Assessment of \textit{l}-arginine asymmetric 1 dimethyl (ADMA) in early-onset and late-onset (severe) preeclampsia

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Preeclampsia (PE) is characterized by hypertension and proteinuria. It has been classified in early or late according to gestational age at the onset of disease. Endothelial dysfunction plays a crucial part in its pathogenesis. NO is a potent vasodilator and ADMA is its endogenous inhibitor. We have assessed maternal ADMA levels. ADMA were increased in early [0.66 \text{\mu mol/L}] versus late sPE [0.47 \text{\mu mol/L}] (P = 0.001) and versus normotensive pregnant [0.48 \text{\mu mol/L}] (P = 0.001). Our findings suggest that high ADMA levels in early sPE could compromise NO synthesis contributing to endothelial dysfunction, leading to impaired placentation and the onset of this disease.

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Introduction

Preeclampsia (PE) is characterized by hypertension and proteinuria occurring after the 20th week of pregnancy in women who have had no previous symptoms. Clinically, it is important to diagnose the severe form of the disease, in which blood pressure and proteinuria are much higher [1]. According to gestational age (GA) at the onset of disease, PE has been classified as early (GA<34 weeks) and late (GA \geq 34 weeks) [2]. Although the etiology of PE is unclear, the predisposition to endothelial dysfunction [3], which may trigger abnormal placentation and haemostatic and inflammatory systems is thought to play a crucial part in its pathogenesis [4,5].

Nitric oxide (NO) is a free radical, produced by several cell types, from \textit{l}-arginine, in a reaction catalyzed by the NO-synthase (NOS). NO produced by endothelial cells has emerged as a versatile mediator involved in many endothelium functions including flow-mediated vasodilatation and platelet activation inhibition [6].

Asymmetric dimethylarginine (ADMA) is synthesized by post-translational modification, involving the addition of two methyl groups from the donor methionine to arginine residues in proteins. This reaction is catalyzed by an enzyme called protein arginine methyltransferase (PRMT) type 1. These methylated proteins are predominantly found in the nucleus and play a role in RNA processing and transcriptional control [7]. ADMA is released into the cytosol when these proteins are hydrolyzed, thereby being an obligatory product of protein turnover. Thus, the amount of ADMA generated depends on the extent of arginine methylation in proteins and on the rate of protein turnover. Although the kidney plays an important role in the elimination of ADMA from the body, by excreting it into the urine [8], the conversion of ADMA by dimethylarginine dimethylaminohydrolase (DDAH I and II) into citrulline and dimethylamine is the most important metabolic pathway [9]. In 1992, Vallance and Leone firstly described that ADMA is an endogenous inhibitor of the arginine-NO pathway [10]. From then, its role in regulating NO production has attracted increasing attention.

Since a role of NO in pathogenesis of PE has been admitted [11,12], we hypothesized that high ADMA levels that compromise the NO synthesis resulting in local endothelial dysfunction might be one important event in PE.

Methods

We have assessed maternal ADMA plasma levels by ELISA (Diagnostika GMBH) in 29 women with early severe PE (sPE), 24 women with late sPE and 50 normotensive pregnant. The inclusion criteria for severe PE were blood pressure \geq 160 \times 110 \text{mmHg}, taken in two different measurements with a minimum interval of 2 h between them and after rest, and proteinuria \geq 2 g in 24 h urine or higher than (++) by the qualitative strip method in random urine sample [1]. Early and late sPE were classified according the GA at the onset of disease (< or \geq 34 weeks, respectively) [2]. For the
normotensive pregnant group the inclusion criteria were systolic/diastolic blood pressure $\leq 120 \times 80$ mmHg, with no former record of hypertension and PE, as well as absence of proteinuria. Exclusion criteria common for the three groups were chronic hypertension, twin pregnancy, haemostatic abnormalities, cancer, diabetes, and cardiovascular, autoimmune, renal and hepatic diseases and anti-coagulant or corticosteroids therapy. Data statistical analysis was performed using SPSS 13.0 software, by Mann–Whitney test.

**Results**

ADMA levels were increased in the early sPE [0.66 μmol/L (0.52–0.78)] compared to late sPE [0.47 μmol/L (0.42–0.60)] ($P = 0.001$) and to normotensive pregnant [0.48 μmol/L (0.42–0.58)] ($P = 0.001$). No difference was observed comparing late sPE and normotensive pregnant.

**Discussion**

Previous studies reported elevated maternal ADMA levels in preeclamptic women [13–17]. Speer et al. 2008 [17] related that pregnant who develop PE had higher ADMA levels in the first half of pregnancy comparing to those that keep normotensives. Savvidou et al., 2003 [14] showed that increased ADMA levels preceded the clinical onset of the disease and these molecule were higher at the 23–25 weeks of gestation which develops PE. Sandrim et al., 2010 [15] found a negative association between ADMA and nitrite (a marker of NO production) levels in preeclamptic Brazilian women, comparing to normotensive pregnant.

It is known that in healthy pregnancy, maternal ADMA levels falls until up to 24 weeks of gestation before rising to pre-pregnancy levels towards term [17]. This change mirrors the initial fall and subsequent rise in maternal vascular tone and blood pressure during normal gestation. As the median of GA of women with early PE performed using SPSS 13.0 software, by Mann–Whitney test. It has been admitted that early sPE is associated with ischemic placential lesions, while in late sPE an adequate or slightly impaired placentation occurs [18].

DDAH II is highly expressed in placenta [19] and protein turn-over in developing fetoplacental unit is high [20], which suggest a large local production of ADMA.

Besides, since an oxidative stress occurs in PE during early placental development [21] and there is evidence that DDAH is sensitive to oxidative inactivation [22], it could be admitted a decrease in DDAH leading to systemic endothelial dysfunction.

Additionally, it has been proposed that high ADMA levels could affect angiogenesis, since the growth factors activity such as vascular endothelial growth factor (VEGF), placental growth factor and basic fibroblast growth factor are mediated by NO-dependent mechanisms [23,24]. A decrease in VEGF expression in culture of endothelial cells by ADMA was demonstrated, suggesting that this protein may affect not only growth factors activity but also their production [25].

To the best of our knowledge, it is the first study assessing ADMA levels in early and late sPE. Our findings suggest that high ADMA levels in early sPE could compromise NO synthesis contributing to endothelial dysfunction, leading to impaired placentation and the onset of this disease. Besides, our results also corroborate to admit that physiopathology of early and late PE is distinct, as suggested by reports on the literature.

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**References**