Early-Pregnancy Circulating Antioxidant Capacity and Hemodynamic Adaptation in Recurrent Placental Syndrome: An Exploratory Study

Carmen A.H. Severens-Rijvers^a Salwan Al-Nasiry^b Annemiek Vincken^c
Guido Haenen^d Bjorn Winkens^e Chahinda Ghossein-Doha^b
Marc A.E. Spaanderman^b Louis L.H. Peeters^f

^aDepartment of Pathology, Maastricht University Medical Centre, Maastricht, The Netherlands; ^bDepartment of Obstetrics and Gynecology, Maastricht University Medical Centre, Maastricht, The Netherlands; ^cDepartment of Family Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands; ^dDepartment of Pharmacology and Toxicology, Maastricht University Medical Centre, Maastricht, The Netherlands; ^eDepartment of Methodology and Statistics, Maastricht University Medical Centre, CAPHRI Research School, Maastricht, The Netherlands; ^fDepartment of Obstetrics, UMC Utrecht, Division "Vrouw and Baby", Utrecht, The Netherlands

Keywords

Preeclampsia · Hypertension in pregnancy · Pregnancy · Antioxidants · Trolox equivalent antioxidant capacity · Uric acid · Longitudinal

Abstract

Background/Aims: Placental syndromes (PS) refer to pregnancy complications that include gestational hypertension, (pre)eclampsia, HELLP syndrome, and/or placental insufficiency-induced fetal growth restriction. These disorders are characterized by increased oxidative stress. This study aims to test the hypothesis that the abnormal hemodynamic adaptation to pregnancy, typical for early PS pregnancy, is accompanied by abnormal maternal levels of antioxidants relative to those in normal pregnancy. **Methods:** Before, and at 12, 16, and 20 weeks pregnancy, we measured trolox equivalent antioxidant capacity (TEAC), uric acid (UA), and TEACC (TEAC corrected for UA) in maternal serum of former PS patients, who either developed recurrent PS (rPS; $n = 16$) or had

a normal next pregnancy (non-rPS; $n = 23$). Concomitantly, we also measured various hemodynamic variables. **Results:** rPS differed from non-rPS by higher TEACC levels before pregnancy (178 vs. 152 μM ; $p = 0.02$) and at 20 weeks pregnancy (180 vs. 160 μM ; $p = 0.04$). Only non-rPS responded to pregnancy by significant rises in hemodynamic measures. **Conclusion:** These data indicate that rPS pregnancies are preceded by an increase in antioxidant capacity, presumably induced by subclinical vascular injury and low-grade chronic inflammation.

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Introduction

Preeclampsia (PE) is a common placental syndrome (PS) and contributes importantly to maternal and perinatal morbidity and mortality worldwide. Its overall prevalence is about 4.6% [1] with a recurrence rate of about

25% [2]. Other PSs are gestational hypertension, HELLP syndrome, eclampsia, and fetal growth restriction caused by placental insufficiency. Although the clinical signs of PS usually only develop in the second half of pregnancy, PS is often preceded by impaired placental development in early pregnancy [3].

During early placentation, trophoblast cells (TBs) migrate upstream into the lumen of the spiral arteries, not only increasing their functional capacity but also forming plugs that impede maternal blood to reach the intervillous spaces. The latter not only secures embryonic development in a low oxygen environment. It also provides protection of proliferating and differentiating TBs against damage by reactive oxygen species (ROS) [4]. Defective spiral artery remodeling increases the risk of premature dislocation of TB plugs, and with it, premature rise in local tissue pO_2 [5]. Therefore, the length of the period of hampered spiral artery blood flow and associated preservation of the low-oxygen milieu at the fetal-maternal interface contributes importantly to the embryo's defense against ROS, more so as fetal antioxidant defenses are relatively weak in early gestation [6]. These inferences support the view that circulating maternal antioxidant levels in early pregnancy are likely to provide an important back-up system in the defense of the developing concept against ROS. However, it is unknown whether this premature unplugging is the only cause of increased oxidative stress and development of clinical disease or whether other factors play a role as well. Antioxidant capacity also provides an indirect estimate for the amount of oxidative stress [7].

Antioxidants have been shown to be decreased in both plasma [8–10] and placentas [9] of women with PE. Unfortunately, in the preclinical phase of PE, only a limited number of antioxidant markers were reported instead of total antioxidant capacity [9]. Therefore, we evaluated total antioxidant capacity by measuring trolox equivalent antioxidant capacity (TEAC), in serum of former PS patients before pregnancy, and again at 12, 16, and 20 weeks of pregnancy.

Methods

We performed a retrospective longitudinal study at the Maastricht University Medical Center in Maastricht, the Netherlands. Although serum samples were collected prospectively, the samples were analyzed retrospectively. From 2002 to 2010, women who had a severe PS in the preceding pregnancy underwent serial monitoring in a specialized obstetrical unit before and during the first half of their next pregnancy as part of their high-risk obstetrical

care. Exclusion criteria for this study were multiple pregnancies and presence of hypertension at the time of initial screening, since preexistent hypertension is not only treated in most cases but also associated with subnormal antioxidant levels [11]. The study protocol was approved by the institutional Medical Ethics reviewing Committee (MEC 10-4-049). All women gave informed consent and participated consecutively.

During the study period, we followed women prospectively with serial examination of various hemodynamic parameters and serum markers of the metabolic and the oxidative status. Post hoc, we subdivided these women into subgroups based on whether they did recurrent PS (rPS) or did not develop non-rPS later on in that pregnancy.

We defined PE and gestational hypertension, as described in detail previously [12]. A newborn was considered small-for-gestational-age, when birthweight was below the 10th centile, based on the most recent Dutch birth weight reference curves [13]. PS was considered severe, when its clinical onset was early (<34 weeks) and/or the infant's birthweight was low (<2.3rd centile) due to placental dysfunction as diagnosed on the basis of abnormal fetal biometry (asymmetric growth) and/or abnormal Doppler tracings of the uterine arteries (resistance or pulsatility index >90th centile).

After an overnight fast, we collected a venous blood sample, which was immediately centrifuged and the serum stored at -30°C for later analysis. A technician blinded for pregnancy outcome measured all serum TEAC and uric acid (UA) levels after completion of data collection. We obtained ABTS (2,2'-azinobis [3-ethylbenzthiazoline-6-sulfonic acid]) and UA from Sigma Chemical Co. (St. Louis, MO, USA), and 2,2'-azino-bis(2-amidinopropane) and dihydrochloride from Brunschwig Chemicals (Amsterdam, The Netherlands). Finally, Merck Biochemica (Darmstadt, Germany) provided $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, and trichloroacetic acid, and NaOH, which were of analytical grade purity. For measuring total serum antioxidant status, we used the TEAC assay. This assay determines the capacity of all antioxidants present in blood serum to scavenge $\text{ABTS}^{+\bullet}$. Serum deproteinization was carried out by adding an equal volume of a 10% (w/v) trichloroacetic acid solution. Serum samples were placed on ice for 5 min to complete deproteinization, followed by centrifugation at $14,000\text{ g}$ at 4°C for 5 min. In the TEAC decolorization assay, $\text{ABTS}^{+\bullet}$ is generated chemically. $\text{ABTS}^{+\bullet}$ was produced by incubating a solution of 0.23 mM ABTS and 2.3 mM 2,2'-azino-bis(2-amidinopropane) dihydrochloride in 100 mM sodium phosphate buffer, pH 7.4 at 70°C until the absorption of the solution reached 0.70 ± 0.02 at 734 nm. The antioxidants in the serum react with $\text{ABTS}^{+\bullet}$ which leads to decolorization of the initially blue/green sample. The fall in the $\text{ABTS}^{+\bullet}$ concentration in a given time period, due to the reaction of $\text{ABTS}^{+\bullet}$ with the antioxidants present in the sample, is used to quantify the antioxidant capacity of the sample. So, the degree of decolorization reflects the amount of $\text{ABTS}^{+\bullet}$ that has been scavenged and can be determined spectrophotometrically. The resulting value was compared with the TEAC value for the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman 2-carboxylic acid). The TEAC value of a serum sample is expressed in μM and gives the level of a trolox solution, which corresponds to the antioxidant capacity in the serum sample.

Given that UA is a major determinant of TEAC [14] and also strongly associated with the development of PE [15], we also measured UA concentrations and calculated antioxidant capacity in serum attributable to other antioxidants than UA (TEACC). For

Table 1. Baseline demographic characteristics and outcome of current pregnancy in both study groups

	Non-rPS (<i>n</i> = 18)	rPS (<i>n</i> = 21)	<i>p</i> value
Age, years	33 (30–36)	32 (29–36)	0.57
BMI (at 12 weeks GA)	27 (23–31)	27 (23–28)	0.47
Obstetric history			
GH/PE, <i>n</i> (%)	15 (83)	20 (95)	0.22
SGA/stillbirth, <i>n</i> (%)	3 (17)	1 (5)	0.22
Birth weight, g	1,630 (974–2,509)	1,283 (670–1,841)	0.22
GA, weeks	33 (31–36)	31 (28–34)	0.14
Current pregnancy			
GH, <i>n</i> (%)	–	10 (48)	
GA at onset, weeks		34.1 (27–38)	
PE, <i>n</i> (%)	–	6 (29)	
GA at onset, weeks		36.0 (33–37)	
SGA, <i>n</i> (%)	–	8 (38)	
Birth weight, g	3,417 (3,149–3,675)	2,756 (2,278–3,365)	0.003
GA at birth, weeks	39 (38–40)	37 (36–39)	0.01

Data are presented as median (IQR), unless noted otherwise.

BMI, body mass index; GA, gestational age; GH, gestational hypertension; PE, preeclampsia; SGA, small-for-gestational age; Non-rPS, no recurrent placental syndrome; rPS, recurrent placental syndrome; IQR, interquartile range.

measuring UA in supernatant after denaturation, reversed-phase high-performance liquid chromatography was used. TEACC was calculated by subtracting UA from TEAC to provide an indication of the change in other important contributors to overall antioxidant capacity.

Since oxidative stress not only impairs hemodynamic function [16] but also associates with increased vascular injury- and inflammatory markers [17], we measured these components as well. PV was estimated as detailed previously [18]. Markers of vascular injury and inflammation (serum fibronectin and CRP, mg.L⁻¹), parameters of maternal hemodynamic adaptations (mean arterial pressure [mm Hg], heart rate [bpm], cardiac output [L.min⁻¹], stroke volume [mL], creatinine clearance [mL.min⁻¹]) [19], as well as uterine artery Doppler indices – as a measure of placental hemodynamic development – were performed as previously described [19–21].

We performed statistical analyses using IBM SPSS Statistics for Windows (version 23.0, Armonk, NY, USA). Data are presented as median with interquartile range for numerical variables and as number (%) for categorical variables. Interquartile ranges are presented as 25th and 75th percentiles. Intergroup differences were tested using Mann-Whitney U test for numerical variables and Fisher's exact test for categorical variables. Spearman's rho correlations were used for associations between numerical variables. The differences in longitudinal trends in outcome parameters between groups (rPS vs. non-rPS) were assessed using linear mixed models with time (categorical: prepregnancy, 12, 16, and 20 weeks of pregnancy), group and group * time as fixed factors. Different covariance structures for repeated measures ([heterogeneous] first-order auto-regression, compound symmetry, diagonal, or independence) were considered, where the final structure was based on the smallest Akaike's information criterion. Missing outcome data are assumed to be missing at random, where the likelihood-based approach (no multiple imputation) was used. Results from

linear mixed models are presented as estimated means with standard error. A two-sided *p* value ≤0.05 was considered to be statistically significant.

Results

Serum samples were available from 48 women with a severe PS in their preceding pregnancy along with the intention to conceive again. We excluded 9 women because of preexistent hypertension, thus enrolling 39 women in our study. From these women, 16 developed rPS and 23 had a normal next pregnancy (non-rPS). Median time interval between pregnancies was in rPS 36 months (range 19–96 months) and in non-rPS 29 months (range 17–92 months). The difference was not statistically significant (*p* = 0.866).

Table 1 lists baseline characteristics and pregnancy outcomes of both study groups. All women were Caucasian. There were no statistically significant differences between the 2 study groups, besides current pregnancy outcome in which rPS differed from that in non-rPS by an about 2 weeks shorter pregnancy duration (*p* = 0.003) and a 20% lower birthweight (*p* = 0.01).

Table 2 shows the levels of TEAC, UA, and TEACC at the 4 consecutive measurement points. There were no significant interaction effects of group with time. However, there was a higher TEACC level at 12 weeks

Table 2. (Pre-)pregnant circulating levels of TEAC, UA, and TEACC

		Prepregnancy	12 weeks GA	16 weeks GA	20 weeks GA
		<i>n</i> = 13 <i>n</i> = 9	<i>n</i> = 17 <i>n</i> = 20	<i>n</i> = 18 <i>n</i> = 20	<i>n</i> = 13 <i>n</i> = 16
TEAC, mM	Non-rPS	465 (19)	389 (15)***	394 (16)***	398 (23)**
	rPS	466 (23)	397 (15)***	405 (16)**	437 (24)
UA, mM	Non-rPS	312 (20)	217 (14)***	233 (15)***	238 (19)***
	rPS	287 (24)	223 (15)**	230 (15)*	258 (20)
TEACC, mM	Non-rPS	152 (7)	171 (6)***	161 (6)	160 (7)
	rPS	178 (8) [†]	181 (7)	179 (7)	180 (7) [†]

Data are presented as estimated means (SE), obtained from linear mixed model analysis.

[†] Significant difference ($p < 0.05$) compared to non-rPS.

* Significant within-group change ($p < 0.05$) compared to prepregnant value.

** Significant within-group change ($p < 0.01$) compared to prepregnant value.

*** Significant within-group change ($p \leq 0.001$) compared to prepregnant value.

Non-rPS, no recurrent placental syndrome; rPS, recurrent placental syndrome; Pre, prepregnant; GA, gestational age; TEAC, trolox equivalent antioxidant capacity; UA, uric acid.

Fig. 1. Circulating TEACC concentrations in women with rPS and those without (non-rPS). Data are presented as estimated means (SE), obtained from LMM analysis. [†] Significant difference ($p < 0.05$) compared to non-rPS. GA, gestational age; rPS, recurrent placental syndromes; non-rPS, no recurrent placental syndrome; TEACC, trolox equivalent antioxidant capacity.

relative to prepregnancy, only in non-PS ($p = 0.001$). TEACC levels in rPS tended to be higher than in non-rPS, the difference only being statistically significant before pregnancy ($p = 0.019$) and at 20 weeks ($p = 0.041$), as illustrated in Figure 1. Meanwhile, UA and TEAC levels did neither differ appreciably between the 2 groups at the 4 measurement points nor did these levels change appreciably during the measurement period, except for temporarily lower levels in both groups

at 12 and 16 weeks, relative to prepregnancy (all $p < 0.05$).

Table 3 shows maternal parameters related to inflammation and to the hemodynamic and placental adaptation to pregnancy in both study groups. Although the hemodynamic response to pregnancy was similar in both study groups, the magnitude of the systemic changes was larger in non-rPS. Fibronectin levels were higher in rPS at 16 weeks pregnancy than in non-rPS ($p = 0.008$), this difference being only borderline significant at 12 and 20 weeks ($p = 0.059$ and $p = 0.056$, respectively). Prepregnant CRP seemed higher in rPS, but because there were only 2 women with data at that time-point in the rPS group, it was not possible to test for a between-group difference. However, in both groups we observed a rise in CRP in response to pregnancy (non-rPS $p \leq 0.001$ at 12 weeks, rPS $p = 0.042$ at 20 weeks of gestation), reaching a comparable plateau by 12 weeks. Interestingly, the highest uterine PI and RI levels at 12 weeks of pregnancy in the rPS group were observed in women who also had the highest TEACC levels ($r = 0.736$, $p = 0.028$ and $r = 0.788$, $p = 0.025$, respectively; online suppl. Table. 1, see www.karger.com/doi/10.1159/000501254).

Discussion

In this study, we demonstrated that antioxidant capacity measured as TEACC levels was higher in rPS as compared to non-rPS in the prepregnant state and at 20 weeks

Table 3. (Pre)pregnant maternal parameters for inflammation, hemodynamic, and placental adaptation to pregnancy in women with rPS and those without (non-rPS)

	Prepregnancy	Number	12 weeks GA	Number	16 weeks GA	Number	20 weeks GA	Number
Fibronectin, mg/L								
Non-rPS	262 (23)	10	269 (21)	15	257 (19)	13	250 (22)	13
rPS	285 (31)	5	331 (24)	16	335 (20) ^{††}	16	313 (24)	15
CRP								
Non-rPS	1.3 (1.1)	6	8.6 (1.8) ^{***}	12	9.2 (1.5) ^{***}	13	7.5 (1.3) ^{***}	12
rPS	3.8 (2.4)	2	8.4 (1.9)	15	8.4 (1.5)	16	8.8 (1.3) [*]	15
PV, mL								
Non-rPS	2,357 (140)	10	2,520 (124)	13	2,723 (124) [*]	13	2,845 (124) ^{**}	13
rPS	2,073 (219)	5	2,667 (140) [*]	14	2,733 (136) ^{**}	15	2,644 (140) [*]	14
MAP, mm Hg								
Non-rPS	92 (3)	10	82 (2) ^{***}	15	82 (2) ^{***}	4	82 (2) ^{***}	13
rPS	94 (3)	5	85 (2) ^{**}	16	79 (2) ^{***}	8	82 (2) ^{***}	15
HR (beats/min)								
Non-rPS	67 (3)	10	69 (3)	15	72 (2) ^{**}	15	74 (2) ^{***}	13
rPS	71 (3)	5	76 (3)	16	73 (2)	16	75 (2)	15
CO, L/min								
Non-rPS	5.4 (0.2)	10	5.7 (0.2)	15	6.0 (0.2) ^{**}	15	6.1 (0.2) ^{***}	13
rPS	5.3 (0.3)	5	5.8 (0.2)	16	5.6 (0.2)	16	5.9 (0.2)	15
SV, mL								
Non-rPS	78 (3)	10	77 (3)	15	80 (3)	15	79 (3)	13
rPS	68 (4)	5	73 (3)	16	74 (3)	16	76 (3)	15
CC, mL/min								
Non-rPS	113 (8)	10	150 (7) ^{***}	14	144 (6) ^{***}	15	144 (7) ^{***}	13
rPS	124 (12)	5	135 (7)	16	135 (7)	16	135 (7)	15
Uterine artery PI								
Non-rPS	2.9 (0.2)	9	1.9 (0.2) ^{**}	12	1.4 (0.2) ^{***}	13	1.0 (0.1) ^{***}	11
rPS	3.4 (0.3)	5	1.9 (0.3) ^{**}	12	2.0 (0.2) ^{**}	12	1.1 (0.1) ^{***}	14
Uterine artery RI								
Non-rPS	0.91 (0.0)	9	0.72 (0.0) ^{***}	12	0.65 (0.0) ^{***}	13	0.58 (0.0) ^{***}	11
rPS	0.92 (0.0)	5	0.75 (0.0) ^{**}	12	0.75 (0.0) ^{**}	12	0.59 (0.0) ^{***}	14

Data are presented as estimated means (SE) obtained from linear mixed model analysis.

* Significant within-group change ($p < 0.05$) compared to prpregnant value.

** Significant within-group change ($p \leq 0.01$) compared to prepregnant value.

*** Significant within-group change ($p \leq 0.001$) compared to prepregnant value.

†† Significant difference ($p < 0.01$) compared to Non-rPS.

CRP, C-reactive protein; PV, plasma volume; MAP, mean arterial pressure; PI, pulsatility index; RI, resistance index; PS, placental syndrome; non-rPS, no recurrent PS; rPS, recurrent PS; HR, heart rate; CO, cardiac output; SV, stroke volume; CC, creatinine clearance.

pregnancy. An increase was also seen in fibronectin levels, but these were only significantly higher in rPS at 16 weeks. Maternal hemodynamic response to pregnancy was similar in both groups, but the magnitude of the systemic changes was larger in non-rPS. CRP levels in pregnancy did not differ appreciably between both groups. We had insufficient pre-pregnant CRP data in this study to enable intergroup comparison.

Changes in circulating biomarkers for oxidative stress and antioxidant capacity as indicators of normal preg-

nancy adaptation as well as predictors of the later development of PE have been debated for decades [22, 23]. We previously observed higher prepregnant CRP and fibronectin levels in a large fraction of former PS women, indicative of chronic low-grade inflammation and subclinical endothelial activation [24]. Together with the observation in the present study of higher prepregnant TEACC levels, this suggests that the higher prepregnant TEACC levels in rPS patients in this study could be an adaptive response to the previously reported chronic changes. This

adaptive response is supported by the strong positive correlation between TEACC and uterine artery Doppler PI/RI values, since increased uterine PI and RI are consistent with defective spiral artery remodeling [25] and therefore with increased oxidative stress. Such a redox mediated, adaptive response is not unusual in nature [26].

Reports on the antioxidant levels in relation to PS are conflicting with some studies showing decreased [8–10] and others increased circulating antioxidant levels [27, 28] in PS pregnancies. Similarly, studies measuring antioxidant status in the preclinical phase of the disorder showed either decreased [29] or equal [30] levels. The conflicting reports are probably related to the use of different methods to assess the antioxidant status, for example, by determining distinct antioxidative enzymes or total antioxidant capacity or by correcting or not correcting the antioxidant status for UA levels, as the endocrine environment of pregnancy is known to influence UA levels [31].

Our findings do not support a solitary link between pregnancy-dependent changes in circulating antioxidant capacity and abnormal placental development in rPS. At any rate, it is conceivable that the preexistent subclinical vascular damage together with the chronic low-grade inflammation are responsible for both the trend to higher antioxidant capacity and the defective placental development, which precedes symptomatic rPS. Thus, rPS may continue to develop in spite of the already activated antioxidant system. Apparently, the activated antioxidant system in rPS pregnancies does not play a protective role, but is merely an epiphenomenon of some other latent cardiovascular or metabolic abnormality that interferes with early placental development. It is now widely accepted that PS is indeed related to (sub)clinical cardiovascular and metabolic changes. It is interesting to interpret these results in the context of larger randomized controlled trials which did not show any effect of antenatal antioxidant therapy with vitamin E and/or C in preventing PE [32, 33].

Strengths of this study are the inclusion of longitudinal data on antioxidant capacity from preconception until mid-pregnancy and the simultaneous measurement of various circulatory parameters. This allowed us to study the association between oxidative stress and maternal hemodynamic adaptation in a group of women at high risk of developing PS. Yet, we are aware of the modest sample size that limits the power of this study. On the other hand, the demonstration of statistically significant differences in this exploratory study is encouraging and may enable sample size calculations for larger prospective studies. We are also aware of the consequence of studying this particular high-risk population, which is known to contain a relatively large fraction of women with some subclinical disorder that may influence the endpoints of this study.

In conclusion, this study showed that rPS pregnancies are preceded by an increase in antioxidant capacity, presumably induced by subclinical vascular injury and low-grade chronic inflammation. More research is needed to determine how these phenomena are interconnected to improve the management strategy of high-risk pregnancies.

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