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Elevated circulating adenosine deaminase activity in women with preeclampsia: association with pro-inflammatory cytokine production and uric acid levels

Vanessa S. Giorgi [a], Steven S. Witkin [b], Camila F. Bannwart-Castro [c], Maria Salete Sartori [c], Mariana Romão-Veiga [a], Vera Therezinha M. Borges [c], José Carlos Peraçoli [c], Maria Terezinha Peraçoli [a,*]

[a] Department of Microbiology and Immunology, Institute of Biosciences, São Paulo State University, Botucatu, São Paulo, Brazil
[b] Department of Obstetrics and Gynecology, Weill Cornell Medicine, New York, New York, USA
[c] Department of Gynecology and Obstetrics, São Paulo State University, Botucatu, São Paulo, Brazil

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ABSTRACT

Objective: Preeclampsia is a specific disorder of human pregnancy that is associated with hyperuricemia and higher levels of pro-inflammatory cytokines. Adenosine deaminase (ADA) is an enzyme present in all human tissues, and is considered an indicator of cellular inflammation. In the present study we assess whether adenosine deaminase (ADA) activity is altered in women with preeclampsia (PE) and contributes to elevated levels of uric acid and pro-inflammatory cytokine production.

Study design: The population studied consisted of 60 women with PE, 30 normotensive pregnant women (NT) and 20 non-pregnant women (NP). Uric acid concentration and ADA activity were determined in the serum. Peripheral blood mononuclear cells (PBMCs) were isolated and evaluated for intracellular nuclear transcription factor kappa B (NF-κB) levels and for endogenous tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) production. The data were evaluated with parametric or non-parametric tests with significance set at $P < 0.05$.

Results: ADA levels were higher in the PE group compared with the NT and NP groups ($P < 0.001$). A positive correlation between ADA and uric acid levels was identified in women with PE ($P < 0.001$). Endogenous production of IL-1β and TNF-α, as well as intracellular NF-κB levels, were higher in PBMCs from the PE group than from NT and NP women ($P < 0.01$) and correlated with the ADA concentration in preeclamptic women ($P < 0.01$).

Conclusion: An elevation in ADA activity in women with PE may contribute to their increased levels of uric acid and pro-inflammatory immune activity.

1. Introduction

Preeclampsia (PE) is a dangerous complication of pregnancy that is responsible for a high proportion of maternal and infant morbidity and mortality [1]. The disease affects 2–10% of pregnant women [2], and was recently redefined as hypertension present after 20 weeks of gestation combined with proteinuria (>300 mg/-day) and other maternal organ dysfunction, such as renal insufficiency, liver involvement, neurological or haematological complications, uteroplacental dysfunction, or fetal growth restriction [3,4].

Although the etiology of PE remains unclear, the associated pathogenesis has been extensively studied. Excessive activation of leukocytes in PE is associated with exaggerated innate and adaptive immune responses that may interfere with normal pregnancy progression [5]. Increased production of pro-inflammatory cytokines and activation of maternal endothelial cells result in a systemic and diffuse endothelial cell dysfunction that is the fundamental pathophysiological feature of PE [1]. Another important contributor to development of this disorder is oxidative stress; the up-regulated production of reactive oxygen species (ROS), superoxide anion and hydrogen peroxide induces enhanced lipid peroxidation [6]. A higher level of activation of nuclear transcription factor-κB

* Corresponding author at: Department of Microbiology and Immunology, Institute of Biosciences of Botucatu, Rubião Junior s/n, UNESP, Botucatu, São Paulo CEP 18618-970, Brazil.
E-mail address: peracoli@ibb.unesp.br (M.T. Peraçoli).

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(NF-κB), responsible for the transcription of genes related to inflammation, is also evident in mononuclear lymphoid cells of preeclamptic women and results in enhanced release of the primary pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [7,8]. Several studies have suggested that a high concentration of circulating TNF-α is associated with more severe clinical manifestations of PE [9,10].

To down-regulate deleterious increases in immune responses, adenosine nucleotides are released at inflammatory and ischemic sites into the extracellular milieu where they are converted to adenosine by ectonucleases. Concomitantly, intracellular ATP is also dephosphorylated and adenosine is released from the cells. The accumulated adenosine binds to specific receptors on macrophages, neutrophils and T lymphocytes triggering an increase in intracellular levels of cyclic AMP and results in the inhibition of pro-inflammatory cytokine production [11,12]. Adenosine deaminase (ADA) is the enzyme that decreases extracellular adenosine levels by catalyzing its hydrolytic deamination to inosine [13]. Addition of ADA to plasma from newborn infants has been shown to result in an enhanced capacity of mononuclear cells to produce TNF-α [14].

Serum ADA activity is reduced in pregnant as compared to non-pregnant women [15] suggesting an increased need for adenosine-mediated down-regulation of pro-inflammatory immunity during gestation. The association between elevated ADA activity and PE was first reported by Yoneyama et al. [16–18]. Elevated levels of ADA in maternal plasma, umbilical cord blood and the placenta were associated with development of PE, but not with the severity of the disease [19]. A correlation between elevated ADA and myeloperoxidase activities with disease severity and neonatal outcomes in preeclamptic women has also been reported [20].

In pregnant women with PE an elevated serum level of uric acid has been associated with increased production of pro-inflammatory cytokines by mononuclear cells in peripheral blood [21]. Superoxide anion, the inducible 70 kDa heat shock protein and TNF-α production by monocytes have also been associated with uric acid levels in PE [7,9]. Thus, high circulating uric acid levels may directly contribute to the pathogenesis of PE by its promotion of inflammation [22].

Uric acid is the final breakdown product from metabolism of the purines ATP, ADP, AMP, adenosine, inosine and hypoxanthine. The origins of high purine levels, increases in purine metabolism as well as the mechanism leading to elevated levels of uric acid in preeclamptic women are unknown. It is possible that trophoblast microparticles released into the circulation as a consequence of placental damage in preeclamptic women may provide the major substrate for purine metabolism and uric acid formation [23,24].

The present study evaluated whether ADA activity in sera of preeclamptic women was related to enhanced intracellular NF-κB activity in peripheral blood mononuclear cells (PBMCs), endogenous TNF-α and IL-1β production by PBMCs and serum levels of uric acid. Elucidation of these associations would strongly suggest the major involvement of adenosine metabolism in development of PE-associated pathology.

2. Materials and methods

2.1. Patients and controls

The study group consisted of 60 primiparous women without a previous history of hypertension or obstetric and medical complications, admitted to the Obstetric Unit of Botucatu Medical School, Botucatu, SP, Brazil, with a diagnosis of PE. Preeclampsia was defined as a persistent elevated blood pressure value (⩾140/90 mmHg) evaluated on two occasions 2 h apart after 20 weeks of gestation and proteinuria (⩾300 mg/24 h in urine collected during 24 h) in women with no previous history of hypertension [2]. Thirty normotensive (NT) primiparous women with an uncomplicated pregnancy who were non-proteinuric were recruited and matched for gestational age at time of sampling with the preeclamptic group. These women remained normotensive and non-proteinuric until the end of gestation. Gestational age was calculated from the last menstrual period and confirmed by early (<12 weeks gestation) ultrasound examination. Twenty non-pregnant (NP) women were volunteer donors of the Blood Bank from the Hemocenter of the Botucatu Medical School, Botucatu, SP, Brazil and were included as additional controls. Proteinuria in 24-h urine was measured by a colorimetric method, the Technicon RAXT automation system, and uric acid in serum was assessed by uric acid enzymatic Trinder (Biotrol Diagnostic, Bridgewater, NJ, USA) in the Clinical Laboratory of Botucatu Medical School, Botucatu, SP, Brazil. Exclusion criteria included multiple gestation, illicit drug use, and preexisting medical conditions such as diabetes, chronic hypertension, and renal disease. For NP women the same exclusion criteria of pregnant women was utilized, except for gestation characteristics. The study was approved by the Ethics Committee of the Botucatu Medical School, and the written informed consent was obtained from all women involved in the study. (Protocol number 3443/10). For pregnant women with age below 18 years old the written informed consent was obtained from their parents or guardians.

2.2. Determination of adenosine deaminase serum activity

Ten milliliters of blood was collected by venipuncture from the antecubital vein of PE, NT and NP women, and after clot formation the samples were centrifuged at 4 °C for 10 min at 1200 g. The serum fraction was stored in aliquots at −80 °C until the analyses were performed. For ADA activity evaluation preeclamptic patients were also classified as early-onset PE (n = 30) or late-onset PE (n = 30) according to whether disease manifestations occurred before or from the 34th week of gestation, respectively. ADA activity was determined by automation (Vitros System Model 5.1/FS) and a commercial kit (Diagnostica Genbiotech, Brazil) following the manufacturer's instructions. The kit, a non-radioactive colorimetric method, is based on the enzymatic deamination of adenosine to inosine to hypoxanthine by the enzyme purine nucleoside phosphorylase. Hypoxanthine is converted into uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase. H_2O_2 reacts with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-amino antipyrine (4-AA) in the presence of peroxidase and generates the quinone dye, which is measured by a colorimetric method. One ADA unit is defined as the amount required to generate 1 umol of inosine per minute at 37 °C. Results were expressed in IU/L.

2.3. Peripheral blood mononuclear cell (PBMC) culture

PBMCs were isolated from heparinized venous blood by density gradient centrifugation on Histopaque, as described previously [7]. PBMC at a concentration of 5×10^6 cells/mL in RPMI 1640 tissue culture medium (Gibco Laboratories, Grand Island, NY, USA) containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum, 20 mM HEPES, and 40 μg/mL gentamicin were dispensed (100 μL per well) into 24-well sterile flat bottom plates (Nalge Nunc Int, Rochester, NY, USA). The cells were cultured without exogenous stimulation at 37 °C and 5% CO2 for 30 min to assay for intracellular NF-κB activity and for 18 h to assay for cytokine release into the culture medium.

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