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ORIGINAL ARTICLE

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Analysis of plasma elastase levels in early and late onset preeclampsia

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Abstract Background: Circulatory neutrophils have been reported to be activated in preeclampsia. It has been suggested that maternal plasma levels of elastase may serve as a possible cell-free marker to quantify such activation. Although plasma elastase levels have been found to be elevated in cases with manifest preeclampsia and eclampsia, this has not yet been examined in cases with early and late onset preeclampsia. We have now examined this aspect. Methods: In this retrospective study, maternal plasma samples were examined from eight cases with early onset preeclampsia (< 34 weeks of gestation), eight cases with late onset preeclampsia (> 34 weeks of gestation) and an equal number of gestational age matched normotensive term controls. Plasma concentrations of elastase were measured by ELISA using a commercially available assay. Results: Plasma elastase concentrations were significantly elevated the preeclampsia study group when compared to the normotensive control group (median = 139.2 ng/ml versus median = 72.1 ng/ml; P = 0.0025). These elevations remained significant when the preeclampsia study group was stratified into case with early onset preeclampsia (median = 118.8 ng/ml versus median = 62.2 ng/ml; P = 0.03), but jailed failed to attain significance for those cases with late onset preeclampsia (median = 181.3 ng/ml versus median = 86.3 ng/ml; P = 0.061). Conclusions: Our data indicate that elastase levels are elevated in both early and late onset forms of preeclampsia, and imply that the activation of neutrophils may be more acute in the former than in the latter (238 words).

Keywords Neutrophils · Elastase · Preeclampsia

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Introduction

Despite intensive research, preeclampsia continues to be a leading cause of feto-maternal mortality and morbidity in the developed and developing world [1, 2]. Current consensus is that the disorder is initiated by a placental defect, which triggers a cascade of events leading to the maternal syndrome [1, 2]. These events include an overt activation of the maternal innate immune system, possibly due the elevated shedding of syncytiotrophoblast-derived microparticles (frequently termed STBM), as well as an imbalance in placenta-derived angiogenic factors [1, 2].

Neutrophils have previously been characterized as one of the major cell types of the maternal innate immune system to be activated in preeclampsia [3–5]. Characteristically these cells play a major role in the clearing of bacterial and viral infections, and are predominant in the immediate cellular infiltration of inflammatory sites. Neutrophils are capable of phagocytosing foreign elements such as bacteria, destroying them via the expression of a number of bacteriocidal substances, which include highly reactive toxic oxygen species, proteases, phospholipases and defensins. Their uncontrolled activation and degranulation can, however, lead to considerable tissue damage, and this feature has been implicated in the wide-spread damage of the maternal endothelium which occurs in preeclampsia [5].

Evidence for the activation of neutrophils in preeclampsia has been obtained using flow cytometry to measure the expression of cell surface activation markers, such as CD11b [4], or the intracellular expression of reactive oxygen species [6]. Soluble markers of neutrophil activation include elastase and lactoferrin [3, 7], the

levels of which have been shown to be significantly elevated in cases with preeclampsia, especially those with eclampsia. Additional evidence for aberrant neutrophil activation in preeclampsia includes data obtained using in vitro models where placentally derived syncytiotrophoblast microparticles have been shown to induce the expression of superoxide radicals in peripheral neutrophils [8]. In this context we have recently observed that STBM will induce the expression of CD11b on isolated circulatory neutrophils, and that such treatment also triggers the formation of extracellular DNA lattices (neutrophil extracellular traps termed NETs) by these cells [9]. We also found evidence for the dramatic increase in the presence of such NETs directly in the intervillous space of preeclamptic placentae when compared to placentae from normal term deliveries [9].

As it has recently been suggested that preeclampsia be stratified into two distinct categories [10], namely those with early onset of the disorder (defined as symptoms prior to 34 weeks of gestation) and late onset (defined as symptoms prior to 34 weeks of gestation), we wished to assess whether neutrophil activation differs between these two groups. For our pilot examination we selected a soluble marker, plasma elastase, which can be readily measured by commercial ELISA technology [3, 7].

Materials and methods

Study and control cohorts

This study was performed in a retrospective manner using samples, which had previously been collected for the analysis of cell-free fetal nucleic acids [11, 12]. Approval for this study was granted by the respective Institutional Review Boards (Basel and Stellenbosch), and export of biological material was approved by the South African Department of Health. Written informed consent was obtained in all instances.

In the present analysis 16 cases with manifest preeclampsia were stratified according to early (n = 8) and late onset (n = 8) forms of the disorder [10]. These were matched with an equal number of normotensive controls. As in our previous examinations, early onset preeclampsia was defined as onset of disease after 20 weeks but before 34 weeks of pregnancy, while those with late onset disease were defined as onset of clinical symptoms after 34 weeks of an otherwise uneventful pregnancy [11, 12]. Preeclampsia was defined by a blood pressure measurement of at least 140/90 mm Hg in two determinations at least 4 h apart, using Korotkoff V, or by a single diastolic blood pressure measurement of more than 110 mm Hg as well as associated proteinuria of at least 2+ on diagnostic urine strips (again, two measurements at least 4 h apart) or ≥300 mg protein loss in a 24 h urine collection [11, 12]. The blood samples from the normotensive control pregnancies were taken at gestational ages matched to those of the two preeclampsia study groups (n = 16).

Plasma elastase analysis

The plasma samples were separated by centrifugation and stored frozen as described previously [11, 12]. All samples were shipped by air freight on dry ice from Cape Town to Basel for analysis. Plasma elastase was determined using a commercial ELISA kit (Bender MedSystems, Vienna, Austria), with a sensitivity of 0.156–10 ng/ml. Absorbance was recorded on spectro-photometer (Molecular Devices, Sunnyvale, CA, USA) using 450 nm as the primary wave length and 620 nm as the reference wavelength. Data was analyzed using SoftMax Pro 3.1 software (Molecular Devices).

Statistical analysis

Data were analyzed using SPSS for Windows, using the Mann–Whitney U-test. P values of less than 0.05 were considered significant. Data are presented by box plots (Fig. 1), indicating the median value (line in the box), the 75th and 25th percentiles (limits of box), and the 10th and 90th percentiles (upper and lower horizontal bars), respectively. One outlier in the cases with late onset preeclampsia with a value of 575.1 was excluded from the figure. This value was not excluded from the statistical analysis.

Results

Our analysis confirms previous reports [3, 7] that the levels of plasma elastase are elevated in cases with preeclampsia when compared to the control group (median = 139.2 ng/ml; range = 53.1–575.15 ng/ml; n = 16 versus median = 72.1 ng/ml; range = 36.6–108.6 ng/ml; n = 16; P = 0.0025). This also held true when the preeclampsia study group was stratified into cases with early and late onset forms of the disorder (Table 1 and Fig. 1) [13, 14]. In this instance, however, the elevations were only significant in the group with early onset preeclampsia (P = 0.03), and just failed to attain significance in the group with the late onset form of the disorder (P = 0.06).

Discussion

Part of the motivation for stratifying preeclampsia into early and late onset forms, is that the former are characterized by higher rates of neonatal mortality and are associated with a greater degree of maternal morbidity than the latter form [10]. It has also been determined that the maternal symptoms associated with the early form are more severe than those with late onset pre-

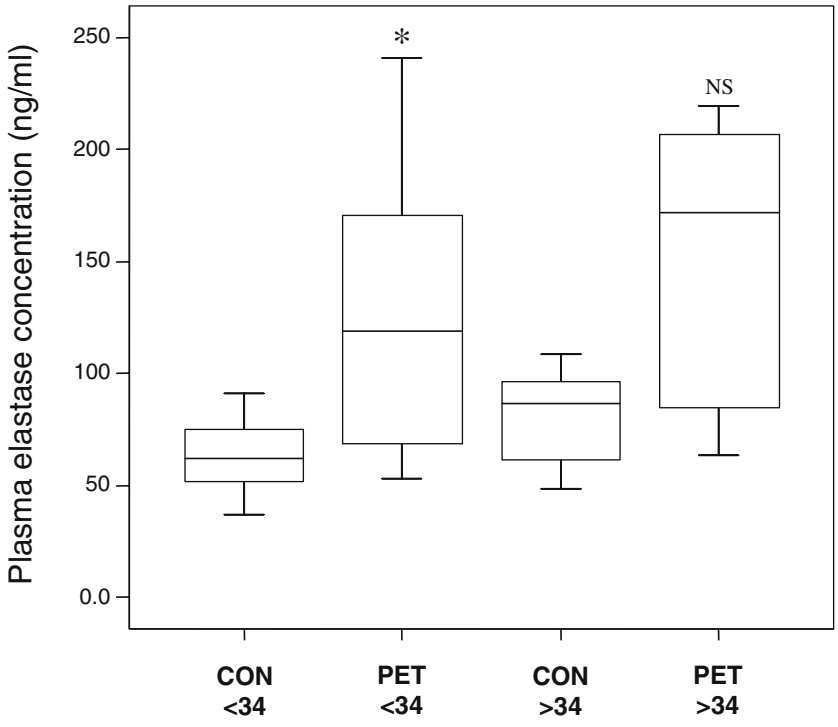

Fig. 1 Box plot illustrating plasma elastase concentrations in cases with early onset preeclampsia (PET < 34), late onset preeclampsia (PET > 34) and, respectively, matched control groups (CON < 34 and CON > 34). Significance is indicated by *

Table 1 Clinical characteristics of the preeclampsia study and gestational age matched control cohorts

	Early pregnancy controls	Early onset preeclampsia	Late pregnancy controls	Late onset preeclampsia
n	8	8	8	8
Blood pressure: systolic	135 (110–150)	170 (150–220)	120 (100–130)	150 (150–190)
Blood pressure: diastolic	80 (70–80)	105 (90–160)	72.5 (60–80)	100 (90–110)
Gestational age at the time of blood collection	30 weeks (24–32 weeks)	31 weeks (24–32 weeks)	36 weeks (35–38 weeks)	34.5 weeks (34–38 weeks)
Gestational age at the time of delivery	39 weeks (37–41 weeks)	31.5 weeks (24–33 weeks)	38 weeks (36–41 weeks)	35 weeks (34–38 weeks)
Fetal weight (g)	3180 (2820–4352)	1425 (476–1760)	3119 (2490–3418)	1880 (1200–2565)
Plasma elastase concentration (ng/ml)	62.2 (47.0–91.3)	118.8 (48.6–108.6)	86.3 (53.1–240.8)	181.3 (63.3–575.1)

eclampsia, a feature also evident in the current study (Table 1) [15]. Evidence for the latter was also seen in our recent studies where we examined the levels of cell-free fetal and maternal nucleic acids in these conditions, where these levels were generally higher in the cases with early onset preeclampsia than in those with late onset [11].

In our analysis we confirm that plasma elastase levels are elevated in preeclampsia when compared to control normotensive pregnancies, thereby providing continuing evidence for the activation of neutrophils in this disorder. A new aspect of our study is that we examined cases with early and late onset forms of preeclampsia. This analysis indicated that although plasma elastase levels were elevated in both forms, these elevations only attained significance in the cases with early onset preeclampsia. This latter result may possibly be due to the small size of our study cohorts (eight cases per group), as the extent of the elevations in each study group in comparison to the matched control group was of the order of twofold. Consequently, it will be necessary to examine this aspect in a larger study.

Although a host of evidence exist implicating the activation of neutrophils in preeclampsia, ranging from the up-regulated expression of cell surface or intracellular activation markers on peripheral circulatory neutrophils [4, 6], in vitro models [8], as well as the recent detection of neutrophil NETs directly in preeclamptic placentae [9], it is currently not clear whether the activation of this cell type is an early or late event in the cascade leading to the development of maternal symptoms.

In order to study this particular facet in a large scale prospective study it will be necessary to apply tools, which permit the ready assessment of neutrophil activation in a simple and reliable manner. Although it is

highly probably that flow cytometry will provide a more accurate assessment of the degree of neutrophil activation in the maternal periphery, such an analysis would not be feasible for a large scale examination, especially for samples collected distal to the site of analysis. In this context, our present data, as well as those of a number of previous studies indicate that the analysis of plasma elastase may serve as a useful marker to quantify the extent of neutrophil activation and degranulation in preeclampsia. It is therefore possible that such measurements may help address this important question and thereby assist in increasing our understanding of this enigmatic disorder.

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