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Placental exosomes and pre-eclampsia: Maternal circulating levels in normal pregnancies and, early and late onset pre-eclamptic pregnancies

Preenan Pillay [a], Niren Maharaj [d], Jagidesa Moodley [c], Irene Mackraj [b, *]

[a] Discipline of Human Physiology, School of Basic Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa
[b] Nelson R Mandela School of Medicine, School of Laboratory Medicine & Medical Sciences, University of KwaZulu-Natal, Durban, South Africa
[c] Women's Health and HIV Research Group, University of KwaZulu-Natal, Durban, South Africa
[d] Prince Mshiyeni Memorial Hospital, Department of Obstetrics and Gynaecology, Durban, South Africa

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ABSTRACT

Introduction and aim: Exosomes are a subtype of extracellular vesicle (20–130 nm) released by biological cells under normal and pathological conditions. Although there have been reports of circulating exosomes in normal pregnancy, the relevance of placental-derived exosomes in normal and abnormal pregnancies still needs to be elucidated. The aim of this study was to quantify total and placental-derived exosomes in maternal plasma from normal (N), early onset- and late onset-preeclampsia (PE).
Method: Plasma samples were obtained from pregnant women in the third trimester, for the isolation of exosomes by differential ultracentrifugation. Total exosomes were quantified using nanoparticle tracking analysis and immuno-reactive exosomal CD63 quantification. Placental-derived exosomes were quantified using placental alkaline phosphatase (PLAP) as a specific marker. The contribution of placental-derived exosomes to total exosomes in maternal plasma was determined by the ratio of $PLAP^+$ exosomes to $CD63^+$ exosomes.
Results: The concentration of total exosomes significantly increased in early onset-PE and late onset-PE compared to N (≤33 weeks) and N (≥34 weeks). The relative concentration of placental-derived exosomes significantly increased in early onset-PE but decreased in late onset-PE compared to N. The ratio of $PLAP^+$ exosomes to total number of exosomes significantly decreased in early onset-PE and late onset-PE. A positive correlation between total and placental-derived exosomes were obtained in N (≤33 weeks: Pearson's $r = 0.60$, ≥34 weeks: Pearson's $r = 0.67$) and early onset-PE (Pearson's $r = 0.51$, $p < 0.05$) with the inverse in late onset-PE (Pearson's $r = -0.62$, $p < 0.01$).
Conclusion: The differences in the contribution of placental-derived exosomes to total exosomes in maternal circulation suggests a possible pathophysiological role of placental-derived exosomes in pre-eclampsia.

1. Introduction

Pre-eclampsia (PE) is a hypertensive pregnancy disorder unique to human pregnancies. It is defined as new onset hypertension (systolic blood pressure of ≥140 mm Hg and diastolic blood pressure of ≥90 mm Hg), proteinuria (≥300 mg) [1]. Pre-eclampsia is reported to occur in approximately 5–7% of pregnant woman globally [2] and is a major cause of maternal and neonatal morbidity and mortality [3].

The recent classification of hypertensive disorders of pregnancy, recommends that the categorization of PE is categorised into mild to moderate PE, severe preeclampsia, eclampsia and early onset-PE & late onset-PE [1]. Early onset-PE is associated with greater risks of maternal and fetal complications than late onset-PE [4–6]. The distinction in severity between early onset-PE and late onset-PE may reflect the differences in the etiopathogenesis of the subtypes of PE which adds a new dynamic to a disorder with existing complexities. The exact etiology of PE is not known, however, the

* Corresponding author.
E-mail address: mackraji@ukzn.ac.za (I. Mackraj).

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pathophysiology is thought to be a two-stage disorder originating in the placenta [7]. The first stage is theorised to be due to defective trophoblastic invasion of the uterine spiral arterioles, which leads to decreased uteroplacental blood flow and a reduction in oxygenation [7]. Subsequently, the second stage is one in which decreased cellular oxygenation results in the release of proangiogenic factors and an imbalance between pro- and anti-angiogenic factors leading to widespread endothelial damage and the clinical manifestations of PE [7–10]. The placenta, therefore, plays an important role in the pathophysiology of PE. Both early onset-PE and late onset-PE are of placental origin with early onset-PE believed to be superimposed on underlying medical conditions such as diabetes [11,12]. Therefore the severity of placental changes and maladaptation in the uterine spiral arterioles may be greater than that which occurs in late onset-PE.

The aetiology of preeclampsia still remains unknown due to the multifactorial nature of the disorder. Angiogenic, antiangiogenic, *cff*DNA, *cf*DNA, proteins and vasoactive factors have been implicated in the pathogenesis PE [13–15]. More recently, studies have identified syncytiotrophoblast microparticles (STBM) as factors that are immune-stimulatory, antiangiogenic, pro-coagulant and involved in endothelial dysfunction, a key pathological feature of preeclampsia [16–18]. Syncytiotrophoblast microparticles are directly released from the placenta into maternal circulation and consist of vesicles ranging from 20 to 3000 nm in size [19].

Exosomes are a key constituent of STBMs which fall within the smaller size spectrum of 20–130 nm and have a latent role in fetal-maternal immune tolerance and endothelial cell migration [20–22]. Exosomes are formed as products of the lysosomal pathway and secreted by most cell types due to the fusion of intracellular multivesicular bodies with the plasma membrane. These vesicles consist of a bi-lipid membrane containing a variety of signalling molecules including cell adhesion molecules and growth factor receptors [23]. In addition, exosomes contain mRNA and miRNA [24], which are involved in immune function [21,25,26]. Exosomes function primarily by communicating with adjacent or distal cells to re-programme their phenotype and regulate cell function [24]. Reports suggest that placental-derived exosomes play a role in the regulation of immune tolerance during normal and complicated pregnancies [21,23–26]. The variations in the contribution of placental-derived exosomes and bioactivity in normal pregnancies are suggestive of the immune regulatory role of exosomes in a successful pregnancy. The probable role of placental-derived exosomes is to regulate the activity of both proximal and distal target cells, translation, angiogenesis, proliferation, metabolism, and apoptosis [21,27–31].

Exosomes may have a potential role as activating agents in endothelial dysfunction, a hallmark of PE [30]. It has been established that total and placental-derived exosomes with no associated pathologies are biologically active molecules with a regulatory role in endothelial cell migration [27]. Normally, placental exosomes in maternal circulation increase with gestational age which inversely correlates to endothelial cell migration [27]. In the third trimester of pregnancy, however, the lower contribution of placental-derived exosomes in relation to total exosomes contributes to the inhibition of endothelial cell migration [27].

Ex-vivo and *in-vitro* studies have been used to examine enriched preparations (supraphysiological levels) of placental exosomes from placental perfusates [31]. However, the relevance of placental-derived exosomes in pathological pregnancies has not yet been elucidated. Therefore the aim of this study was to determine the levels of placental-derived exosomes from maternal plasma in normal, early onset-PE and late onset-PE pregnancies.

2. Materials and methods

2.1. Ethics statement

Regulatory ethical and institutional approval were obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE310/15), South Africa.

2.2. Study group and samples

Normotensive (N), early onset-PE and late onset-PE patients, in the third trimester were recruited (n = 15 per group). Normotensive patients were classified by a blood pressure of 120 ± 10/80 ± 5 (systolic/diastolic mm Hg) and absent proteinuria as detected by a rapid urine dipstick test (Markomed®, South Africa). Normotensive patients were matched for gestational age according to early onset-PE and late onset-PE patients. Early onset pre-eclampsia was defined by new onset hypertension (diastolic blood pressure of ≥90 mm Hg and systolic blood pressure of ≥140 mm Hg) and proteinuria (≥300 mg) at < 33 weeks plus 6 days of gestation. Late onset-pre-eclampsia was defined by new onset hypertension (diastolic blood pressure of >90 mm Hg and systolic blood pressure of >140 mm Hg) and proteinuria (≥300 mg) at > 34 weeks gestational age. All patients had singleton pregnancies and those with evidence of any infections or medical, surgical or other obstetric complications were excluded. Blood samples were collected [BD Vacutainer Tubes (EDTA), Becton Dickinson and Company, South Africa] and the plasma samples were stored at −80 °C for analyses [27,28].

2.3. Isolation and purification of exosomes from maternal circulation

Exosomes were isolated according to the method described by Théry et al., 2006 (Fig. 1) [32]. Plasma (1 ml) was diluted with an equal volume of phosphate buffered saline (PBS; pH 7.4). Exosomes were isolated and purified by differential ultracentrifugation using a 30% sucrose cushion. In brief, centrifugation was initially performed at 2000 × g at 4 °C for 30 min, followed by 12 000 × g at 4 °C for 45 min. The supernatant was centrifuged at 110 000 × g at 4 °C for 120 min (Optima™ MAX-XP Ultracentrifuge, fixed angle MLA-55 rotor, Beckman Coulter Inc., Brea, CA, USA). The pellet was suspended in PBS and filtered through a 0.22 μm filter (Cellulose acetate, GVS™, Europe). The filtrate was centrifuged at 110 000 × g at 4 °C for 70 min, the pellet re-suspended in PBS (pH 7.4) and centrifuged at 110 000 g for 70 min, 4 °C. The exosome pellet was suspended in 1 ml of PBS and subsequently purified using a 30% sucrose cushion as described by Théry et al., 2006 [32]. The final pellet was resuspended in 100 μl of PBS and stored at −80 °C. The exosomal protein concentration was determined as described by Salomon et al. (2014) using the RC DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) [27].

2.4. Nanoparticle tracking analysis

Quantification and size distribution of exosomes were determined using the NS500 equipped with a 405 nm laser and sCMOS camera (NanoSight NTA 3.0 Nanoparticle Tracking and Analysis Release, Version Build 0069). Samples were diluted with PBS prior to analysis in order to obtain particle distribution of 10 and 100 particles per image (optimal, 50 particles per image) before the analysis with NTA system. Samples were introduced into the sample chamber using the following script: PUMPLOAD, REPEAT-START, PRIME, DELAY 10, CAPTURE 60, REPEAT 5. Videos were recorded at a camera level of 10, camera shutter speed of 20 ms and