ORIGINAL SCIENTIFIC ARTICLE

ASSESSMENT OF THE SYSTEMIC OXIDATIVE STRESS IN PREECLAMPSIA

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ABSTRACT

Preeclampsia (PE) is a major complication of pregnancy with both mother and fetal adverse outcomes. Pregnancy is a state of increased oxidative stress that has been reported to be exacerbated when complicated with preeclampsia. However, conflicting data are available in literature regarding the systemic oxidative stress in PE pregnancies. The present pilot study was purported to assess systemic oxidative stress in preeclamptic vs healthy pregnancies. To this aim plasma derived compounds of reactive oxygen metabolites (d-ROMs) and the biological antioxidant potential (BAP) were determined in mild and severe preeclamptic pregnancies using the Diacron equipment. Both healthy and preeclamptic pregnancies showed high levels of systemic oxidative stress. Paradoxically, significantly higher values of d-ROMs were found in healthy pregnancies as compared to the PE ones. At variance, in preeclamptic pregnancies, a major increase in the plasma antioxidant capacity occurred. In this pilot study, we report an increase in the systemic antioxidant capacity in preeclamptic pregnancies.

Keywords: *preeclampsia, plasma reactive oxygen species, d-ROM, biological antioxidant potential, BAP, Diacron.*

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INTRODUCTION

Preeclampsia (PE), the most frequent complication of pregnancy, is a multisystem disorder diagnosed after 20 weeks of gestation. The increased systolic (≥ 140 mmHg) and/or diastolic (≥90 mmHg) blood pressure at two repeated measurements is associated with proteinuria (≥300 mg/24 hours), and/or at least one of the following new-onset dysfunctions: hepatocytolysis, new-onset headache unresponsive to medication, visual symptoms, thrombocytopenia < 100,000/mm3, hemolysis, high risk for disseminated intravascular coagulation, acute kidney injury, pulmonary edema, neurological complications, and also uteroplacental dysfunction (fetal growth restriction, abnormal Doppler ultrasound findings) (1).

The pathogenesis of preeclampsia has been extensively studied, yet the precise underlying molecular mechanism is only partially elucidated. However, oxidative stress has been unequivocally reported to play a major role in both endothelial and placental dysfunction systemic from PE ultimately leading to the specific clinical syndrome (2).

Oxidative stress is defined as the imbalance between reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) generation and the antioxidant defense systems. Preeclampsia has been systematically associated with both increased ROS production and impaired antioxidant capacity (3).

ROS denomination include both free radicals and their non-radical intermediates, which playing important roles in cell physiology as second messengers in many signaling pathways when generated in small amounts. When excessively produced, they become harmful molecules, the most common deleterious free radical being the superoxide anion (O2-) originating from several sources: mitochondrial electron transport chain, NADPH oxidase (nicotinamide adenine dinucleotide phosphate), xanthine oxidase, uncoupled endothelial nitric oxide synthase (eNOS), cytochrome P450 etc.

Antioxidants comprise both enzymatic and non-enzymatic compounds aimed at counteracting the deleterious effects of excessive ROS. The most important enzymatic antioxidants are: superoxide dismutase (SOD), hemoxygenase (HO-1), catalase (CAT), glutathione peroxidase (GPx), and thioredoxin (TRX) (4). Superoxide dismutase enzyme catalyzes the dismutation of superoxide anion (O2-) into hydrogen peroxide, finally, catalase and glutathione peroxidase, will convert it into water (5,6).

It has to be mentioned that increased ROS generation occurs in both hyperoxic and hypoxic states or their alternance as it happens in PE where repetitive episodes of tissue hypoxia-reoxygenation have been described (7).

While pregnancy itself is associated with increased oxidative stress, ROS generation is further exacerbated in all pregnancy complications, such as: PE, gestational diabetes, spontaneous pregnancy loss and fetal growth restriction (4).

The pathogenesis of preeclampsia has been classically described as evolving according to the “two-stage” model, where the stressed syncitiotrophoblast releases factors into the maternal circulation which stimulates the inflammatory response and activation of the maternal endothelial cells (8).

Both local and systemic oxidative stress have been incriminated as underlying pathomechanisms in PE, yet data in the literature are controversial.

Local oxidative stress can be directly assessed in confocal microscopy with the aid of fluorescent probes: 2′,7′-dichlorofluorescein (DCF) and dihydroethidium (DHE) stainin. Systemic oxidative stress can be quantified using a spectrophotometric method of measuring the derivates of reactive oxygen metabolites (d-ROM) using the Diacron equipment. The latter also allows the global assessment of the biological antioxidant potential (BAP) (9,10).

Indirect markers of oxidative stress in plasma (eg, lipid hydroperoxides, malondialdehyde) or urine (F2-isoprostanes and uric acid) were reported to be increased in pregnancies complicated by preeclampsia (11-13).

In preeclampsia, it is the systemic oxidative stress that leads to endothelial dysfunction, generalized vasoconstriction, which are further amplified via the release into the maternal circulation of pro-inflammatory cytokines, exosomes, and cell-free fetal DNA (14-16).

The present study was aimed to quantify the systemic oxidative stress by measuring the plasma reactive oxygen metabolites (d-ROMs) and the biological antioxidant capacity (BAP) in pregnancies complicated or not by preeclampsia.

PATIENTS AND METHODS

2.1 Study population

This cross-sectional, single-site study was conducted according to the Helsinki declaration (1975), as revised in 2008 and its later amendments, and was approved by the Committee for Research Ethics of "Victor Babeș" University of Medicine and Pharmacy, Timișoara, RO (no. 7/30.01.2019 and 28/25.06.2020). All participants provided a written informed consent.

The systemic oxidative stress was assessed in 20 participants evaluated in the third trimester of pregnancy (28-40 weeks of pregnancy), divided into healthy (n=10) and preeclamptic pregnancies (n=10), both mild (n=8) and severe forms (n=2) of preeclampsia were included in the study.

2.2 Systemic oxidative stress

Peripheral blood samples were collected in the third trimester of pregnancy (28-40 weeks of gestation). One-step centrifugation was performed and the resulting plasma stored at -80 degrees Celsius for a maximum of 12 months. Systemic oxidative stress was determined in plasma using the Free Radical Analytical System 4 (Diacron, Grosseto, Italy), which evaluates changes in the reactive oxygen metabolites (d-ROMs) and the plasma antioxidant capacity (BAP) as a measure of the non-enzymatic scavengers. d-ROMs are derivates generated from the interaction of free radicals with organic molecules, being more stable. The method assumes biochemical reactions between the hydroperoxides and the reagents, resulting in colored compound which is further measured spectrophotometrically. The results were expressed in Carratelli units (CARR U).

The antioxidant capacity of the plasma is quantified by the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), by adding a biological sample which possesses antioxidative defense capacity.

To express differences between antioxidant and pro-oxidant potential, the ratio of BAP to d-ROMs was calculated (BAP/d-ROMs) (17) .

2.3 All chemicals were purchased from Diacron International SRL (Ital.)

2.4 Statistics

Statistical data processing was performed with the GraphPad Prism Software Version 9.3.1 (GraphPad, USA). Data are presented as mean ± SEM and were analyzed using Student t-test. Values of $P < 0.05$ were considered statistically significant.

The diagnosis of preeclampsia was based on the criteria proposed by The International Society for the Study of Hypertension in Pregnancy (ISSHP), as previously defined (18). The exclusion criteria were: diagnosis of chronic hypertension, coexistent diabetes mellitus, chronic kidney disease, twin pregnancy, inflammatory disorders, malignancies, cardiovascular disease and coagulation disorders. All pregnant women were evaluated in the third trimester (28-40 weeks of pregnancy).

All preeclamptic patients underwent antihypertensive treatment with Methyldopa and/or Nifedipine. Patients who received antiplatelet drugs, anticoagulants or magnesium sulphate therapy were excluded from the study.

RESULTS

Characteristics of the study groups

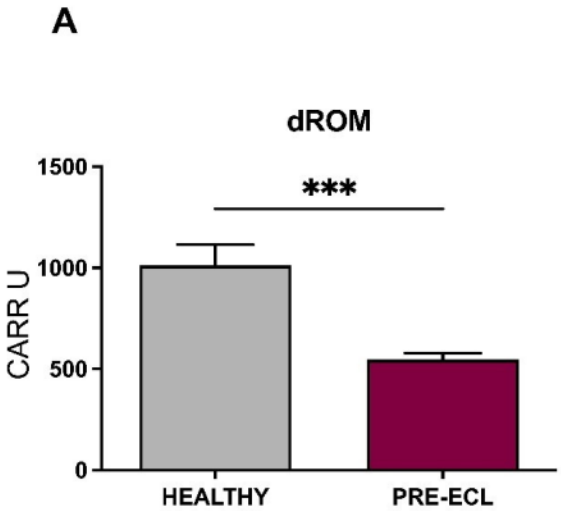
The following demographic and laboratory characteristics of pregnant women (PE and healthy pregnancies) were included: maternal age, gestational age, maternal blood pressure, fetal weight (Table 1). Additionally, the mean arterial pressure - MAP = (Syst BP + 2 X Diast BP)/3 was calculated. The mean arterial pressure (MAP) was significantly increased in the PE pregnancies, as the main diagnostic criteria.

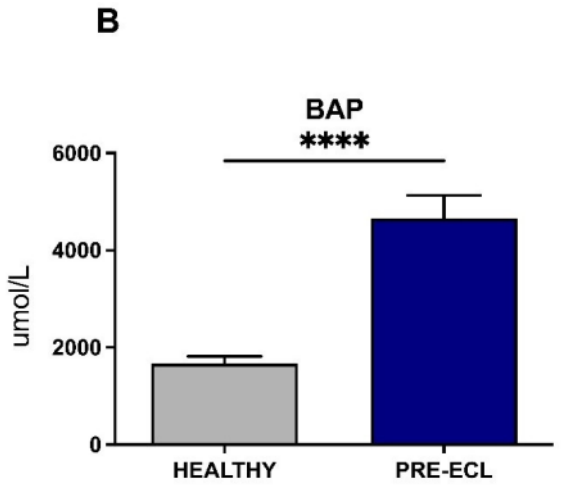
Table 1. Patients characteristics

Parameter	HEALTHY	PREECLAMPSIA
Gestational age	35.37 ± 0.33	32.66 ± 2.6
Systolic BP	123.7 ± 3.52	144.3 ± 2.33 **
Diastolic BP	77.33 ± 1.45	93.33 ± 1.66 **
MAP	92.67 ± 1.45	110.3 ± 1.45 **
Proteinuria	-	0,76
Fetal weight	3423 ± 132.8	2350 ± 419.3

** $p < 0.01$

Figure 1. A) Concentrations of derivatives of reactive oxygen metabolites (d-ROMs) and (B) biological antioxidant potential (BAP) in plasma collected from healthy pregnancies and pregnancies complicated by preeclampsia. (Data is presented as mean±SEM. Unpaired t- was applied. *** p<0.001, **** p<0.0001)





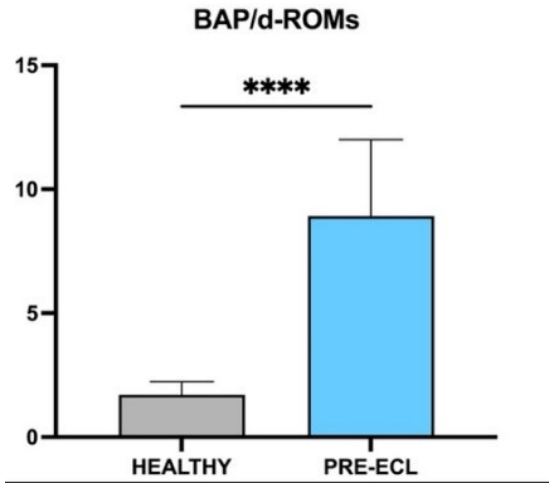
Preeclampsia was associated with decreased plasma reactive oxygen species and increased antioxidant capacity

ROS production and the plasma antioxidant capacity were evaluated in preeclamptic pregnancies (mild, n=8 and severe, n=2 forms) and healthy pregnancies. Interestingly, both healthy and preeclamptic pregnancies presented higher levels of oxidative stress, the values being almost double in the control group. Thus dROMs values were 1012±103.3 CARR U in the control group as compared to 546.8 ± 32.22 CARR U in the PE group. At variance, the BAP test was low in the control group (1666 ± 151.3 umol/L) and high in preeclamptic pregnancies (4651 ± 479.9 umol/L). The antioxidant capacity in plasma was significantly increased in the preeclamptic group. No differences were noticed in both dROMs and BAP values between the mild and severe forms of preeclampsia.

The ratio antioxidants to reactive oxygen metabolites was significantly increased in preeclampsia

The ratio of BAP to d-ROMs has been calculated (BAP/d-ROMs) and showed significantly increased values in pregnancies complicated by preeclampsia.

Figure 2. The ratio BAP test to d-ROMS in healthy vs preeclamptic pregnancies. (**** p<0.0001)



DISCUSSION

The present study was purported to assess the systemic oxidative stress in pregnancies complicated with preeclampsia by measuring the plasma reactive oxygen metabolites and the antioxidant status.

Preeclampsia is a serious complication of pregnancy remaining a major cause of perinatal morbidity and mortality globally, with an unpredictable evolution. Upon severity, mild and severe forms of preeclampsia are recognized; the latter presents with values of the SBP 160–180mmHg SBP and/or DBP>110 mmHg (19).

The correlation oxidative stress – preeclampsia has been extensively studied in past decades and placenta is the main site of ROS generation with subsequent release into the maternal circulation leading to deleterious effects (4).

The main findings can be summarized as follows: i) the levels of plasma reactive oxygen metabolites (d-ROMS) were above normal limit in all evaluated pregnancies, with significantly higher values in healthy pregnancies; ii) the plasma antioxidant capacity (BAP) was in the normal to high range in preeclampsia but healthy pregnancies associated a deficiency and iii) for a better evaluation the BAP test/d-ROMS ratio was calculated, reflecting a significantly higher state of oxidative stress in healthy pregnancies. No differences were noticed between the mild and severe forms of preeclampsia.

Since ROS have a very short lifespan, the evaluation of reactive oxygen metabolites in form of hydroperoxides, which are stable molecules and can be stored in blood samples is a reliable method of oxidative stress evaluation, being in the last years evaluated in several pathologies. A meta-analysis of four population-based cohort studies suggest that increased d-ROMs levels were associated with cardio-vascular disease and cancer mortality (20). Of note, values above the normal upper limit were also reported in heavy drinkers (10).

In a recent study it has been reported that ultramarathon running induces systemic oxidative stress since the ratio BAP/d-ROMS was significantly decreased 24 hours after the race, due to the proinflammatory state and subsequent muscular and renal injury (21).

The literature regarding the antioxidant capacity (measured globally or as specific enzymes) in preeclampsia is inconclusive, with several studies reporting high values while in others a significant reduction was observed (22). Aris et al established an increased H_2O_2 level in plasma and placenta of preeclamptic pregnancies correlated with decreased levels of NO due to the cytotrophoblasts NO synthesis inhibition by H_2O_2 (2).

Watanabe et al reported increased levels of dROM in the severe forms of preeclampsia, an observation that was not recapitulated in the mild forms or healthy pregnancies. The

same study found no change in plasma antioxidant capacity in the three study groups (23). In contrast, Kinoshita et al evaluated only the dROM and found significantly higher values in preeclampsia vs normal pregnancies; however, this study included only mild forms of preeclampsia at term (24).

Oxidative stress in preeclampsia was also evaluated in the early *vs* late onset disease, with higher concentration of dROMs in both groups as compared to the control, while no changes in the antioxidant capacity was found (25).

In women with recurrent PE, Rijvers et al determined the plasma antioxidant capacity before pregnancy and at 20 weeks of gestation reporting higher values; however, in this study a latent cardiovascular or metabolic abnormality appeared to interfere with early placental development (26). Specific antioxidant enzymes suc as reduced glutathione, superoxide dismutase, catalase and vitamin E were increased in preeclampsia as compared to healthy pregnancies (27).

Conflicting results reported in the literature are most probably related to the use of different methods to assess the reactive oxygen species (direct or indirect markers) and antioxidant status (specific antioxidative enzymes or total antioxidant capacity).

Last but not least, we should mention that a major influence has also the severity of PE (mild *vs* severe). The lack of higher systemic oxidative stress in the PE group might be related to the fact that most of the consecutive cases were mild (not severe) forms. Of note in a parallel study (under review), we have also assessed the local oxidative stress (placental tissue) and reported it was increased in severe (but not in mild) preeclamptic pregnancies.

We cannot exclude the fact that the higher antioxidant capacity in pregnancies complicated with preeclampsia, is a compensatory mechanism of the triggered as a consequence of placental oxidative stress (and subsequent release of various ROS in the circulation).

CONCLUSION

Pregnancy is associated with an increased systemic oxidative stress. In this pilot study higher values of d-ROMs were found out in healthy pregnancies as compared to preeclamptic ones whereas the antioxidant capacity was higher in preeclamptic pregnancies. Future studies in larger groups of patients aimed at simultaneously assessing both local and systemic oxidative stress in relation with the severity of the disease are worth to be designed.

AUTHOR CONTRIBUTIONS:

Conceptualization, A.M.B., C.B. and D.M.M.; Methodology, A.M.B., A.I.A.; Validation, A.M.B., A.I.A..; Formal Analysis, A.M.B, A.V.L; Investigation, D.V.C., A.G.M., E.B., Z.L.P.; Resources, M.L.C..; Data Curation, A.M.B, A.V.L.; Writing - Original Draft Preparation, A.M.B, A.V.L..; Writing - Review & Editing, D.M.M.; Visualization, E.B., Z.L.P.; Supervision, C.B., M.L.C., Z.L.P.; Project Administration, O.M.C; Funding Acquisition, O.M.C.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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