brito CF, Carvalho LH: Augmented plasma microparticles during acute Plasmodium vivax infection. Malar J 2010:9:1-8.

27) Dey-Hazra E, Hertel B, Kirsch T, Woywodt A, Lovric S, Haller H, Haubitz M, Erdbruegger: Detection of circulating microparticles by cytometry: influence of centrifugation, filtration of buffer, and freezing. Vasc Health Risk Manag 2010:6:1125-1133.

28) Barry OP, Pratico D, Lawson JA, Fitzgerald GA: Transcellular activation of platelets and endothelial cells by bioactive lipids in platelets microparticles. J Clin Invest 1997:99:2118-2127.

29) Nomura S, Tandon NN, Nakamura T, Cone J, Fukuhara S, Kambayashi J: High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in THP-1 and endothelial cells. Atherosclerosis 2001:158:277-287.

30) Han KH, Hong KH, Park JH, Ko J, Kang DH, Choi KJ, Hong MK, Park SW, Park SJ: C-reactive protein promotes monocyte chemoattractant protein-1-mediated chemotaxis through upregulating CC chemokine receptor 2 expression in human monocytes. Circulation 2004:109:2566-2569.

31) Mesri M, Altieri DC: Endothelial cell activation by leukocyte microparticles. J Immunol. 1998:161:4382-4387.

32) Bizios R, Lai LC, Cooper JA, Del Vecchio PJ, Malik AB: Thrombin-induced adherence of neutrophils to cultured endothelial monolayers: increased endothelial adhesiveness. J Cell Physiol 1988:134:275-280.

33) Owens A, Mackman N: Microparticles in hemostasis and thrombosis. Circ Res 2011:108:1284-1297

34) Bach R: Tissue factor encryption. Arterioscler Thromb Vasc Biol 2006:26:456-461.

35) Bajaj MS, Ghosh M, Bajaj SP: Fibronectin-adherent monocytes express tissue factor and tissue factor pathway inhibitor whereas endotoxin-stimulated monocytes primarily express tissue factor: physiologic and pathologic implications. J Thromb Haemost 2007:5:1493-1499.

36) Solovey A, Kollander R, Shet A: Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. Blood 2004:104:840-846.

37) Sibai B, Dekker G, Kupferminc M: Pre-eclampsia. Lancet 2005:365:785-795.

38) Szaba FM, Smiley ST: Roles for thrombin and fibrinogen in cytokine/chemokine production and macrophage adhesion in vivo. Blood 2002:99:1053-1059.

39) Walter JJ: Pre-eclampsia. Lancet 2000:356:1260-1265.

40) Roberts JM, LAIN KY: Recent insights into the pathogenesis of pre-eclampsia. Placenta 2002:23:359-372.

41) Meziani F, Tesse A, David E, Martinez CM, Wangsteen R, Schneider F, Andriantsitohaina R: Shed membrane particles from preeclamptic women generate vascular wall inflammation and blunt vascular contractility. Am J Pathol 2006:169:1473-1483.

42) Lock CA, Nieuwland R, Sturk A, Hau CM, Boer K, Vanbavel E: Microparticle-associated P-selectin reflects platelet activation in preeclampsia. Platelets 2007:18:68-72.

43) Tesse A, Meziani F, David E: Microparticles from preeclamptic women induce vascular hyporeactivity in vessels from pregnant mice through an overproduction of NO. Am J Physiol Heart Circ Physiol 2007:293:520-525.

44) Redman CW, Sargent IL: Circulating microparticles in normal pregnancy and pre-eclampsia. Placenta 2008:29:73-77.

45) Kupferminc MJ, Fait G, Many A, Lessing JB, Yair D, Bar-Am A, Eldor A: Low molecular weight heparin for the prevention of obstetric complications in women with thrombophilia. Hypertens Pregnancy 2001:20:35-44.

46) Paternoster DM, Fantinato S, Manganelli F, Nicolini U, Milani M, Girolami A: Recent progress in the therapeutic management of pre-eclampsia. Expert Opin Pharmacother 2004:5:2233-2239.

47) González-Quintero VH, Smarkusky LP, Jiménez JJ: Elevated plasma endothelial microparticles: Preeclampsia versus gestational hypertension. Am J Obstet Gynecol 2004:191:1418-1424.

48) Austgulen R, Lien E, Vince G, Redman C: Increased maternal plasma levels of soluble adhesion molecules (ICAM-1, V-CAM-1, E-selectin) in preeclampsia. Eur J Obstet Gynecol Reprod Biol 1997:71:53-58.

49) Clausen T, Djurovic S, Brosstad F, Berg K, Henriksen T: Altered circulating levels of adhesion molecules at 18 week's gestation among women with eventual preeclampsia: Indicators of disturbed placentation in absence of evidence of endothelial dysfunction? Am J Obstet Gynecol 2000:182:321-325.

50) Walsh SW, Vaughan JE, Wang Y, Roberts LJ: Placental isoprostane is significantly increased in preeclampsia. FASEB J 2000:14:1289-1296.

51) Lok CAR, Van Der Post JAM, Sargent IL: Changes in Microparticle Numbers and Cellular Origin During Pregnancy and Preeclampsia. Hypert Pregn 2008:27:344-360.

52) Cockell AP, Learmont JG, Smárason AK, Redman CW, Sargent IL, Poston L: Human placental syncytiotrophoblast microvillous membranes impair maternal vascular endothelial function. Br J Obstet Gynaecol 1997:104:235-240.

53) Redman CW, Sargent IL: Placental debris, oxidative stress and preeclampsia. Placenta 2000:597-602.

54) Aharon A, Brenner B: Microparticles and pregnancy complications. Thromb Res 2011:127:67-71.

55) Reister F, Frank HG, Kingdom JCP, Heyl W, Kaufmann P, Rath W: Macrophage-induced apoptosis limits endovascular trophoblast invasion in the uterine wall of preeclamptic women. Lab Invest 2001:81:1143-1152.

56) Makrides M, Duley L, Oslen SF: Fish oil, and other prostaglandin precursor supllementation during pregnancy for reducing pre-eclampsia, preterm birth, low birth weight and intrauterine growth restriction. Cochrane Database Syst Rev 2001:4:CD003402.

57) McKay DG: Hematologic evidence of disseminated intravascular coagulation in eclampsia. Obstet Gynecol Surv 1972:27:399-417.

58) Raijmakers MTM, Dechend R, Poston L: Oxidative stress and preeclampsia. Rationale for antioxidant clinical trials. Hypertension 2004:44:374-380.

59) Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ, Parmar K, Bewley SJ, Shennan AH, Steer PJ, Poston L: Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet 1999:354:810-816.

60) Sibai BM: Prevention of preeclampsia: a big disappointment. Am J Obstet Gynecol 1998:179:1275-1278.

61) Aagaard-Tillery KM, Belford MA: Eclampsia: Morbidity, Mortality, and Management. Clin Obst Gynecol 2005:48: 12-23.

62) Messerli M, May K, Hansson SR, Schnneider H, Holzgreve W, Hahn S, Rusterholz C: Feto-maternal interactions in pregnancies: Placental microparticles activate peripheral blood monocytes. Placenta 2010:31:106-112.

63) Reister F, Frank HG, Heyl W, Kosanke G, Huppertz B, Schroder W: The distribution of macrophages in spiral arteries of the placental bed in pre-eclampsia differs from that in healthy patients. Placenta 1999:20:229-233.

64) Huppertz B, Kadyrov M, Kingdom JCP: Apoptosis and its role in the trophoblast. Am J Obstet & Gynecol 2006:195: 29-39.

65) Redman CWG, Sargent IL: Microparticles and immunomodulation in pregnancy and pre-eclampsia. J Reprod Immunol 2007:76:61-67.

66) Stallmach T, Hebisch G, Orban P, Lu X: Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. Virchows Arch 1999:434:207-211.

67) Redman CW, Sacks GP, Sargent IL: Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999:180:499-506.

68) Mellembakken JR, Aukrust P, Olafsen MK, Ueland T, Hestdal K, Videm V: Activation of leukocytes during the uteroplacental passage in preeclampsia. Hypertension 2002:39:155-160.

69) Luppi P, Deloia JA: Monocytes of preeclamptic women spontaneously synthesize pro-inflammatory cytokines. Clin Immunol 2006:118:268-275.

70) Salomon O, Katz B, Dardik R, Livnat T, Steinberg DM, Achiron R, Seligsohn U: Plasma levels of microparticles at 24 weeks of gestation do not predict subsequent pregnancy complications. Fertil Steril 2009:92:682-687.

OBJETIVOS

1 Objetivo geral

Avaliar a origem e o número de micropartículas e associá-los ao desenvolvimento da pré-eclâmpsia grave e suas complicações clínicas e laboratoriais.

2 Objetivos específicos

- Padronizar e validar, por citometria de fluxo, a análise das MPs originadas de: plaquetas, endotélio, leucócitos, eritrócitos, neutrófilos, células trofoblásticas, monócitos e linfócitos.

- Identificar a origem celular das MPs e quantificá-las em mulheres com PE grave, comparado a um grupo composto por gestantes normotensas e um grupo formado por não-gestantes.

- Relacionar a contagem de micropartículas e os aspectos clínicos e laboratoriais apresentados pelas gestantes com PE grave.

CAPÍTULO 2

Artigo original intitulado: MICROPARTICLES IN SEVERE PREECLAMPSIA

MICROPARTICLES IN SEVERE PREECLAMPSIA

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CONDENSATION

Microparticles are associated with severe preeclampsia, and those derived from erythrocytes and endothelial cells seem to be good markers for the diagnosis of preeclampsia.

SHORT VERSION OF THE ARTICLE TITLE

Microparticules and preeclampsia

ABSTRACT

Objective: The present study aimed to evaluate microparticles (MPs) from different sources in women with severe preeclampsia (PE) compared with normotensive pregnant women and non-pregnant women.

Study Design: This case-control study evaluated 28 pregnant women with severe PE, 30 normotensive pregnant women, and 29 non-pregnant women. MPs from neutrophils, endothelial cells, monocytes, platelets, leukocytes, erythrocytes, and syncytiotrophoblasts were evaluated using flow cytometry.

Results: A higher total number of MPs was observed in women with severe PE compared with normotensive pregnant women and non-pregnant women ($P = 0.004$ and $P = 0.001$, respectively). MPs derived from erythrocytes were increased in women with severe PE compared with normotensive pregnant women ($P = 0.002$). A positive correlation was observed between platelet count and the number of MPs derived from platelets ($P = 0.05$). A positive correlation was also found between the number of endothelial cell-derived MPs and the number of platelet-derived MPs, leukocyte-derived MPs, neutrophil-derived MPs, and lymphocyte-derived MPs ($P < 0.05$).

Conclusion: MP counts can be helpful for the diagnosis of severe PE, and erythrocyte-derived MPs seem to be a good marker for severe PE. Moreover, endothelial cell-derived MPs are associated with inflammation and coagulation in severe PE.

Keywords: coagulation, inflammation, microparticles, preeclampsia

INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific syndrome characterized clinically by hypertension and proteinuria after 20 weeks' gestation.[1,2] The etiology of PE remains unknown, but it is a multifactorial disorder. The clinical spectrum ranges from mild to severe.[3,4] In its severe form, PE is an important cause of maternal and fetal morbidity and mortality worldwide.[3,5] The origin of PE remains enigmatic despite considerable research, but the placenta undoubtedly plays a role in its pathogenesis because delivery inevitably leads to recovery.[6,7]

Pregnancy is a controlled inflammatory state. It is believed that an excessive systemic inflammatory response is the basis of clinical manifestations of PE, but the causes of this inflammatory response in normal pregnancy and PE are not known.[8,9] Some studies have shown that all network components of intravascular inflammation (leukocytes, endothelial cells, and the coagulation cascade) contribute to exacerbation of the inflammatory response in PE.[10] In addition to placental cytokines and angiogenic factors, apoptotic fragments released into the maternal blood are candidates that trigger this systemic inflammatory process.[9]

Microparticles (MPs) are vesicles (0.05–1 μm) that are shed from the plasma membranes of several cell types in response to activation or apoptosis. The initial step in their formation is membrane remodeling with the formation of blebs. This step requires increased intracellular calcium levels resulting in the rearrangement and loss of phospholipidic membrane asymmetry with externalization of phosphatidylserine (PS). Concomitant to the loss of membrane asymmetry, calcium-sensitive enzymes are activated and promote cleavage of the cytoskeletal filaments, leading to bleb formation on the membrane and MP release.[11,12]

MPs are considered potent vectors of biological information and protagonists of cellular communication networks, such as the induction of endothelial modifications, inflammation, differentiation, and angiogenesis, because they mediate cell–cell communication by transferring through their surface receptor mRNAs and microRNA from the cell of origin to the target cells.[11,12,13]

MPs of various cellular origins are found in the plasma of healthy subjects, and their amounts increase under pathological conditions.[12] Several groups have reported elevated circulating levels of MPs during pregnancy, but this increase is especially important in preeclampsia, suggesting their involvement in the hypertension associated with this

disease.[14,15] Measurement of MP phospholipid content (mainly PS) has allowed their quantification and characterization.[12]

Few studies have evaluated the MPs of different cells in severe PE. Because severe PE is associated with procoagulant and pro-inflammatory states, studies involving MP pathways should be conducted to clarify a possible role of MPs in PE.

The present study aimed to evaluate MPs from different sources in pregnant women with severe preeclampsia compared with normotensive pregnant women and non-pregnant women.

MATERIAL AND METHODS

Study design

This study included 87 women: 28 pregnant women with severe PE, 30 normotensive pregnant women, and 29 non-pregnant women. Women with severe preeclampsia were selected from Maternidade Odete Valadares, Santa Casa de Misericórdia de Belo Horizonte, and Hospital Municipal Odilon Behrens - Belo Horizonte/Brazil. Normotensive pregnant women and non-pregnant women were selected from Centro de Saúde Guanabara, Betim/Brazil. Clinical data were obtained from the patients' medical records.

Inclusion criteria

Severe PE was defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on at least 2 consecutive occasions, 4 h apart; and proteinuria ≥2 g/L or at least 3+ protein by dipstick. The normotensive pregnant women had systolic/diastolic blood pressure ≤120/80 mmHg and no history of hypertension or proteinuria. The non-pregnant women had neither clinical alterations nor a history of PE or hypertension.

Exclusion criteria

Exclusion criteria common to the 3 groups were chronic hypertension, haemostatic abnormalities, cancer, diabetes, obesity, and cardiovascular, autoimmune, renal, and hepatic diseases.

Ethical aspects

This study was approved by the Ethics Committee of Universidade Federal de Minas Gerais (COEP), No. ETIC 0343.0.203.000-10, and informed consent was obtained from all participants.

Blood samples

Blood samples were drawn in sodium citrate (0.129 mol/L) in a 9:1 volume ratio. The samples were centrifuged at 2,500 × *g* for 15 min to obtain plasma. Samples were aliquoted and stored at -70°C until analysis.

Flow cytometry assay

MPs were prepared as described elsewhere.[16] Briefly, samples were centrifuged at 13,000 × *g* for 3 min to obtain platelet-free plasma, which was then diluted 1:3 in citrated phosphate buffered saline (PBS) containing heparin and centrifuged at 14,000 × *g* for 90 min at 15°C. The subsequent MP pellet was resuspended in 1× annexin V binding buffer (Sigma-Aldrich, MO, USA).

MPs isolated from plasma were gated on the basis of their forward (FSC) and side (SSC) scatter distribution of synthetic 0.7–0.9 μm SPHEROTM Amino Fluorescent Particles (Spherotech Inc., Libertyville, IL, USA) (Figure 1). Taking into account the presence of phosphatidylserine residues on the MP surfaces, events present in the gate were assessed for their positive staining for annexin V (Sigma-Aldrich) - a classical marker for microparticles - using fluorescein isothiocyanate (FITC) - conjugated monoclonal antibodies. Labeling with cell-specific monoclonal antibodies was corrected for isotype-matched control antibodies.

FITC-labeled immunoglobulin G1 (IgG1) and PE-labeled IgG1 isotype controls, monoclonal antibodies directed against neutrophils (CD66-PE), endothelial cells (CD51-PE), monocytes (CD14-PERCP), platelets (CD41-PERCP), leukocytes (CD45-APC), and erythrocytes (CD235a-PECy5), were purchased from BD Biosciences® (CA, USA). Monoclonal antibody directed against lymphocytes (CD3-PE) was purchased from Beckman Coulter Immunotech (Marseille, France).

A trophoblast-derived MP assessment was performed using an indirect staining procedure. NDOG2 (a trophoblast - specific primary antibody) and a goat anti-mouse IgM secondary antibody PE-conjugate (Thermo Scientific®, IL, USA) were used. MPs were

incubated with unlabeled NDOG2, washed with PBS, and incubated with secondary antibody PE.

The samples were analyzed for 60 s in a Flow Cytometry FACSCalibur (Becton-Dickinson®, CA, USA). The following final dilutions of antibodies were used: anti-CD235a-PECy5 (1:400), NDOG2 (1:20), and anti-mouse IgM secondary antibody (1:25). The other antibodies were used in concentrations according to each manufacturer's instructions.

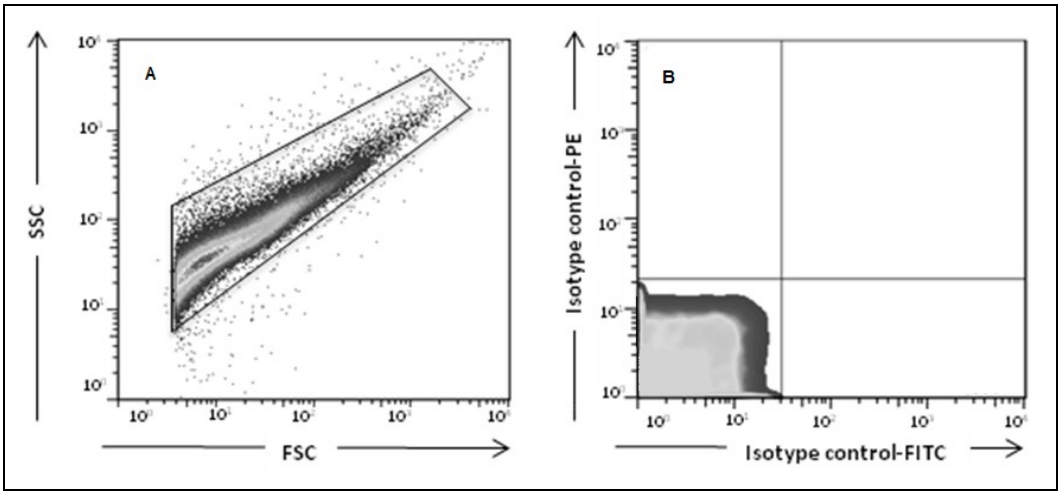

Figure 1. (A) MPs isolated from the plasma were gated based on the basis of their forward (FSC) and side (SSC) scatter distribution. (B) Mouse IgG FITC and PE conjugated isotype control monoclonal antibodies were used to accurately place the gates.

Determination of MP plasma levels

To investigate the absolute MP plasma levels and to determinate the numbers of plasma MPs per microliter (MPs/μL), the cytometer was set to operate at a high flow rate setting for 60s for each sample. The MPs/μL of plasma was calculated as described elsewhere[17]: MPs/μL = (N × 400)/(60 × 100), in which N = number of events, 400 = total volume of sample in the tube before analysis, 60 = sample volume analyzed, and 100 = original volume of MP suspension.

Statistical analysis

Statistical analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk tests were used to verify if the variables were normally

distributed. Data not normally distributed were compared using the Kruskal-Wallis test. Comparison between 2 groups was done using the Mann-Whitney *U* test with Bonferroni's correction (non-normal data) or *t*-test (normal data). Normal data are presented as mean and standard deviation, while non-normal variables are presented as median and interquartile range (25th–75th percentiles). Correlations were analyzed using the Pearson or Spearman two-sided test. Differences were considered significant when $P < 0.05$.

RESULTS

The characteristics of the women enrolled in this study are summarized in Table 1.

All women with severe PE had significantly increased systolic ($P < 0.001$) and diastolic blood pressure ($P < 0.001$) compared with the 2 other groups. The mean proteinuria value (g/L/24 h) for pregnant women with PE was 4.16_± 2.1, confirming the presence of severe PE.

No differences in gestational age were noted between the women with severe PE and the normotensive pregnant women. Body mass index (BMI) before pregnancy did not differ among the 3 groups. Differences were found in age among the 3 groups ($P = 0.008$).

Most participants in the 3 groups were multiparous. Eleven (39%) of the 28 women with severe PE were nulliparous. Five of the multiparous women had PE in their previous pregnancy. Eleven women with PE had abnormal liver function markers or decreased platelets counts but did not fulfill the criteria for HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count). The most common symptom among women with PE was headache (21 women), scotomata (9), epigastric pain (3), and patellar reflex alteration (1).

Table 2 summarizes the cellular origin and number of circulating MPs studied. A higher total number of MPs was observed in women with severe PE compared with normotensive pregnant women and non-pregnant women ($P = 0.004$ and $P = 0.001$, respectively). However, the 2 last groups did not display different numbers of MPs ($P = 0.154$). Individual data points for total circulating MPs in each group are presented in Figure 2.

Table 1: Characteristics of the women studied

Characteristic	Severe PE (n=28)	Normotensive pregnant (n=30)	Nonpregnant women (n=29)	p Value
Age (years)	29.0(26.0-34.5)	24.7(20.7-28.0)	22.0(18.5-30.0)	0.008*
GA (weeks)	33.5±3.7	33.9±3.9	-	0.493**
Platelets (/mm3)	193,741±75,586	-	-	
Parity				
Nulliparous	11 (39%)	10 (33%)	12 (41%)	
Multiparous	17 (61%)	20 (67%)	17 (59%)	

Age – Values are presented as median (25th-75th percentiles). GA: gestacional age, platelets – Values are presented as mean ± standard deviation. * Kruskal-Wallis test, ** T test, (-): does not apply.

Normotensive pregnant and non-pregnant women showed higher levels of circulating platelet-derived MPs. Unlike these 2 groups, most circulating MPs in women with severe PE originated from the endothelial cells. Numbers of erythrocyte-derived MPs were increased in women with severe PE compared with normotensive pregnant women (P = 0.002) and were higher in normotensive pregnant women compared with non-pregnant women (P = 0.005) (Figure 3A).

Trophoblast-derived MPs (NDOG2-positive) were detected in the circulation of women with severe PE and in normotensive pregnant women. Curiously, some trophoblast-derived MPs were detected in non-pregnant women. However, those levels were lower than what was seen in the women with severe PE or normotensive pregnant women (P = 0.002 in both cases) (Figure 3B).

Table 2. Cellular origin and numbers of circulating microparticles

MPs	Severe PE	Normotensive pregnant	Non-pregnant women	P Value*
Total	8.43 (1.60-30.48)	4.87 (1.23-19.20)	3.53 (0.80-14.73)	**0.004**[a] **0.001**[b] 0.154[c]
Platelet	30.93 (11.08-86.92)	38.27 (11.43-132.93)	60.13 (11.87-129.80)	0.726[a] 0.798[b] 0.915[c]
Endothelial cell	36.77 (5.48-73.03)	28.67(3.55-95.48)	7.93 (2.77-38.90)	0.453[a] 0.132[b] 0.468[c]
Leukocyte	19.76 (5.20-63.77)	16.57 (2.70-58.07)	16.67 (4.43-79.70)	0.474[a] 0.873[b] 0.565[c]
Erythrocyte	12.77 (1.87-37.40)	5.27 (1.22-10.08)	2.73 (1.23-14.20)*	**0.002**[a] 0.814[b] **0.005**[c]
Neutrophil	9.13 (1.37-17.78)	3.00 (1.42-9.25)	3.47 (0.63-8.60)	0.123[a] 0.133[b] 0.808[c]
Trophoblast	6.37 (1.62-12.45)	5.00 (1.00-13.08)	2.00 (0.17-3.03)	0.555[a] **0.002**[b] **0.002**[c]
Monocyte	1.93 (0.55-5.40)	1.53 (0.48-2.70)	1.00 (0.23-3.60)	0.259[a] 0.238[b] 0.879[c]
Lymphocyte	0.90 (0.15-3.37)	1.20 (0.22-4.20)	0.73 (0.13-2.53)	0.674[a] 0.527[b] 0.215[c]

Dat

a are presented as median (25th-75th centiles), MPs/μL. *Differences between two groups (Mann-Whitney U test and Bonferroni correction).
a= group 1 x group 2
b= group 1 x group 3
c= group 2 x group 3

* Two outliers were excluded in this analysis

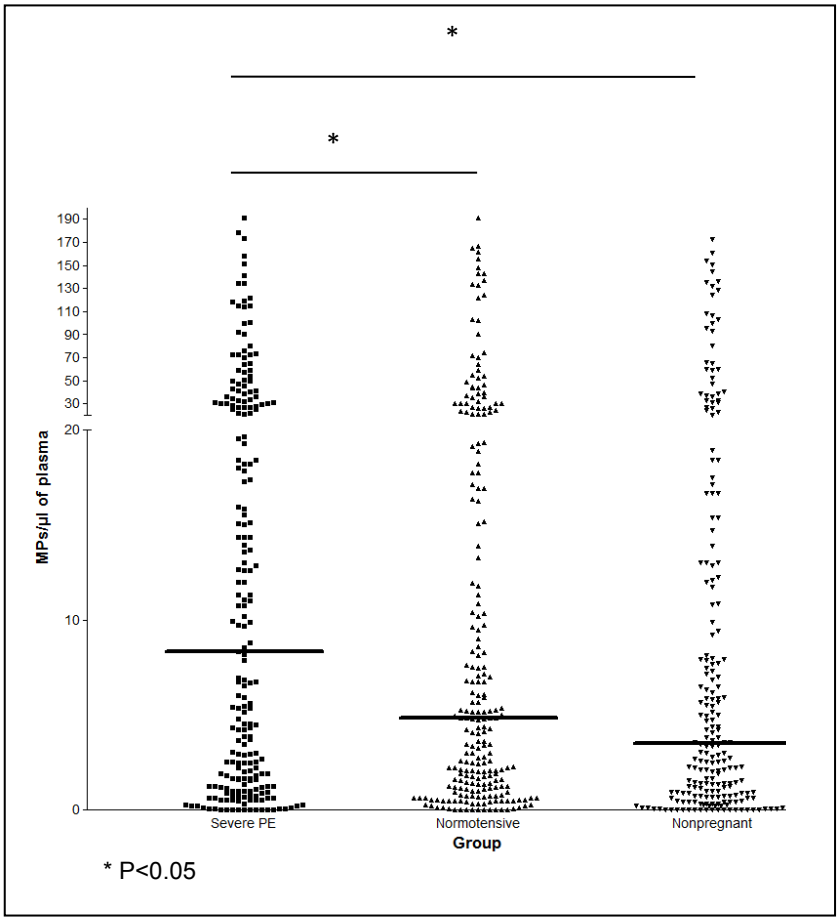

Figure 2. Data points and medians for total numbers of microparticles in women with severe preeclampsia, normotensive pregnant women, and non-pregnant women.

No significant differences were observed among the 3 groups regarding numbers of platelet-, endothelium-, leukocyte-, neutrophil-, monocyte-, and lymphocyte-derived MPs. Nevertheless, there was a clear reduction in platelet-derived MP levels in women with severe PE and normotensive pregnant women but an increase in neutrophil- and endothelial cell-derived MPs in women with severe PE (Figure 4).

Correlation analysis showed no correlation between MP levels and gestational age considering all types of MPs in women with PE ($P > 0.05$). No correlation was observed between trophoblast-derived MPs and systolic or diastolic blood pressure ($P > 0.05$). Similarly, no correlation was found among trophoblast-, endothelial cell-, and platelet-derived MPs ($P > 0.05$). No correlation was found between platelet-, erythrocyte-, and leukocyte-derived MPs and their respective cell numbers in the circulation of women with

PE. However, we did find a positive correlation between platelet count (categorized according to cutoff = 150,000/mm^3) and the number of MPs derived from platelets (categorized considering the median of the control group) (r = 0.380; P = 0.05).

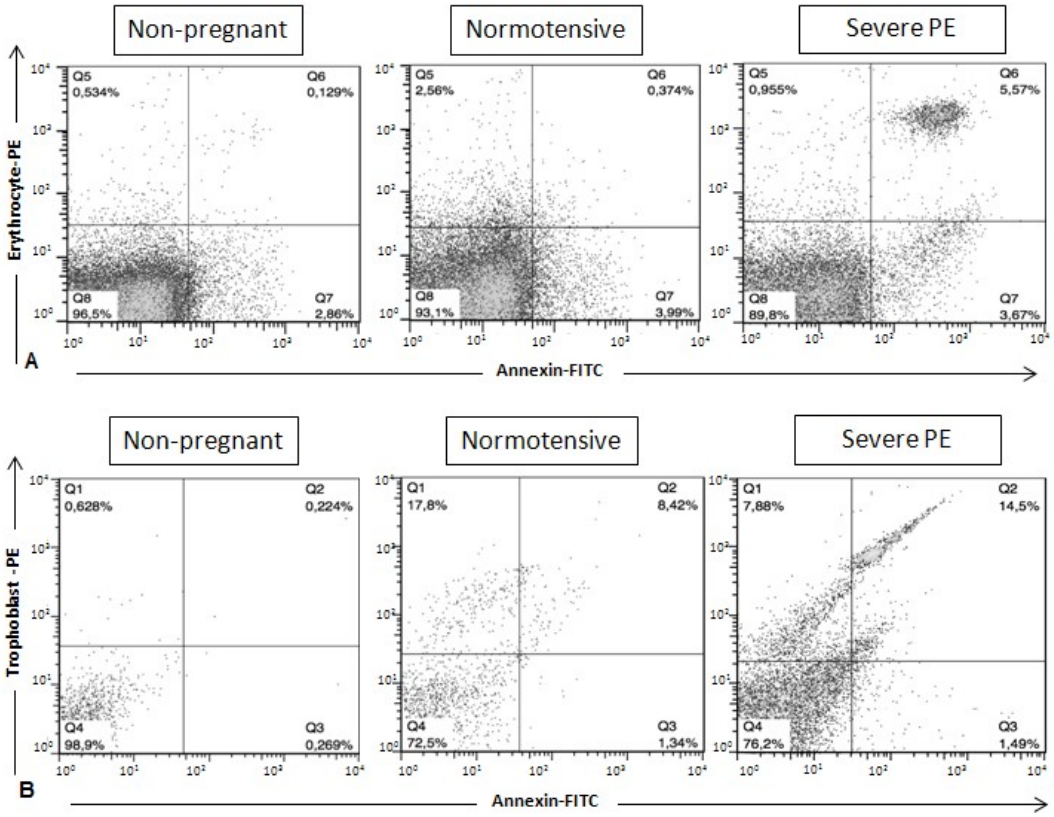

Figure 3. Flow cytometry plots of microparticles derived from erythrocytes (A) and trophoblasts (B) in non-pregnant woman, normotensive pregnant women, and women with severe preeclampsia.

Positive correlations were found between numbers of endothelial cell-derived MPs and plate .005), neut 3; P < 0.00

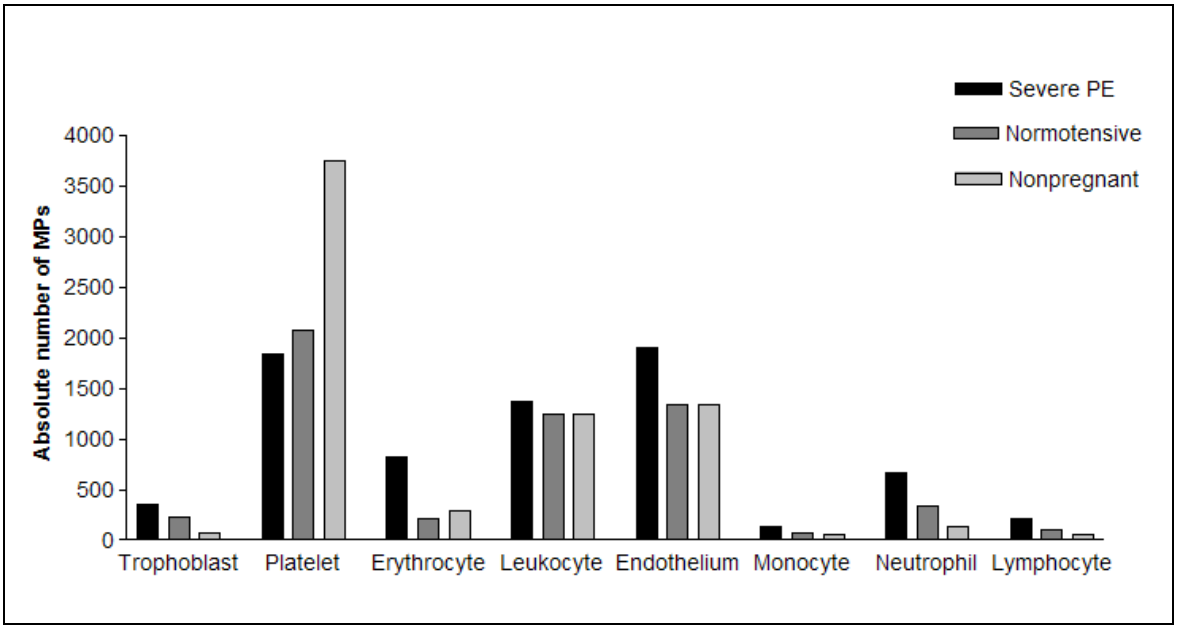

Figure 4. Absolute number of MPs in women with severe PE, normotensive pregnant women, and non-pregnant women.

DISCUSSION

This study showed that MPs were significantly increased in women with severe PE compared with normotensive pregnant women and non-pregnant women. Similarly, Lok et al.[18] and Orozco et al.[19] demonstrated higher numbers of MPs in women with PE compared with normotensive pregnant women.

The majority of circulating MPs detected in severe PE were derived from endothelial cells, while most MPs in normotensive pregnant women and non-pregnant women were derived from platelets. Although we observed reduced numbers of platelet MPs in women with severe PE compared with non-pregnant women, this difference was not significant, probably due to the high dispersion of the data in this variable. Similarly, Alijotas-Reig et al.[20] found no difference in platelet-derived MPs in women with severe PE vs. non-pregnant women. However, there was a positive correlation between platelet-derived MPs and platelet cell count when these variables were categorized. Lok et al.[21] also noted a reduction in platelet-derived MPs in women with severe PE and a correlation with platelet count.

The number of platelet-derived MPs may reflect the turnover of platelets in the plasma. Although platelet activation has been observed in PE, we were not able to identify increased numbers of platelet-derived MPs. A possible explanation for this finding could be

that platelet MPs would remain trapped in the fibrin clots that are frequently evidenced in the placental microvasculature of women with severe PE.[20] Therefore, a lower platelet count in severe PE is associated with exacerbated platelet activation and high consumption.[20,22] Thus, the decreased platelet counts in PE may explain the decreased number of this MP type.[21]

PE is believed to be a disorder of the maternal endothelium.[6] Although there was a tendency for higher numbers of endothelial cells in women with severe PE compared with normotensive pregnant women and non-pregnant women, the difference was not significant. Contrarily, González-Quintero et al.[23] documented higher numbers of endothelial cell-derived MPs in women with PE compared with women with gestational hypertension and non-pregnant women. Endothelial cell activation may contribute to both inflammatory response and vasoconstriction. In the kidney, the endothelial defect can cause proteinuria and endothelium-dependent dilatation failure, which can contribute to hypertension and intense vasoconstriction in different organs.[6] Therefore, endothelium activation should be detectable by an increased number of endothelial cell-derived MPs in the circulation.[24] Although we were not able to show a significant increase in numbers of endothelial cell-derived MPs in women with severe PE compared with the other groups, the number of endothelial cell-derived MPs was associated with higher levels of lymphocyte-, leukocyte-, and platelet-derived MPs, which suggests a correlation between endothelium activation and these cell types.[12]

Our data showed increased numbers of erythrocyte-derived MPs in women with severe PE. This finding could be explained by hemolysis, which is commonly observed in this disease.[25] Because fibrin clots have been observed in the microvasculature of women with PE, one hypothesis is that erythrocytes are lysed by colliding with such clots and result in MP release.[26] However, no correlation between erythrocyte-derived MPs and erythrocyte numbers in the circulation was found.

Our data do not reveal significant differences in leukocyte-, monocyte-, lymphocyte-, and neutrophil-derived MPs, although there was a tendency toward increased numbers of neutrophil-derived MPs in women with severe PE. In contrast, monocyte-, lymphocyte-, and neutrophil-derived MPs were previously determined to be associated with PE.[21,27,28] Elevated numbers of leukocyte-derived MPs may reflect activation of these cells because this disease is associated with the local inflammatory response that results in an enhanced leukocyte–endothelial interaction.[29]

Stallmach et al.[30] observed higher numbers of activated lymphocytes in the placentas of women with severe PE, which could generate increased numbers of MPs released into the maternal circulation. Leukocyte-derived MPs induce endothelial cell and cytokine gene activation. This may be a mechanism for amplification of the local

concentration of inflammatory and chemotactic cytokines and induction of adhesion molecule facilitating intercellular communication and cross-signaling between leukocytes and endothelial cells.[31] Leukocyte-derived MPs cause endothelial damage, which could explain the correlation between neutrophil-, leukocyte-, and lymphocyte-derived MPs and endothelial-derived MPs observed in this study.[27]

The placenta has been shown to play an important role in the pathogenesis of PE. Trophoblast invasion is impaired, which results in placental factor release in the maternal circulation that causes generalized vascular dysfunction.[32] Syncytiotrophoblast (STBM)-derived MPs have been considered a candidate for this factor, mainly because increased trophoblast apoptosis was observed in PE.[33,34] Our data showed that numbers of placenta-derived MPs were not significantly elevated in women with severe PE compared with normotensive pregnant women. However, there were elevated numbers of placenta-derived MPs in women with severe PE and normotensive pregnant women compared with non-pregnant women. Considering the gestational age of women evaluated in this study, this result is not surprising because placenta size increases during pregnancy. This finding is in contrast to the findings of other authors, who reported elevated numbers of placenta-derived MPs in women with PE compared with normotensive pregnant women.[14,35]

NDOG-2 is a trophoblast-specific antibody that recognizes placental alkaline phosphatase.[36] Similarly to the study of Vanwijk et al.[32], we used the NDOG2 antibody to detect and quantify STBM. These antibodies bound to placenta-derived MPs in both women with severe PE and normotensive pregnant women. However, some NDOG-2 was detected in non-pregnant women, even in women who had never been pregnant. This finding suggests that NDOG2 is not STBM-specific.[32] Despite this low specificity, the high capacity of these MPs to damage the vascular endothelium or to activate neutrophils should be considered.[37] Moreover, trophoblast-derived MPs bind to monocytes and B cells, stimulate the production of inflammatory cytokines, and may be related to placental ischemia and oxidative stress.[35,38]

Taken together, our data suggest that MP count could be helpful for the diagnosis of severe PE. Higher numbers of endothelial cell-derived MPs in women with severe PE suggest endothelium activation. Erythrocyte-derived MPs seem to be a good marker for severe PE. In the future, new therapeutic targeting erythrocyte-derived MPs could be proposed. However, considering the limited sample of the current study, other studies are needed to elucidate the mechanisms involved in their effects to contribute to additional intervention strategies for the management of severe PE.

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REFERENCES

1. Wang A, Rana S, Karumanchi SA. Preeclampsia: The Role of Angiogenic Factors in Its Pathogenesis. Physiology 2009;24:147-158.

2. Trogstad L, Magnus P, Stoltenberg C. Pre-eclampsia: Risk factors and causal models. Best Pract Res Clin Obstet and Gynaecol 2011;25:329-3.

3. 3. Turner JA. Diagnosis and management of pre-eclampsia: an update. International Journal of Women's Health 2010;30: 327-337.

4. 4. Young BC, Levine RJ, Karumanchi SA. Pathogenesis of Preeclampsia. Annu Rev Pathol Mech Dis 2010;5:173-192.

5. Roberts JM, LAIN KY. Recent insights into the pathogenesis of pre-eclampsia. Placenta 2002;23:359-372.

6. Poston L. Endothelial dysfunction in pre-eclampsia. Pharmacol Rep 2006;58:69-74.

7. Walsh SW, Vaughan JE, Wang Y, Roberts LJ. Placental isoprostane is significantly increased in preeclampsia. FASEB J 2000;14:1289-1296.

8. Roveri-Querini P, Castiglioni MT, Sabbadini MG, Manfredi AA. Signals of cell death and tissue turnover during physiological pregnancy, pre-eclampsia, and autoimmunity. Autoimmunity. 2007;40(4): 290-294.

9. Messerli M, May K, Hansson SR, et al. Feto-maternal interactions in pregnancies: Placental microparticles activate peripheral blood monocytes. Placenta. 2010;31: 106-112.

10. Redman CW, Sargent IL. Circulating microparticles in normal pregnancy and pre-eclampsia. Placenta 2008;29:73-77.

11. Meziani F, Tesse A, Andriantsitohaina R. Microparticles are vectors of paradoxical information in vascular cells including the endothelium: role in health and diseases. Pharmacol Rep 2008;60:75-84.

12. Chironi GN, Boulanger CM, Simon A, George FD, Freyssinet JM, Tegui A. Endothelial microparticles in diseases. Cell Tissue Res 2009;335:143-151.

13. Mause SF, Weber C. Microparticles: Protagonists of a Novel Communication Network for Intercellular Information Exchange. Circ Res 2010;107:1047-1057.

14. Goswami D, Tannetta DS, Magee L, et al. Excess Syncytiotrophoblast Microparticle Shedding is a Feature of Early-onset Pre-eclampsia, but not Normotensive Intrauterine Growth Restriction. Placenta 2006;27:56-61.

15. Tesse A, Meziani F, David E. Microparticles from preeclamptic women induce vascular hyporeactivity in vessels from pregnant mice through an overproduction of NO. Am J Physiol Heart Circ Physiol 2007;293:520-525.

16. Combes V, Taylor T, Juhan-Vague I, et al. Circulating endothelial microparticles in Malawian children with severe falciparum malaria complicated with coma. JAMA 2004;291:2542-2544.

17. Faille D, Combes V, Mitchell A, et al. Platelet microparticles: a new player in malaria parasite cytoadherence to human brain endothelium. FASEB J 2009;23:3449-3458.

18. Lock CA, Nieuwland R, Sturk A, Hau CM, Boer K, Vanbavel E. Microparticle-associated P-selectin reflects platelet activation in preeclampsia. Platelets 2007;18:68-72.

19. Orozco AF, Jorgez CJ, Horne C, et al. Membrane Protecded Apoptotic Trophoblast Microparticles Contain Nucleic Acids. Am J Pathol 2008;173:1595-1608.

20. Alijotas-Reig J, Palacio-Garcia C, Farran-Codina I, Ruiz-Romance M, Llurba E, Vilardell-Tarres M. Circulating Cell-Derived Microparticles in Severe Preeclampsia and in Fetal Growth Restriction. Am J Reprod Gynecol

2011;67:140-151.

21. Lok CA, Van Der Post JAM, Sargent IL. Changes in Microparticle Numbers and Cellular Origin During Pregnancy and Preeclampsia. Hypert Pregn 2008;27:344-360.

22. Redman CW, Bonnar J, Beilen L. Early platelet consumption in preeclampsia. Br Med J 1978;1:467-469.

23. González-Quintero VH, Smarkusky LP, Jiménez JJ. Elevated plasma endothelial microparticles: Preeclampsia versus gestational hypertension. Am J Obstet Gynecol 2004;191:1418-1424.

24. Brodsky SV, Zhang F, Nasjletti A, Goligorsky MS. Endothelium-derived microparticles impair endothelial function in vitro. Am J Physiol Heart Circ Physiol 2004;286:H1910-915.

25. Sarrel PM, Lindsay DC, Poole-Wilson P A, et al. Hypothesis: inhibition of endothelium-derivate relaxing factor by haemoglobin in the pathogenesis of pre-eclampsia. Lancet 1990; 336: 1030-1032.

26. Silva RFN, Resende LSR, Cardoso BR, Abbade JF, Peraçou JC. Significado da presence de esquizócitos no sangue periférico de gestantes com pré-eclâmpsia. Rev Bras Ginecol Obstet 2008;30(8):406-12.

27. Meziani F, Tesse A, David E, et al. Shed membrane particles from preeclamptic women generate vascular wall inflammation and blunt vascular contractility. Am J Pathol 2006;169:1473-1483.

28. VanWijk M J, Nieuwland R, Boer K, Van der Post JAM, VanBavel E, Sturk A. Microparticle subpopulations are increased in preeclampsia: Possible involvement in vascular dysfunction? Am J Obstet Gynecol 2002;187:450-456.

29. Mellembakken JR, Aukrust P, Olafsen MK, Ueland T, Hestdal K, Videm V. Activation of leukocytes during the uteroplacental passage in preeclampsia. Hypertension 2002;39:155-160.

30. Stallmach T, Hebisch G, Orban P, Lu X. Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. Virchows Arch 1999; 434:207-211.

31. Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. J Immunol. 1998;161:4382-4387.

32. VanWijk MJ, Kublickiene KR, Boer K, VanBavel E. Vascular function im preeclampsia. Cardiovasc Res 2000;47:38-48.

33. Redman CW, Sargent IL. Placental debris, oxidative stress and preeclampsia. Placenta 2000;21(7):597-602.

34. Redman CW, Sargent IL. The pathogenesis of pre-eclampsia. Gynecol Obstet Fertil 2001;29:518-22.

35. Germain SJ, Sacks GP, Soorana SR, Sargent IL, Redman CW. Systemic Inflammatory Priming in Normal Pregnancy and Preeclampsia: The Role of Circulating Syncytiotrophoblast Microparticles. The Journal of Immunology. 2007; 178: 5949-5956.

36. Davies JO, Davies ER, Howe K, et al. Practical applications of a monoclonal antibody (NDOG2) against placental alkaline phosphatase in ovarian cancer. J R Soc Med 1985;78: 899–905.

37. von Dadelszen P, Hurst G, Redman CW. Supernatants from co-cultured endothelial cells and syncytiotrophoblast microvillous membranes activate peripheral blood leukocytes in vitro. Hum Reprod 1999;14:919-24.

38. Southcombe J, Tennetta D, Redman C, Sargent I. The Immunomodulatory Role of Syncytiotrophoblast Microvesicles. PLoS ONE 2011;6(5):e20245.

DISCUSSÃO

A PE caracteriza-se pelo aparecimento de hipertensão e proteinúria após a 20ª semana de gestação em mulheres até então normotensas. A etiologia da doença ainda não foi elucidada e não foram descritos biomarcadores de diagnóstico ou prognóstico amplamente aceitos. Desta forma, o presente estudo objetivou avaliar se as micropartículas (MP) constituem possíveis biomarcadores da forma grave da doença.

Todas as gestantes incluídas neste estudo apresentando PE grave tinham valores de pressão arterial (sistólica e diastólica) significativamente aumentados quando comparados aos dois outros grupos, bem como proteinúria na urina de 24 horas (média de 4,16±2,1), confirmando o critério diagnóstico da PE grave (ACOG, 2002). Segundo Turner et al (2010) e Trogstad et al (2011), estes achados podem ou não estar acompanhados por oligúria, distúrbios do sistema nervoso, dor epigástrica, disfunção hepática, plaquetopenia e restrição ao crescimento fetal. Os sintomas apresentados pelas gestantes com PE grave avaliadas foram principalmente a cefaléia, seguida por escotomas, dor epigástrica e alteração no reflexo patelar.

Uma classificação mais recente da PE baseia-se na idade gestacional na qual surgem os sintomas. Desta forma, a PE é classificada em precoce, quando ocorre antes da 34ª semana de gestação, e tardia quando diagnosticada após 34 semanas (Turner et al., 2010). Há evidências de que a PE precoce seja a forma mais severa da doença, indicando que sua etiologia pode ser diferente da PE tardia (Sibai et al., 2003). A PE precoce parece ser mediada pela placenta e está associada a um Doppler anormal da artéria uterina e restrição ao crescimento fetal. A forma tardia da PE tem sido ligada a fatores constitucionais maternos, como o índice de massa corporal (IMC) e parece estar associada a resultados mais favoráveis (Lindheimer et al., 2010; Valensine et al., 2008). Cumpre ressaltar que no presente estudo, a idade gestacional média foi 33,5±3,66 e não foi observada diferença na idade gestacional entre gestantes com PE grave e gestantes normotensas.

Dentre as 28 mulheres com PE grave, 11 (39%) eram primigestas e cinco (29,4%) dentre as 17 multíparas tiveram PE em gestação anterior. Apesar das primigestas não

representarem a maioria no grupo de PE grave, nesse grupo há dois fatores de risco para PE: a primiparidade e o histórico de PE em gestação anterior (Trogstad et al., 2011).

Nesse grupo houve dois relatos de histórico de PE na família. Uma gestante com PE grave informou que sua mãe teve eclâmpsia na primeira gestação, enquanto outra relatou que sua irmã também teve PE. Embora a maioria dos casos de PE ocorra sem conhecido histórico familiar, já foi observado que a presença de PE em parentes de primeiro grau aumenta o risco para a PE grave (Young et al., 2010). Mulheres nascidas de uma gestação pré-eclâmptica também têm um risco aumentado de ter PE. Além disso, fatores maternos e paternos contribuem para o risco de desenvolvimento da PE. No entanto, o risco de mães afetadas é maior, pois estas carregam genes de susceptibilidade e também transmitem fatores de risco genético independentes para seus fetos. O risco através de pais nascidos de gestações pré-eclâmpticas é menor, pois estes transmitem apenas fatores de risco fetais. Irmãs de homens e mulheres afetados têm um risco aumentado para PE, mesmo que nenhuma delas tenha nascido de uma gestação afetada (Esplin et al., 2001; Skjaerven et al., 2005; Carr et al., 2005).

Sabe-se que a gestação é um estado clínico associado à adaptação anatômica e funcional do sistema vascular da gestante para acomodar as novas demandas fisiológicas. Estudos têm mostrado que as MP estão normalmente aumentadas durante a gestação, o que pode resultar deste novo estado de homeostase observada neste período. Entretanto, esta elevação torna-se especialmente importante em gestantes com PE, na qual se observa uma extensa ativação de células envolvidas nos sistemas de coagulação e inflamatório (Meziani et al., 2006; Lok et al., 2007). Em conformidade com estas observações, foi demonstrado, no presente trabalho, um aumento significativo no número total de MP nas gestantes com PE comparado às gestantes normotensas e mulheres não gestantes. No entanto, não foi observada diferença quando o grupo de gestantes normotensas foi comparado ao grupo de não gestantes com relação ao número total de MP.

As MP derivadas de plaquetas são as mais frequentes dentre todas as MP circulantes, tanto em indivíduos saudáveis quanto em condições patológicas (Mause et al., 2010), incluindo a PE (Lok et al., 2008). Neste estudo, foi observado que as MP derivadas de plaquetas são as principais encontradas no grupo de gestantes normotensas e mulheres não gestantes. Na PE grave, foi observada uma predominância de MP derivadas de células endoteliais.

Acredita-se que as plaquetas tenham um papel crucial na fisiopatologia da PE por exarcebar a coagulação e formar trombos, especialmente na microcirculação (Lyall et al., 1996; Italiano et al., 2010).

Um estudo *in vitro* demonstrou aumento no número de agregados plaquetas-leucócitos em gestantes com PE comparado a gestantes normotensas (Holthe et al., 2005). As plaquetas ativadas expressam P-selectina na sua superfície e têm a capacidade de se ligar a neutrófilos e monócitos (Harlow et al., 2002; Holthe et al., 2004; Macey et al., 2010). Os neutrófilos ativados, por sua vez, liberam radicais livres e aumentam a produção de superóxidos (Tsukimori et al., 1993). Estes radicais livres agridem o endotélio, induzem a expressão de fator tissular e integrina pelos leucócitos e levam à ativação plaquetária (Konijnenberg et al., 1997), sendo, portanto, causa e consequência da formação dos trombos. Sabe-se ainda que a interação entre plaquetas ativadas e monócitos ativados induz a formação de MP que expressam o fator tissular (FT) (Del Conde et al., 2005).

O número de MP derivadas de plaquetas pode refletir o *turnover* destas células no plasma. Assim, a plaquetopenia frequentemente observada na PE pode explicar a redução destas MP. No presente estudo, foi observada uma redução no número de MP derivadas de plaquetas na PE grave, principalmente quando comparado ao grupo de mulheres não gestantes, mas esta diferença não foi significativa, provavelmente em função da grande amplitude dos valores que compõem esta variável. Da mesma forma, Alijotas-Reig et al (2011) não encontraram diferença no número de MP derivadas de plaquetas entre gestantes com PE grave e mulheres não gestantes. Apesar da ativação plaquetária já ter sido admitida na PE, a redução do número de MP derivadas de plaquetas pode ser explicada pela retenção dessas em coágulos de fibrina, frequentemente observados na microvasculatura da placenta na PE (Redman et al., 1978; Konijnenberg et al., 1997; Alijotas-Reig et al., 2011). Corroborando com esta hipótese, Lok et al (2008) observaram redução deste tipo de MP nas gestantes com PE e uma correlação positiva dessas com a contagem de plaquetas. No presente estudo, foi encontrada uma correlação positiva entre o número de MP derivadas de plaquetas (categorizado de acordo com a mediana do grupo controle) e a contagem de plaquetas (categorizada segundo o ponto de corte de 150.000/mL). Esta correlação mostra que quanto menor o número de plaquetas, menor será o número de MP derivadas de plaquetas.

Barry et al (1997) observaram que quando as MP derivadas de plaquetas são tratadas com fosfolipase A_2 ocorre a liberação do ácido aracdônico, que é subsequentemente metabolizado a tromboxano A_2. Isto resulta em ativação de plaquetas e células endoteliais, além de promover a interação destas com monócitos (Barry et al., 1998). No presente estudo, encontrou-se uma correlação positiva entre o número de MP derivadas de plaquetas e o número de MP derivadas de células endoteliais.

As MP derivadas de plaquetas têm um papel importante na função vascular durante a PE e estão associadas às propriedades pró inflamatórias e à capacidade de induzir a ativação da enzima óxido nitrico sintase induzível (iNOS) e da cicloxigenase-2 (COX-2) na parede vascular. Embora estas MP induzam a superprodução do óxido nítrico com subsequente redução na contração vascular, ao mesmo tempo aumentam a produção de metabólitos vasoconstritores de COX-2, que possui um papel relevante na elevação da pressão arterial observada na PE (Meziani et al., 2006).

Neste estudo foi observado um aumento no número absoluto de MP derivadas de células endoteliais nas gestantes com PE grave comparado às gestantes normotensas e principalmente às mulheres não gestantes, no entanto, esta diferença não foi significativa. Este aumento também foi observado quando comparadas gestantes normotensas e mulheres não gestantes. González-Quintero et al (2003) observaram um aumento de MP derivadas de células endoteliais em gestantes com PE comparado a gestantes saudáveis, mas a diferença também não foi significativa.

Evidências importantes apontam a injúria do endotélio como ponto chave para o desenvolvimento da PE. As manifestações clínicas da doença sugerem que a disfunção vascular generalizada poderia explicar o vasoespasmo, edema, proteinúria, coagulopatia e anormalidades hepáticas e renais observados com freqüência na PE (Roberts et al., 1989). Estudos demostraram o aumento de MP derivadas de células endoteliais em doenças relacionadas à injúria endotelial, como a púrpura trombocitopênica trombótica (TTP) (Jimenez et al., 2001) e a esclerose múltipla (Minagar et al., 2001). A injúria endotelial na TTP promove ativação de plaquetas, levando à formação de trombos em pequenos vasos. Estes trombos na microcirculação levam à plaquetopenia grave, anemia hemolítica microangiopática e disfunção transitória do sistema nervoso central (Moake, 1997). Similarmente, a plaquetopenia, a ativação plaquetária e a hemólise ocorrem na PE grave (González-Quintero et al., 2003).

A gestação normal é considerada um estado de inflamação subclínico, mas na PE este estado é exacerbado, o qual está associado à ativação materna de leucócitos, liberação de citocinas e interação leucócito/endotélio (Redman & Sargent, 2003). Assim, a ativação de células endoteliais é parte importante da resposta inflamatória e seria resultante da ativação de linfócitos e neutrófilos (Poston, 2006). Isto pode explicar a correlação positiva observada entre o número de MP derivadas de neutrófilos e linfócitos e as MP derivadas de células endoteliais. No entanto, os fatores responsáveis pela liberação de MP derivadas de células endoteliais na PE ainda não foram totalmente elucidados. Sabe-se que o fator de necrose tumoral α (TNF-α) e a interleucina 1β são indutores da liberação destas MP *in vitro*

(Heyl et al., 1999; Serin et al., 2002). Provavelmente, diversos fatores devem atuar em sinergia para a injúria do endotélio e liberação das MP (González-Quintero et al., 2003).

Neste estudo foi evidenciado um aumento significativo no número de MP derivadas de eritrócitos nas gestantes com PE grave comparadas às gestantes normotensas; e também quando comparadas gestantes normotensas e mulheres não gestantes. Entretanto, não foi observada diferença significativa quando comparadas gestantes com PE grave e mulheres não gestantes; o que deve-se provavelmente a grande amplitude dos valores que compõem esta variável. A lise e fragmentação dos eritrócitos, decorrente da presença de fibrina na micorcirculação, poderia explicar o elevado número destas MP na PE grave (Lok et al., 2008). Uma hipótese para explicar esta hemólise intravascular seria por atrito mecânico, resultante da passagem dos eritrócitos pelos vasos sanguineos constritos ou que sofreram lesão endotelial. Em tais lesões também é comum ocorrer deposição de fibrina em grau variável, decorrente da ativação da cascata da coagulação. Quando presentes, os depósitos intravasculares de fibrina seccionam eritrócitos, contribuindo para a hemólise na microcirculação (Silva et al., 2008).

Estudos *in vivo* comprovaram que as MP derivadas de eritrócitos estão relacionadas com a coagulação, fibrinólise e ativação endotelial (van Berrs et al., 2009). A relação dos eritrócitos e suas MP com o estado de hipercoagulabilidade observado na PE pode ser decorrente da geração de trombina, desencadeada pela presença da fosfatidilserina exposta na sua membrana (McDonald et al., 2006).

O número elevado de MP derivadas de leucócitos observado em alguns trabalhos pode refletir a ativação destas células, que é uma característica da PE, principalmente a ativação de monócitos e neutrófilos (Mellembakken et al., 2002). O aumento no número de MP derivadas de linfócitos T e granulócitos pode ser um possível mecanismo para o desenvolvimento da disfunção vascular na PE e deve refletir a ativação alterada do sistema imune e da resposta inflamatória aumentada (VanWijk et al., 2002).

No presente trabalho, o número de MP derivadas de neutrófilos foi cerca de três vezes maior nas gestantes com PE grave comparado ao grupo de gestantes normotensas e mulheres não gestantes, mas este aumento não foi significativo. Também não foi observada diferença no número de MP derivadas de leucócitos, monócitos e linfócitos quando comparados os três grupos. Diferentemente do presente resultado, VanWijk et al (2002) observaram aumento significativo de MP derivadas de linfócitos T e neutrófilos em gestantes com PE comparado a gestantes normotensas e mulheres não gestantes. Já foi demonstrado que os monócitos circulantes de gestantes com PE produzem níveis elevados de citocinas inflamatórias como IL-1β, IL-6 e IL-8 (Luppi et al., 2006).

O aumento no número de MP derivadas de leucócitos induz a expressão de moléculas de adesão nas células endoteliais e podem induzir disfunção endotelial na PE, bem como aumentar a concentração local de citocinas inflamatórias (Carlos et al., 1994). Apesar da fração de MP derivadas de leucócitos em geral ser pequena, o número desta subpopulação de MP pode ser maior, pois estas provavelmente se aderem ao endotélio dos vasos sanguineos maternos, dificultando sua detecção (Furie & Furie, 2004).

Uma hipótese para explicar a ativação de leucócitos observada na PE seria que estes são ativados quando passam pelo espaço interviloso e são expostos aos lípides oxidados secretados pela placenta (Walsh et al., 1993; Walsh et al., 1998). A formação de MP pode então ser desencadeada secundariamente e resultar em disfunção endotelial (VanWijk et al., 2002). Outra possibilidade para explicar este evento seria a ativação pelo ácido aracdônico, que está presente em níveis elevados no plasma de mulheres pré-eclâmpticas (Ogburn et al., 1984).

Cumpre ressaltar que MP liberadas de leucócitos ativados possuem papel importante no mecanismo de comunicação intercelular e sinalização cruzada entre leucócitos e células endoteliais. Esta comunicação célula-célula pode ser explicada pela transferência de mRNA e microRNA entre a célula que originou a MP e a célula alvo, uma vez que estas moléculas são carreadas na superfície da MP (Mause &Weber, 2010). Este processo deve contribuir também para a ativação exacerbada de leucócitos, ativação da protrombina (Robinson et al., 1992), adesão intercelular e migração no início da injúria vascular (Ross, 1993). Stallmach et al (1999) demonstraram que linfócitos ativados presentes em maior número no tecido placentário de mulheres com PE podem explicar o aumento de MP derivadas de linfócitos na circulação materna. Estas MP podem lesar o endotélio diretamente ou induzir a formação de MP de outras células que levam à injúria vascular (VanWijk et al., 2002).

Acredita-se que a ativação de neutrófilos seja um dos principais componentes da resposta inflamatória na PE (Walsh, 1994; Greer et al., 1989; Haeger et al., 1992, Sacks et al., 1998). Alguns trabalhos mostraram extensa infiltração de neutrófilos, mas não de linfócitos e monócitos na vasculatura sistêmica de gestantes com PE (Cadden & Walsh, 2008). Os neutrófilos ficam aderidos ao endotélio e infiltrados no espaço entre este e o músculo vascular liso. Os neutrófilos liberam substâncias tóxicas, tais como o TNFα, espécies de oxigênio reativas (ROS), mieloperoxidase e tromboxano, consideradas pró-inflamatórias. Consistente com este achado, a infiltração de neutrófilos foi associada com marcadores de inflamação no endotélio e músculo vascular liso em mulheres com PE, além do fato de que a via do fator nuclear kappa B (NF-κB) foi ativada e houve aumento da

expressão dos genes envolvidas na tradução das proteínas ICAM-1 (molécula de adesão intercelular 1), IL-8 e COX-2 neste grupo (Leik et al., 2004; Shah & Walsh, 2007).

A COX-2, expressa em neutrófilos infiltrados no tecido vascular, é uma forma induzida da cicloxigenase e atua na produção de prostaglandinas e tromboxano, sendo portanto associada com a inflamação vascular e vasoconstrição (Leik et al., 2004; Shah & Walsh, 2007). Bachawaty et al (2010) observaram que a expressão de COX-2 é significativamente maior em gestantes com PE que em gestantes normais e mulheres não gestantes.

A placenta parece ter um papel importante na patogênese da PE. Nesta condição clínica, a invasão do trofoblasto é prejudicada, resultando em liberação de fatores ainda desconhecidos da placenta na circulação materna, levando à disfunção vascular generalizada (VanWijk et al., 2000). As MP derivadas do sinciciotrofoblasto (STBM) têm sido consideradas candidatas promissoras para este fator, pois são liberadas a partir da apoptose aumentada do trofoblasto observada na PE (Redman & Sargent, 2001).

No presente estudo, foram detectadas STBM nas amostras das gestantes com PE grave e nas gestantes normotensas, mas não houve diferença significativa. Intrigantemente, este tipo de MP foi também detectado nas amostras de mulheres não gestantes, embora em número significativamente menor. VanWijk et al (2002), usando o anticorpo NDOG2 (o mesmo usado neste estudo), detectaram STBM nas gestantes com PE, nas gestantes normotensas, em mulheres não gestantes, além de amostras de homens saudávies. Em ambos os trabalhos, estas MP foram detectadas em amostras de mulheres não gestantes e nulíparas. O NDOG2 é um anticorpo que reconhece a fosfatase alcalina (FA) da placenta (Davies et al., 1985), mas mostrou ser inespecífico para STBM. Acredita-se que a FA originada de outras fontes, como músculo e próstata, apresenta constituição antigênica semelhante àquela presente na placenta, o que explicaria a baixa espeficidade deste marcador, inclusive o reconhecimento em indivíduos do sexo masculino.

Durante a gestação normal, as STBM estão presentes na circulação materna e estão associadas a uma resposta inflamatória subclínica e ao dano no endotélio vascular (Hellgren, 2003). Na PE, há aumento de STBM, desencadeando uma maior resposta inflamatória característica desta doença (Redman & Sargent, 2005). O aumento de STBM no plasma de gestantes com PE está relacionado à isquemia da placenta e ao *stress* oxidativo. Foi demonstrado que as STBM se ligam a monócitos, e quando preparadas a partir de perfusões placentárias, foram capazes de estimular a produção de TNFα, IL-12, IL-8 e Interferon γ (INFγ) pelas células mononucleares do sangue de doadoras não gestantes (Germain et al., 2007).

É provável que o aumento no número de STBM, combinado à alta expressão de FT possa contribuir para a inflamação materna e alteração da hemostasia observadas na PE (Gardiner et al., 2011). Na gestação normal há um aumento nos níveis fisiológicos de fatores pró-coagulantes, inibidores de fibrinólise e dos marcadores de geração de trombina (Rosenkranz et al., 2008). Há uma associação entre a ativação da coagulação e a PE, incluindo excessiva ativação de plaquetas, aumento dos produtos de degradação da fibrina e deposição de fibrina na placenta (Bonnar et al., 1971). O trofoblasto tem uma natureza pró-coagulante, caracterizado por níveis elevados de FT (Aharon et al., 2004). O FT é um membro da superfamília de receptores de citocinas constitutivamente expressos pela maioria das células perivasculares e não vasculares, sendo responsável por iniciar a cascata da coagulação após uma injúria vascular (Mackman, 2009). O aumento de MP expressando FT foi observado em vários estados patológicos associados às complicações trombóticas (Mackman et al., 2007). O processo de diferenciação do trofoblasto já está implicado na formação de MP. Então, esta diferenciação pode ser considerada uma fonte potencial de MP expressando FT (Aharon et al., 2009). Gardiner et al (2011) comparando gestantes com PE e gestantes saudáveis, verificaram que as STBM expressam mais FT na PE, como também há maior geração de trombina.

Apesar de inúmeras pesquisas sobre a PE, a etiologia dessa condição clínica ainda é pouco elucidada. Dessa forma, a principal contribuição do presente estudo foi mostrar a potencialidade das MP derivadas de eritrócitos e plaquetas serem utilizadas como marcadores da doença, abrindo perspectivas para outros estudos investigativos envolvendo as MP, a PE e outras doenças relacionadas à gestação.

CONCLUSÕES

Com os resultados deste estudo concluímos que:

- As MP apresentam-se mais elevadas em gestantes com PE grave quando comparadas às gestantes normotensas e não gestantes.

- As gestantes com PE grave apresentam número aumentado de MP derivadas de eritrócitos quando comparado ao de gestantes normotensas.

- A gestação leva a uma redução no número de MP derivadas de plaquetas.

- A contagem de MP derivadas de plaquetas correlaciona-se à contagem destas células.

- As MP derivadas de células endoteliais correlacionam-se ao número de MP derivadas de plaquetas, leucócitos, neutrófilos e linfócitos.

REFERÊNCIAS BIBLIOGRÁFICAS

AHARON, A.; BRENNER, B.; KATZ, T.; MIYAGI, Y.; LANIR, N.Tissue factor and tissue factor pathway inhibitor levels in trophoblast cells: implications for placental hemostasis. *Thrombosis and Haemostasis*, v. 92, n. 4, p. 776–786, 2004.

AHARON, A.; KATZENELL, S.; TAMARI, T.; BRENNER, B. Microparticles bearing tissue factor and tissue factor pathway inhibitor in gestational vascular complications. Journal *of Thrombosis and Haemostasis*, v. 7, n. 6, p. 1047-1050, 2009.

ALIJOTAS-REIG, J.; PALACIO-GARCIA, C.; FARRAN-CODINA, I.; RUIZ-ROMANCE, M.; LLURBA, E.; VILARDELL-TARRES, M. Circulating Cell-Derived Microparticles in Severe Preeclampsia and in Fetal Growth Restriction. *American Journal of Reprodutive Immunology*, v. 67, p. 140-151, 2011.

AMERICAN COLLEGE OF OBSTETRICIANS AND GYNECOLOGISTS (ACOG) PRACTICE BULLETIN. Diagnosis and management of preeclampsia and eclampsia. *Obstetrics and Gynecology*, v. 99, p. 159-167, 2002.

BACHAWATY, T.; WASHINGTON, S.L.; WALSH, S.W. Neutrophil Expression of Cyclooxygenase-2 in Preeclampsia. *Reprodutive Sciences*, v. 17, n. 5, p. 465-470, 2010.

BARRY, O.P.; PRATICÓ, D.; LAWSON, J.A.; FITZGERALD, G.A. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *The Journal of clinical investigation*, v. 99, n. 9, p. 2118-2127, 1997.

BARRY, O.P; PRATICÓ D.; SAVANI, R.C.; FITZGERALD, G.A. Modulation of Monocyte-Endothelial Cell Interactions by Platelet Microparticles. *The Journal of Clinical Investigation*, v. 102, n. 1, p. 136-144, 1998.

BONNAR, J.; MCNICOL, G.P.; DOUGLAS, A.S. Coagulation and fibrinolytic systems in pre-eclampsia and eclampsia. *British Medical Journal*, v. 2, p. 12-16, 1971.

CADDE N, K.A.; WALSH, S.W. Neutrophils, but not lymphocytes or monocytes, infiltrate maternal systemic vasculature in women with preeclampsia. *Hypertens Pregnancy*, v. 27, p. 396-405, 2008.

CARLOS, T. M.; HARLAN, J. M. Leukocyte-endothelial adhesion molecules. *Blood*,v. 84, p. 2068, 1994.

CARR, D.B.; EPPLEIN, M.; JOHNSON, C.O.; EASTERLING, T.R.; CRITCHLOW, C.W. A sister's risk: family history as a predictor of preeclampsia. *American Journal of Obstetrics and Gynecology*, v. 193, p. 965-972, 2005.

DAVIE S JO, DAVIES ER, HOWE K.; JACKSON, P.; PITCHER, E.; RANDLE, B.; SADOWSKI, C.; STIRRAT, G.M.; SUNDERLAND, C.A. Practical applications of a monoclonal antibody

(NDOG2) against placental alkaline phosphatase in ovarian cancer. *Journal of the Royal Society of Medicine*, v. 78, n. 11, p. 899-905, 1985.

DEL CONDE, I.; SHRIMPTON, C.N.; THIAGARAJAN, P.; LOPEZ, J.A. Tissue-factor-bearing microparticles arise from lipid rafts and fuse with activated platelets to iniciate coagulation. *Blood*, v. 106, p. 1604-1611, 2005.

ESPLIN, M.S.; FAUSETT, M.B.; FRASER, A.; KERBER, R.; MINEAU, G.; CARRILO, J.; VARNER, M.W. Paternal and maternal components of the predisposition to preeclampsia. *The New England Journal of Medicine,* v. 344, n. 12, p. 867-872, 2001.

FURIE, B.; FURIE, B.C. Role of P-selectin and microparticle PSGL-1 in thrombus formation. *Trendsin Molecular Medicine*, v. 10, n. 4, p. 171-179, 2004.

GARDINER, C.; TANNETTA, D.S.; SIMMS, C.A.; HARRISON, P.; REDMAN, C.W.G.; SARGENT, I.L. Syncytiotrophoblast Microvesicles Released from Pre-Eclampsia Placentae Exhibit Increased Tissue Factor Activity. *PLoS One*, v. 6, n. 10, p. 1-7, 2011.

GERMAIN, S.J.; SACKS, G.P.; SOORANA, S.R.; SARGENT, I.L.; REDMAN, C.W. Systemic Inflammatory Priming in Normal Pregnancy and Preeclampsia: The Role of Circulating Syncytiotrophoblast Microparticles. *The Journal of Immunology*. v. 178, p. 5949-5956, 2007.

GONZÁLEZ-QUINTERO, V.H.; JIMÉNEZ, J.J.; JY, W.; MAURO, L.M.; HORTMAN, L.; O'SULLIVAN, M.J.; AHN, Y. Elevated plasma endotelial microparticles in preeclampsia. *American Journal of Obstetrics and Gynecology*, v. 189, n. 2, p. 589-593, 2003.

GREER, I.A.; HADDAD, N.G.; DAWES, J.; JOHNSTONE, F.D.; CALDER, A.A. Neutrophil activation in pregnancy-induced hypertension. *British Journal of Obstetrics and Gynaecology*, v. 96, p. 978-982, 1989.

HAEGER, M.; UNANDER, M.; NORDER-HANSSON, B.; TYLMAN, M.; BENGTSSON, A. Complement, neutrophil, and macrophage activation in women with severe preeclampsia and the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *Obstetrics and Gynecology*, v. 79, n. 1, p. 19-26, 1992.

HARLOW, F.H.; BROWN, M.A.; BRIGHTON, T.A.; SMITH, S.L.; TRICKETT, A.E.; KWAN, Y.L.; DAVIS, G.K. Platelet activation in the hypertensive disorders of pregnancy. *American Journal of Obstetrics and Gynecology*, v. 187, p. 688-695, 2002.

HELLGREN, M. Hemostasis during normal pregnancy and puerperium. *Seminars in Thrombosis and Haemostasis*, v. 29, n. 2, p. 125-130, 2003.

HEYL, W.; HANDT, S.; REISTER, F.; GEHLEN, J.; SCHRÖDER, W.; MITTERMAYER, C.; RATH, W. Elevated soluble adhesion molecules in women with preeclampsia: do cytokines like tumour necrosis factor-α and interleukin-1β cause endothelial activation? *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, v. 86, n. 1, p. 35-41, 1999.

HOLTHE, M.R.; LYBERG, T.; STAFF, A.C.; BERGE, L.N. Leucocyte-platelet interactions in pregnancies complicated with preeclampsia. *Platelets*, v. 16, p. 91-97, 2005.

HOLTHE, M.R.; STAFF, A.C.; BERGE, L.N.; LYBERG, T. Different levels of platelet activation in preeclamptic, normotensive pregnant, and nonpregnant women. *American Journal of Obstetrics and Gynecology*, v. 190, p. 1128-1134, 2004.

ITALIANO, J.E. JR; MAIRUHU, A.T.A.; FLAUMENHAFT, R. Clinical relevance of microparticles from platelets and megakaryocytes. *Current Opinion in Hematology*, v. 17, n. 6, p. 578-584, 2010.

JIMENEZ, I.J.; JY, W.; MAURO, I.M.; HORSTMAN, I.I.; AHN, Y.S. Elevated endotelial microparticles in thrombotic thrombocitopenic purpura: finding from brain and renal microvascular cell culture and patients with active desease. *British Journal of Haematology*, v. 112, n. 1, p. 81-90, 2001.

KONIJNENBERG, A.; STOKKERS, E.W.; VAN DER POST, J.A.M.; SCHAAP, M.C.L.; BOER, K.; BLEKER, O.P.; STURK, A. Extensive platelet activation in preeclampsia compared with normal pregnancy: Enhanced expression of cell adhesion molecules. *American Journal of Obstetrics and Gynecology*, v. 176, p. 461-469, 1997.

LEIK, C.E.; WALSH, S.W. Neutrophils infiltrate resistance-sized vessels of subcutaneous fat in women with preeclampsia. *Hypertension*, v. 44, p. 72–77, 2004.

LYALL, F.; GREER, I.A. The vascular endothelium in normal pregnancy and preeclampsia. *Reviews of Reproduction*, v. 1, p. 107-116, 1996.

LINDHEIMER, M.D.; TALER, S.J.; CUNNINGHAM, F.G. Hypertension in pregnancy. *Journal of the American Society of Hypertension*, v. 4, n. 2, p. 68-78, 2010.

LOK, C.A.; NIEUWLAND, R.; HAU, C. M; STURK, A.; BOER, K.; NIEUWLAND, R.; VANBAVEL, E. Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets*, v.18, p. 68-72, 2007.

LOK, C.A.; VAN DER POST, J.A.M.; SARGENT, I.L. Changes in Microparticle Numbers and Cellular Origin During Pregnancy and Preeclampsia. *Hypertens Pregnancy*, v. 27, n. 4, p. 344-360, 2008.

LUPPI, P.; DELOIA, J.A. Monocytes of preeclamptic women spontaneously synthesize pro-inflammatory cytokines. *Clinical Immunology*, v. 118, p. 268-275, 2006.

MACEY, M.G.; BEVAN, S.; ALAM, S.; VERGHESE, L.; AGRAWAL, S.; BESKI, S.; THURAISINGHAM, R.; MACCALLUM, P.K. Platelet activation and endogenous thrombin potential in pre-eclampsia. *Thrombosis Research*, v. 125, n. 3, p. 76-81, 2010.

MACKMAN, N.; TILLEY, R.E.; KEY, N.S. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscleroris,Thrombosis and Vascular Biology*, v. 27, n. 8, p. 1687-1693, 2007.

MACKMAN, N. The many faces of tissue factor. *Journal of Thrombosis and Haemostais*, v.1, p. 136-139, 2009.

MAUSE, S.F.; WEBER, C. Microparticles: Protagonists of a Novel Communication Network for Intercellular Information Exchange. *Circulation Research*, v. 107, p. 1047-1057, 2010.

HORNE, M.K.; CULLINANE, A. M.; MERRYMAN,P. K.; HODDESON, E.K. The effect of red blood cells on thrombin generation. *British Journal of Haematology*, v. 133, n. 4, p. 403-408, 2006.

MELLEMBAK

KEN, J.R.; AUKRUST, P.; OLAFSEN, M.K.; UELAND, T.; HESTDAL, K.; VIDEM, V. Activation of leukocytes during the uteroplacental passage in preeclampsia. *Hypertension*, v. 39, p.155-160, 2002.

MEZIANI, F.; TESSE, A.; DAVID, E.; MARTINEZ, C.M.; WANGESTEEN, R.; SCHNEIDER, F.; ANDRIANTSITOHAINA R. Shed membrane particles from preeclamptic women generate

vascular wall inflammation and blunt vascular contractility. *The American Journal of Pathology*, v. 169, n. 4, p. 1473-1483, 2006.

MINAGAR, A.; JY, W.; JIMENEZ, J.J.; SHEREMATA, W.A.; MAURO, L.M.; MAO, W.W.; HORSTMAN, L.L.; AHN, Y.S. Elevated plasma endothelial microparticles in multiple sclerosis. *Neurology*, v. 56, n. 10, p. 1319-1324, 2001.

MOAKE, J.I. Studies on the pathophysiology of thrombotic thrombocytopenic purpura. *Seminars in Hematology*, v. 34, n. 2, p. 83-89, 1997.

OGBURN, P.L. JR; WILLIAMS, P.P.; JOHNSON, S.B.; HOLMAN, R.T. Serum arachidonic acid levels in normal and preeclamptic pregnancies. *American Journal of Obstetrics and Gynecology*, v. 148, p. 5-9, 1984.

POSTON, L. Endothelial dysfunction in pre-eclampsia. *Pharmacological Reports*, v. 58, p. 69-74, 2006.

REDMAN, C.W.; BONNAR J.; BEILEN L. Early platelet consumption in preeclampsia. *British Medical Journal*, v. 1, p. 467-469, 1978.

REDMAN, C.W.; SARGENT, I.L. The pathogenesis of pre-eclampsia. *Gynécologie, Obstétrique & Fertilité*, v. 29, p. 518-22, 2001.

REDMAN, C.W.; SARGENT, I.L. Pre-eclampsia, the placenta and the maternal systemic inflammatory response-a review. *Placenta*, p. 21-27, 2003.

REDMAN, C.W.; SARGENT, I.L. Latest advances in understanding preeclampsia. *Science*, v. 308, p. 1592-1594, 2005.

ROBERTS, J.M.; TAYLOR, R.N.; MUSCI, T.J.; RODGERS, G.M.; HUBEL, C.A.; MCLAUGHLIN, M.K. Preeclampsia an endothelial cell disorder. *American Journal of Obstetrics an Gynecology*, v. 161, p. 1200-1204, 1989.

ROBINSON, R. A.; WORFOLK , L.; TRACY, P. B. Endotoxin enhances the expression of monocyte prothrombinase activity. *Blood*, v. 79, p. 406, 1992.

ROSENKRANZ, A.; HIDEN, M.; LESCHNIK, B.; WEISS, E.C.; SCHLEMBACH, D.; LANG, U.; GALLISTL, S.; MUNTEAN, W. Calibrated automated thrombin generation in normal uncomplicated pregnancy. *Thrombosis and Haemostasis,* v. 99, n. 2, p. 331–337, 2008.

ROSS, R. The pathogenesis of atherosclerosis: a perspective for the1990s. *Nature*, v. 362, p. 801, 1993.

SACKS, G.P.; STUDENA, K.; SARGEN,T.K.; REDMAN, C.W. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *American Journal of Obstetrics and Gynecology*, v. 179, p. 80-86, 1998.

SE

RIN, Y.S.; ÖZÇELIK, B.; BAPBUÓ, M.; KÝÝ, H.; OKUR, D.; EREZ, R. Predictive value of tumor necrosis factor alpha (TNF-α) in preeclampsia. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, v. 100, n. 2, p. 143-145, 2002.

SHAH, T.J.; WALSH, S.W. Activation of NF-kappaB and expression of COX-2 in association with neutrophil infiltration in systemic vascular tissue of women with preeclampsia. *American Journal of Obstetrics and Gynecology*, v. 196, p. 41-48, 2007.

SIBAI, B.M.; CARITIS, S.; HAUTH, J. What we have learned about preeclampsia. *Seminars in Perinatology*, v. 27, n. 3, p. 239-246, 2003.

SILVA, R.F.N.; RESENDE, L.S.R.; CARDOSO, B.R.; ABBADE, J.F.; PERAÇOU, J.C. Significado da presença de esquizócitos no sangue periférico de gestantes com pré-eclâmpsia. *Revista Brasileira de Ginecologia e Obstetrícia*, v. 30, n. 8, p. 406-412, 2008.

SKJAERVEN, R.; VATTEN, L.J.; WILCOX, A.J.; RONNING, T.; IRGENS, L.M. Recurrence of pre-eclampsia across generation: exploring fetal and maternal genetic components in a population based cohort. *British Medical Journal*, v. 331, p. 1-5, 2005.

STALLMACH, T.; HEBISCH, G.; ORBAN, P.; LU, X. Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. *Virchows Archiv,* p. 434, n. 3, p. 207-211, 1999.

TROGSTAD, L.; MAGNUS, P.; STOLTENBERG, C. Pre-eclampsia: Risk factors and causal models. *Best Practice & Research Clinical Obstetrics and Gynaecology*, v. 25, n.3, p. 329-342, 2011.

TSUKIMORI, K.; HIROTAKA, M.; KIYOSHI, I.; NAGATA, H.; KOYANAGI, T.; NAKANO, H. The superoxide generation of neutrophils in normal and preeclamptic pregnancies. *Obstetrics Gynecology*, v. 81, n. 4, p. 536-540, 1993.

TURNER, J.A. Diagnosis and management of pre-eclampsia: an update. *International Journal of Women's Health*, v. 30, p. 327-337, 2010.

VALENSINE, H.; VASAPOLLO, B.; GAGLIARDI, G.; NOVELLI, G.P. Early and Late Preeclampsia: Two different maternal states hemodynamic in the latent phase of the disease. *Hypertension*, v. 52, p. 873-880, 2008.

VAN BEERS, E. J.; SCHAAP, M.C.L.; BERCKMANS, R.J.; NIEUWLAND, R.; STURK, A.; VAN DOORMAAL, F.F.; MEIJERS,J.C.M.; BIEMOND, B.J. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica*, v. 94, n. 11, p. 1513-1519, 2009.

VANWIJK, M.J.; KUBLICKIENE, K.R.; BOER, K.; VANBAVEL, E. Vascular function in preeclampsia. *Cardiovascular Research*, v. 47, n. 1, p. 38-48, 2000.

VANWIJK, M. J.; NIEUWLAND, R.; BOER, K.; VAN DER POST, J.A.M.; VANBAVEL, E.; STURK, A. Microparticle subpopulations are increased in preeclampsia: Possible involvement in vascular dysfunction? *American Journal of Obstetrics and Gynecology*, v. 187, p. 450-456, 2002.

YOUNG, B.C.; LEVINE, R.J.; KARUMANCHI, S.A. Pathogenesis of Preeclampsia. *Annual Review of Pathology*, v. 5, p. 173-192, 2010.

WALSH, S.W.; WANG, Y. Secretion of lipid peroxides by the human placenta. *American Journal of Obstetrics and Gynecology*, v. 169, p. 1462-1466, 1993.

WALSH, S.W. Lipid peroxidation in pregnancy. *Hypertens Pregnancy*, v. 13, p. 1-32, 1994.

WAL

SH, S.W. Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. *Seminars in Reproductive Endocrinology*, v. 16, n. 1, p. 93-104, 1998.

ANEXOS

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Parecer nº. ETIC 0343.0.203.000-10

Interessado(a): Profa. Karina Braga Gomes Borges
Colégio Técnico - UFMG

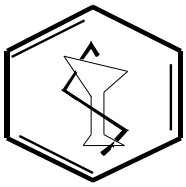
DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 20 de outubro de 2010, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Estudo de micropartículas e mecanismos de apoptose na pré-eclâmpsia grave"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2° andar – Sala 2005 – Cep:31270-901 – BH-MG
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br



UNIVERSIDADE FEDERAL DE MINAS GERAIS

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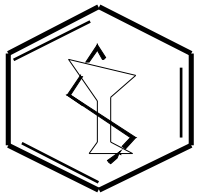
DEPTO. ANÁLISES CLÍNICAS E TOXICOLÓGICAS



TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

PROJETO DE PESQUISA: **"Estudo das micropartículas na pré-eclampsia grave"**

Prezada Sra,

Você está sendo convidada para participar de uma pesquisa que tem por objetivo investigar as alterações que ocorrem na pré-eclâmpsia e,dessa forma, contribuir para o maior entendimento desta doença.

Para realizar este estudo, gostaríamos de colher 10mL do seu sangue para realização dos exames e armazenamento em um banco de amostras biológicas para estudos genéticos futuros. Esclarecemos que este banco de amostras está aprovado e registrado no Comitê de Ética/UFMG sob o nº ETIC 0343.0.203.000-10.

Na coleta de sangue pode ocorrer uma leve dor localizada e formação de um pequeno hematoma. Para minimizar o risco de formação de hematomas, a coleta de sangue será realizada por um profissional experiente. Serão utilizados agulhas e tubos descartáveis.

Seu nome e os resultados dos exames serão mantidos em segredo.

Esclarecemos que caso não queira participar deste estudo, não haverá nenhum problema.

Para qualquer dúvida sobre esta pesquisa você deverá entrar em contato com as pessoas responsáveis pela mesma, cujos nomes estão abaixo relacionados.

Se você estiver de acordo, por favor, assine esta folha.

Professores responsáveis:

Luci Maria Sant'Ana Dusse – telefone: 3409-6880

Karina Braga Gomes Borges – telefone: 3409-4983

Comitê de Ética em Pesquisa – COEP: Av. Antônio Carlos, nº. 6627 – Pampulha – Campus UFMG, Unidade Administrativa II. CEP: 31270-901. Telefone: 3409-4592.

NOME: _____

Carteira de identidade:_____

Assinatura: _____ DATA: ____/____/____

Agradecemos sua valiosa participação!

FICHA CLÍNICA			
Projeto: **Estudo de micropartículas na pré-eclampsia grave**			
Data:			
Paciente nº:			
Grupo: III - Mulheres não gestantes			
1. Identificação			
Nome:			
Nacionalidade:		Naturalidade:	
Data de nascimento:		Idade:	
Estado civil:			
Endereço:			
Rua/Avenida:			
Número:		Complemento:	
Bairro:			
Cidade:		Estado:	
CEP:			
Telefone: ( )			
Escolaridade:			
2. Anamnese			
Presença de doenças intercorrentes? (distúrbios da coagulação, doenças cardiovasculares, doenças renais, doenças autoimunes, doenças hepáticas, diabetes, câncer, sangramento, história familiar)			
Fumante? □ SIM □ NÃO			
Consumo de álcool? □ SIM □ NÃO		Quantidade:	
Pratica exercício físico? □ SIM □ NÃO			
Freqüência:		Modalidade:	

Uso de medicamentos? □ SIM □ NÃO
SE SIM. Quais medicamentos?
Gestações? □ SIM □ NÃO
Se SIM. Quantas?
Intercorrências durante a gestação? (hipertensão, pré-eclâmpsia, aborto, parto prematuro
3. Exame físico
Altura: _____ cm
Peso: _____ Kg
IMC:
Pressão arterial: _____/_____ mmHg

FICHA CLÍNICA	
Projeto: **Estudo das micropartículas na pré-eclampsia grave**	
Data:	
Paciente nº:	
Grupo: □ I - Pré-eclâmpsia	
Diagnóstico de pré-eclâmpsia dado em: _____/_____/_____	
Médico responsável:	
□ II – Normotensas	
1. Identificação	
Nome:	
Prontuário número:	
Nacionalidade:	Naturalidade:
Data de nascimento:	Idade:
Estado civil:	

Número de parceiros:			
Endereço:			
Rua/Avenida:			
Número:		Complemento:	
Bairro:			
Cidade:		Estado:	
CEP:			
Telefone: ( )			
Escolaridade:			
2. Anamnese			
Presença de doenças intercorrentes? (distúrbios da coagulação, doenças cardiovasculares, doenças renais, doenças autoimunes, doenças hepáticas, diabetes, câncer, sangramento, pré-eclâmpsia na família, complicações em gravidez anterior)			
Fumante? □ SIM □ NÃO			
Consumo de álcool? □ SIM □ NÃO	Quantidade:		
Pratica exercício físico? □ SIM □ NÃO			
Freqüência:	Modalidade:		
3. Informações sobre a(s) gestação(ões)			
Idade gestacional**: _____** semanas			
Pré-natal? □ SIM □ NÃO			
Gravidez múltipla? □ SIM □ NÃO			
GPA (Gravidez Parto Aborto): **_____/_____/_____**			
Partos vaginal (PN) ou cirúrgico (PC)?			
Intervalo interpartal (meses):			

Parto prematuro?			
Filhos vivos:			
Prinicipais queixas:			
☐ Cefaléia ☐ Epigastralgia ☐ Escoltoma ☐ Reflexo patelar			
☐ Outros			
4. Uso de medicamentos			
☐ Nifedipina ☐ Metildopa ☐ Sulfato de magnésio			
☐ Outros			
5. Informações clínicas e laboratoriais			
Altura: _____ cm			
Peso: _____ Kg			
Ganho de peso na gravidez:			
Exames laboratoriais: Hm: Hb: Ht: Global: b N E B L M Plaquetas:		TGO: TGP: Bilirrubina total: Bilirrubina direta: Bilirrubina indireta: Ac. Úrico: LDH: Outros:	
Acompanhamento:			
Data	Pressão arterial	Proteinúria (24	Edema

		horas)	

Gmail by Google

Karina Braga <karinabgb@gmail.com>

Submission Confirmation

The American Journal of Obstetrics & Gynecology <ajog@rrohio.com> 2 de maio de 2012 12:23
Para: karinabgb@gmail.com

05-02-2012

Dear Dr. Karina B. Gomes:

This acknowledges the receipt of your submission entitled, "MICROPARTICLES IN SEVERE PREECLAMPSIA," to the American Journal of Obstetrics & Gynecology.

If any items in the submission checklist were omitted, the submission will be considered incomplete and returned to you for resubmission. It is the responsibility of the corresponding author to make sure all authors have been consulted and have approved this submission. We appreciate your attention to these important details.

We will report the results of the manuscript review as soon as possible. Also, you may log onto http://ees.elsevier.com/ajog as an author for details on the processing of your manuscript or to view the new Journal format.

Thank you for your submission to the American Journal of Obstetrics & Gynecology.

Sincerely,

Tom Garite, MD
Editor-in-Chief

Moon Kim, MD
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Cancún · México

April 25-28, 2012

CERTIFICATE OF RECOGNITION

Marques FK*, Carvalho AT**, Nunes FFC**, Carvalho MG***, Dusse LMS***, Gomes KB1***.
*Department of General Biology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; **Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil; ***Department of Clinical and Toxicological Analysis, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

MICROPARTICLES DERIVED FROM PLATELETS AND SEVERE PRE-ECLAMPSIA.

LIII Congreso de la Agrupación Mexicana para el Estudio de la Hematologìa (AMEH) / XXXIV World Congress of the International Society of Hematology (ISH)
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