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Short communication

NAMPT levels are inversely related to nitric oxide formation and positively related to soluble fms-like tyrosine kinase-1 levels in preeclampsia

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ABSTRACT

NAMPT is a biomarker for endothelial dysfunction, but its relationship with nitrite (marker of NO formation) and soluble fms-like tyrosine kinase-1 (sFLT-1) has not been previously evaluated in preeclampsia. Therefore, we measured plasma NAMPT and sFLT-1 levels using enzyme immunoassays and plasma nitrite concentrations using an ozone-based chemiluminescence assay. NAMPT was positively correlated to nitrite ($r = 0.217$; $P = 0.034$) and inversely related to sFLT-1 ($r = -0.340$; $P = 0.029$) in healthy pregnant women, but inversely related to nitrite ($r = -0.259$; $P = 0.035$) and positively correlated to sFLT-1 ($r = 0.326$; $P = 0.007$) in preeclamptic patients, suggesting that NAMPT inhibits NO formation and interacts with the antiangiogenic factor sFLT-1 in preeclampsia.

1. Introduction

Preeclampsia affects up to 7% of pregnant women and is associated with increased maternal and perinatal mortality and morbidity worldwide [1]. Diagnostic for preeclampsia includes new-onset hypertension plus other clinical criteria, which may be proteinuria [2]. Endothelial dysfunction due to increased circulating placental factors is a hallmark of preeclampsia [1,3], which can progress to maternal multi-organ dysfunction [3,4].

Excess production of soluble fms-like tyrosine kinase 1 (sFLT-1) contributes to endothelial dysfunction, hypertension, and proteinuria [5], and is considered a diagnostic marker of preeclampsia [6–8]. Moreover, impaired nitric oxide (NO) bioavailability has been reported in preeclampsia, as reviewed elsewhere [9]. Notably, circulating nitrite concentrations (a marker of endogenous NO formation) were negatively associated with circulating levels of sFLT-1 [9].

Obesity-related metabolic factors also increase the risk for developing preeclampsia [4,10], and dysregulation of adipocytokines has been associated with endothelial dysfunction in preeclampsia [11]. Nicotinamide phosphorybosil transferase (NAMPT) is a potential biomarker for endothelial dysfunction with cardiovascular impact and may play a role in preeclampsia [12–14]. Noteworthy, women with a history of preeclampsia have an elevated risk for cardiovascular diseases many years postpartum [1,7].

However, no previous study has examined the relation of plasma NAMPT with circulating sFLT-1 and nitrite concentrations in preeclampsia and healthy pregnancy. Here, we hypothesized that inverse relationships exist between NAMPT and these markers in healthy pregnancy and preeclamptic women.

2. Methods

2.1. Subjects

Approval for the use of human subjects was obtained from the Institutional Review Board at the Ribeirao Preto Medical School of

Abbreviations: NAMPT, nicotinamide phosphorybosil transferase; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; sFLT-1, soluble fms-like tyrosine kinase-1; VEGF, vascular endothelium growth factor

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University of Sao Paulo (RPMS-USP). All volunteers were consecutively enrolled in the Department of Obstetrics and Gynecology, University Hospital of the RPMS-USP. We studied 110 healthy women with uncomplicated pregnancies and 94 women with preeclampsia defined in accordance to the American College of Obstetricians and Gynecologists report [2], not including women with pre-existing hypertension. Written informed consent and maternal venous blood samples were collected at clinical attendance.

2.2. Visfatin/NAMPT and sFLT-1 measurements

Plasma was obtained from whole blood in EDTA by centrifugation at 2000g for 10 min and stored at −70 °C until assayed. Plasma visfatin/NAMPT levels (RayBiotech, Norcross, USA) and serum sFLT-1 levels (R &D Systems, Minneapolis, USA) were measured by ELISA [9,15], according to manufacturer's instructions.

2.3. Nitrite measurement

Plasma was obtained from whole blood in heparin by immediate centrifugation at 1000g for 3 min. Plasma aliquots were analyzed in triplicate for their nitrite content using an ozone-based chemiluminescence assay, as previously described [9].

2.4. Statistical analysis

The clinical characteristics of healthy pregnant and preeclamptic women were compared by Student's unpaired *t*-test, Mann-Whitney *U* test, or χ^2 as appropriate and reported as mean ± s.e.m. The correlations between NAMPT levels and sFLT-1 levels or nitrite concentrations were analyzed using Spearman's correlation (*r* and *P* values). Statistical analysis was performed with GraphPad Prism 5.0. A *P* value of < 0.05 was considered the level of statistical significance.

3. Results and discussion

Table 1 summarizes the characteristics of the subjects enrolled in this study. Preeclamptic patients presented higher systolic and diastolic blood pressure, higher age and increased body mass index compared with healthy pregnant women (all $P < 0.05$). We found lower gestational age at delivery and at sampling and decreased newborn weights in preeclampsia compared with healthy pregnancy (all $P < 0.05$). Significant proteinuria was found in preeclampsia. Plasma NAMPT levels were similar between groups ($P > 0.05$), but preeclamptic patients showed elevated plasma sFLT-1 levels and reduced plasma nitrite concentrations than healthy pregnant women (both $P < 0.05$), as previously reported [9,15].

Table 1
Demographic and clinical characteristics of study subjects.

Parameters	Healthy pregnant (n = 110)	Preeclampsia (n = 94)	*P*
Age (years)	24.49 ± 0.60	26.50 ± 0.68	**0.028**
Ethnicity (% white)	72.22	72.34	0.994
Current smoking (%)	11.95	13.82	0.145
BMI ($kg\,m^{-2}$)	23.55 ± 0.48	27.81 ± 0.86	**0.000**
SBP (mm Hg)	112.7 ± 1.08	140.1 ± 1.82	**0.000**
DBP (mm Hg)	72.52 ± 0.99	86.98 ± 1.34	**0.000**
HR (beats per min)	82.49 ± 0.78	80.94 ± 0.82	0.177
Fasting glucose ($mg\,dl^{-1}$)	75.64 ± 1.55	78.08 ± 3.73	0.475
Hb ($g\,dl^{-1}$)	11.79 ± 0.17	11.82 ± 0.15	0.924
Hct (%)	35.44 ± 0.63	35.66 ± 0.47	0.774
Creatinine (μmol^{-1})	58.34 ± 1.7	61.88 ± 2.6	0.907
24 h Pr (mg per 24 h)	ND	813.6 ± 128.9	**0.000**
Primiparity (%)	50.0	39.78	0.100
GAD (weeks)	39.71 ± 0.15	35.98 ± 0.41	**0.000**
Newborn wheight (g)	3322 ± 63.29	2571 ± 104.8	**0.000**
GAS (weeks)	36.83 ± 0.37	33.83 ± 0.47	**0.000**
Plasma NAMPT (ng/mL)	21.39 ± 2.08	20.74 ± 2.54	0.290
Serum sFLT-1 (ng/mL)	3.89 ± 0.27	11.20 ± 1.01	**0.000**
Plasma Nitrite (nM)	159.8 ± 11.26	95.90 ± 4.74	**0.000**

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Hb, hemoglobin concentration; Hct, hematocrit; GAD, gestational age at delivery; 24-h Pr, 24-h proteinuria; GAS, gestational age at sampling; ND: not determined (however, negative dipstick test). Values are the mean ± s.e.m. P < 0.05 *versus* healthy pregnant group. Significant *P* values are in bold.

This is the first study to examine the relationship of plasma NAMPT levels with nitrite and sFLT-1 levels in healthy pregnant and preeclampsia (Fig. 1). Our novel findings were that NAMPT and nitrite were positively correlated in healthy pregnancy ($r = 0.317$, 95% CI = 0.110 to 0.497, $P = 0.002$), but inversely related in preeclampsia ($r = -0.257$, 95% CI = −0.477 to 0.007, $P = 0.038$). Moreover, NAMPT and sFLT-1 were inversely related in healthy pregnancy ($r = -0.340$, 95% CI = −0.592 to −0.027, $P = 0.029$), but positively correlated in preeclampsia ($r = 0.326$, 95% CI = 0.084 to 0.532, $P = 0.007$).

NAMPT can stimulate angiogenesis by upregulating vascular endothelium growth factor (VEGF) through activation of PI3K/Akt and induce endothelium-dependent relaxation via the NO pathway [16]. Indeed, NAMPT was shown to activate endothelial NO synthase in endothelial cells, as reviewed elsewhere [12]. However, NAMPT was shown to impair endothelium-dependent relaxation by activation of NADPH oxidase, with intracellular release of superoxide anions and subsequent quenching and inactivation of endothelial NO [23]. It was also shown to be released by inflamed human endothelial cells [24]. The role of sFLT-1 in maternal endothelial dysfunction and the pathogenesis and symptoms of preeclampsia has been reviewed elsewhere [1,3,5,7]. sFLT-1 binds to circulating VEGF and placental growth factor, which results in reduced production of NO [3]. These findings could explain the opposite relationships we found between NAMPT and sFLT-1 and between NAMPT and nitrite (Fig. 1) in preeclamptic patients compared to healthy pregnant women. Noteworthy, it is relevant to consider the interactions of markers within pathways related to NO bioavailability in preeclampsia [17–20].

Adipokines could play a role in trophoblastic invasion and successful placentation, which requires adequate angiogenesis [21]. Indeed, the maternal plasma concentration of NAMPT peaks between 19 and 26 weeks of gestation [22]. Although we found higher BMI in preeclamptic patients than in healthy pregnant women, it is noteworthy that there was no difference between groups in fasting blood glucose and plasma NAMPT levels (Table 1). However, several findings indicate that NAMPT is implicated in the pathophysiology of atherosclerosis, metabolic disorders, and inflammatory diseases [25]. Here we show that plasma NAMPT are positively related to sFLT-1 levels, which may contribute to endothelial dysfunction in preeclampsia. These data suggest that NAMPT may also have a role in long-term complications of preeclampsia [6,7].

In conclusion, we demonstrated that NAMPT has a distinct relationship with nitrite and sFlt-1 in health pregnancy and preeclampsia. Additionally, we found clinical evidence for an inhibitory effect of NAMPT on NO formation in preeclampsia. Moreover, the positive correlations observed between NAMPT and sFLT-1 in preeclampsia suggest that clinical manifestations of the disease may arise from the interaction between these two markers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

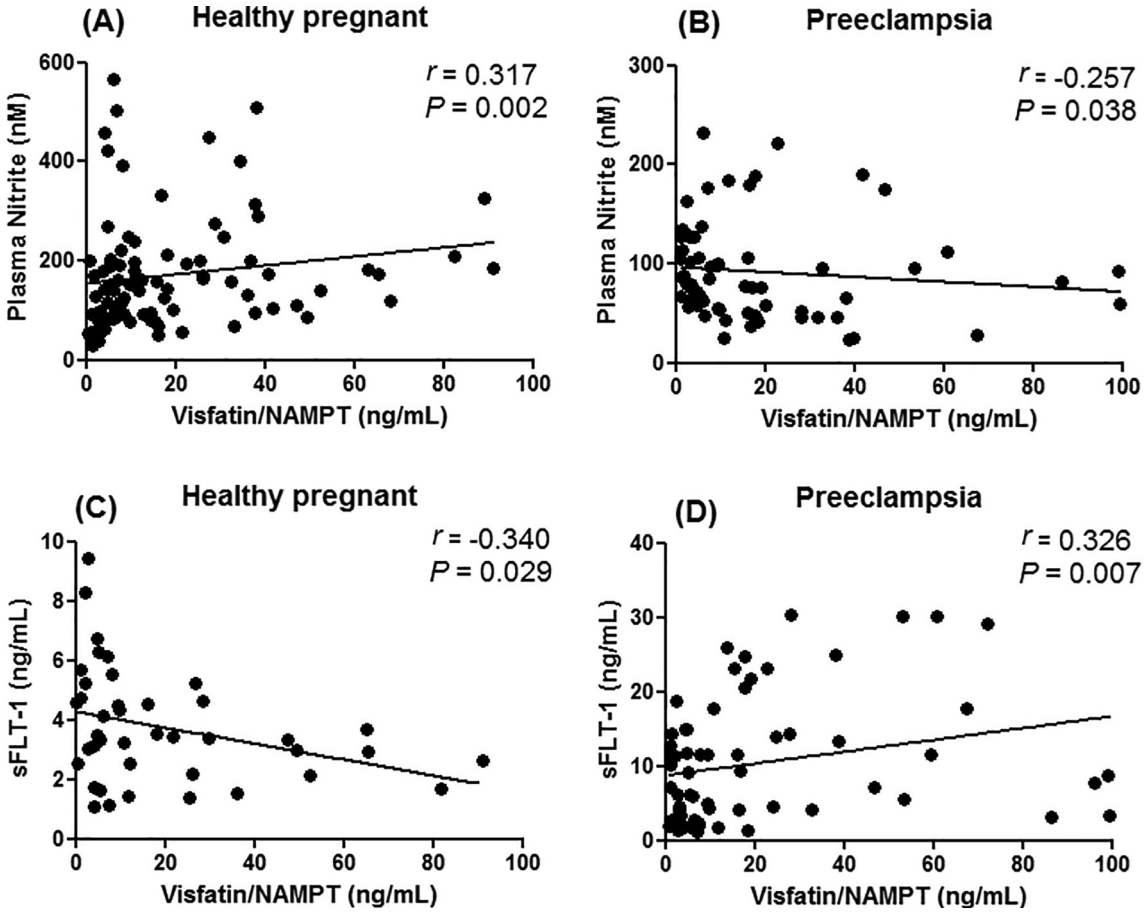

Fig. 1. Correlations between nitrite concentrations and plasma visfatin/NAMPT levels in healthy pregnant (A) and in preeclamptic women (B), and between sFLT-1 and plasma visfatin/NAMPT levels in healthy pregnant (C) and in preeclamptic women (D). The regression lines are plotted. *P* and Spearman's correlation (*r*) values are reported.

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Contributors

VC Sandrim, ACT Palei, Jose E. Tanus-Santos, RC Cavalli, and MR Luizon contributed in the selection of the subjects enrolled in the study. VC Sandrim, ACT Palei, VA Belo and MR Luizon contributed in the measurements of circulating visfatin/NAMPT levels, sFLT-1 levels, and nitrite concentrations. DA Pereira, VC Sandrim, VA Belo and MR Luizon have performed the statistical analysis. DA Pereira, VC Sandrim, ACT Palei and MR Luizon have drafted the manuscript. All authors have seen and approved the final version of the manuscript.

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