Markers of Inflammation in Preeclampsia

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Abstract: Advanced oxidation protein products (AOPP) represent terminal products of proteins exposure to free radicals. The aim of this study was to estimate the serum AOPP levels in preeclamptic patients together with ultrasensitive C-reactive protein and anticardiolipin antibodies (ACA) IgG and IgM. 21 women in the third trimester of pregnancy were included in the study – 10 women with preeclampsia and 11 women with normal outcome of pregnancy. AOPP levels in preeclampsia were higher than those in normal pregnant women in the third trimester, but not statistically significantly. The comparison with AOPP levels in non-pregnant women has shown a significant increase ($P<0.0001$). CRP in preeclampsia was significantly increased in comparison with third trimester levels in normal pregnancy ($P<0.001$) as well as with non-pregnant women ($P<0.0001$). In preeclampsia, the ACA IgG levels were even significantly lower than in normal pregnant women in the same gestation age, but significantly higher than in non-pregnant women ($P<0.001$). No difference was found in ACA IgM in preeclampsia and normal third trimester pregnancy and non-pregnant women. A statistically significant negative correlation was found between AOPP and ACA IgG ($r= -0.708$, $P<0.05$). The results indicate enhanced oxidative and inflammatory reaction of maternal organism to pregnancy, which is more pronounced in preeclampsia than in uncomplicated pregnancy.

Key words: Advanced oxidation protein products - C-reactive protein – Anticardiolipin antibodies – Preeclampsia – Oxidative stress – Pregnancy – Inflammation

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Introduction

Pregnancy is a period when increased oxidative stress may be expected. The disturbed balance between reactive oxygen and endogenous antioxidant defence may damage proteins, lipids and DNA and impair normal cellular functions. Lipid damage by free radicals leads to lipid hydroperoxide and other oxidation products formation. In *in vitro* experiments, the exposure of amino acids, peptides, and proteins to oxidants leads to the alteration in their primary, secondary and tertiary structure. These modifications of proteins can change their function associated with the loss of their enzymatic, hormonal or immune properties [1].

Various markers of oxidative stress had been examined in normal and pathological pregnancies [2–10]. Several studies have shown their elevation both during physiological course of pregnancy and in some diseases in pregnancy [2, 3, 5, 8].

In contrast to lipoperoxidation, which is well understood, the reaction of proteins with various oxidants has been intensively studied in clinical conditions only for a few years. Advanced oxidation protein products [AOPP] were recently described as terminal products of protein exposure to free radicals [11]. The protein electrophoresis analysis showed that AOPP are constituted of two fractions – high-molecular-weight species formed mostly due to albumin in aggregates and low-molecular-weight ones containing albumin in its monomeric form.

Originally, AOPP have been reported as a new marker of oxidative stress and potential mediator of inflammation in uremic patients [11, 12]. Elevated AOPP plasma levels were also found in preterm hypoxic newborns [13, 14], patients with coronary artery disease [15] and in the patients with diabetes mellitus [16]. Recently, we have observed increased AOPP levels in maternal serum in the first and second trimester of normal pregnancy [17, 18].

Increased oxidative stress contributes to the pathogenesis of some diseases in pregnancy. Determination of AOPP in normal and preeclamptic pregnancy can provide further information about the intensity of oxidative stress in these clinical conditions.

It has been observed that a possible link between oxidative stress and inflammation exists in preeclampsia and that these mechanisms may induce endothelial dysfunction [19, 20]. That is why we estimated the maternal serum AOPP levels in preeclamptic patients together with C-reactive protein as a sensitive parameter of systemic inflammation and anticardiolipin antibodies [ACA], which have been detected in a variety of inflammatory and prooxidative conditions [21–23].

Patients

21 women in the third trimester of pregnancy were included in the study – 10 women with preeclampsia (mean age 29±4 years) and 11 women with

normal outcome of pregnancy (mean age 28±3 years). The gestational age was confirmed by ultrasound scan. Preeclampsia was defined as a blood pressure level above 140/90 mm Hg on two or more occasions and proteinuria more than 300 mg protein/day. Three of the preeclamptic pregnancies were twins. The patients delivered between 28th and 38th week of pregnancy. Seven pregnancies were ended by Caesarean section. The birthweight in singleton was from 950 g to 3420 g and in twins from 950 g to 2560 g.

The control group for AOPP contains 26 non-pregnant healthy women (mean age 29±8 years). Ultrasensitive CRP and ACA were determined in a selected group of 14 women.

Venous blood was collected via the cubital vein from each patient during their regular visit or hospitalisation at the Department of Gynaecology and Obstetrics and during routine control examination. All blood samples were centrifuged for 10 minutes at 1450 g (4 °C). The sera were stored at – 20 °C. The study was approved by the local Ethical Committee.

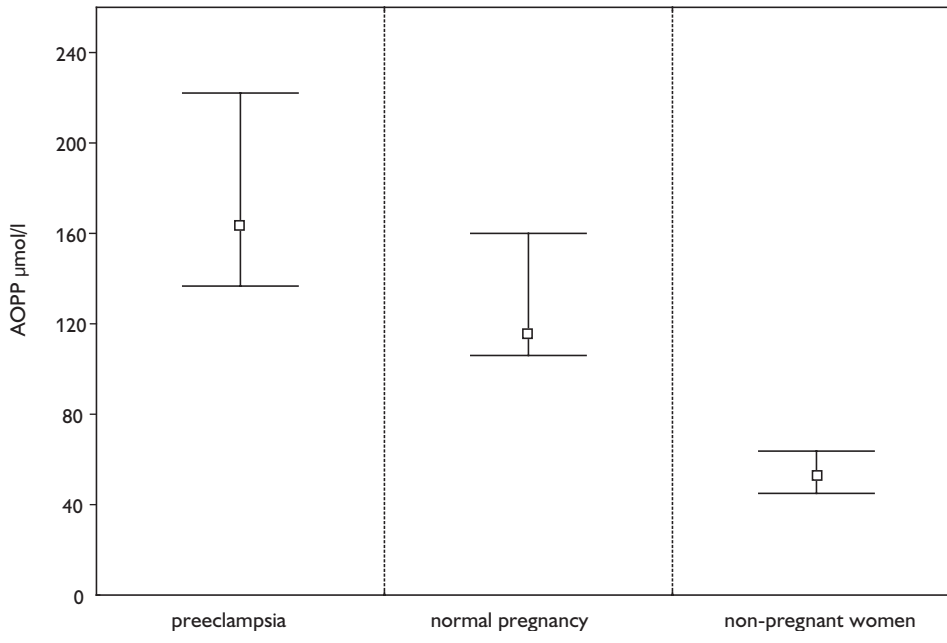

Fig. 1 – Maternal serum AOPP levels in the third trimester in preeclamptic women (n=10), women with normal pregnancy in the same gestational age (n=11) and non-pregnant women (n=26) (non-pregnant women vs preclampsia P<0.0001). Results are shown as medians and interquartile ranges.

Methods

AOPP were determined by a spectrophotometric method according to Witko-Sarsat et al. [11]. 200 μl of plasma diluted 1:5 with PBS, pH 7.4, 200 μl of chloramin T (0–100 μmol/l) for calibration and 200 μl of PBS as blank were applied onto a microtiter plate. 10 μl of 1.16 mol/l potassium iodide and 20 μl of acetic acid were added and absorbance at 340 nm was measured immediately (spectrophotometer Multiscan Ascent, Labsystems, Finland).

Ultrasensitive CRP was measured by TRACE (Time Resolved Amplified Cryptate Emission) technology based on non-radiating energy transfer. Commercial kit KRYPTOR CRP-ultrasensitive (Brahms Diagnostica GmbH, Germany) contains two different monoclonal antibodies – one is conjugated with europium cryptate and another one with fluorescent agents XL 665. The antigens (CRP) present in serum samples are sandwiched between two conjugates. The fluorescent signal measured during the formation of the antigen-antibody complex by analyser KRYPTOR (Brahms Diagnostica GmbH, Berlin, Germany) is proportional to the antigen concentration.

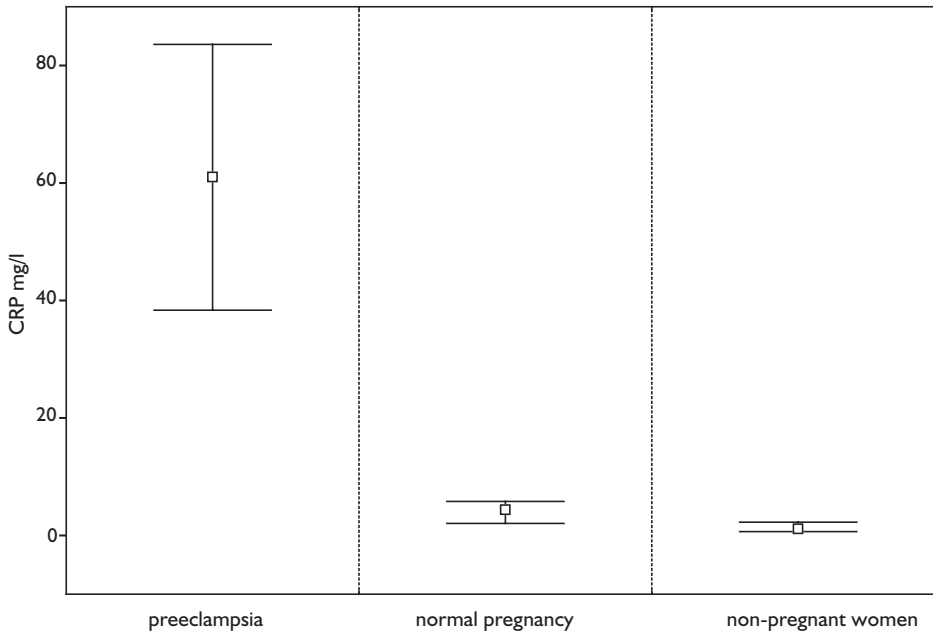

Fig. 2 – Maternal serum CRP levels in the third trimester in preeclamptic women (n=10), in women with normal pregnancy in the same gestational age (n=11) and non-pregnant women (n=14) (normal pregnancy vs preeclampsia P<0.001, non-pregnant women vs preclampsia P<0.0001). Results are shown as medians and interquartile ranges.

Standardised ELISA utilising microplates coated with purified bovine cardiolipin and saturated with human beta$_2$-glycoprotein I (ORGENTEC, Germany) was used for anticardiolipin antibodies determination. The results are expressed in units (U/ml) assigned as GPL for IgG class and MPL for IgM class.

Statistical analysis

The data were analysed by standard statistical methods. The results of analyses were expressed as median and interquartile ranges. A statistical significance between preeclamptic women, normal pregnant women and non-pregnant women was analysed using non-parametric Mann-Whitney U test. Relationship between analysed parameters was assessed by Spearman's and Pearson's correlation coefficients. All results were considered as statistically significant at $P<0.05$.

Results

AOPP levels in preeclampsia were higher than those in normal pregnant women in the third trimester, but not statistically significantly. The comparison with AOPP levels in non-pregnant women has shown a significant increase ($P<0.0001$) (Fig. 1). CRP in preeclampsia was significantly increased in comparison with third trimester levels in normal pregnancy ($P<0.001$) as well as with non-pregnant women ($P<0.0001$) (Fig. 2). We did not observe any outstanding association between the increased AOPP and CRP levels and the course and outcome of pregnancy. In preeclampsia, the ACA IgG levels [3.15 (2.70–3.40) GPL] were even significantly lower than in normal pregnant women in the same gestation age [3.82 (3.3–4.2) GPL, $P<0.05$], but significantly higher than in non-pregnant women [1.5 (1.3–1.6) GPL, $P<0.001$]. In normal pregnancy, ACA IgG was significantly higher than in non-pregnant controls ($p<0.0001$). No difference was found in ACA IgM in preeclampsia [1.25 (0.50–1.60) MPL)], normal third trimester pregnancy [1.3 (1.1–1.7) MPL] and non-pregnant women [1.05 (0.6–2.2) MPL].

The correlation coefficients of AOPP with CRP and ACA IgG and IgM are shown in Tab. 1. Statistically significant negative correlation was found between AOPP and ACA IgG ($r= - 0.708$, $P<0.05$).

Table 1 – Correlation between maternal serum AOPP levels and maternal serum CRP, ACA IgG and IgM levels in third trimester in preeclampsia

	Correlation between		
AOPP	n	r	p
CRP	10	0.018	n.s.
ACA IgG	10	–0.708	0.022
ACA IgM	10	–0.304	n.s.

Our results show that oxidative stress and inflammation are increased in the third trimester of pregnancy and even more pronounced in preeclampsia.

Discussion

Pathogenesis of preeclampsia, which represents a serious complication of pregnancy, is not fully understood, but it is obvious that oxidative stress and inflammation are important mechanisms in the development of this disease. It is undisputed that placenta plays a central role. The superoxide level is significantly increased in the placental tissue of preeclamptic women [10]. Analysis of the oxidatively damaged placental proteins revealed a relative increase of altered proteins in preeclamptic placenta, probably due to reduced proteasomal activity. Decreased metabolization of oxidatively modified proteins is associated with their accumulation in placenta, which may interfere with its metabolism and may contribute to the development of preeclampsia [24]. Apoptotic or necrotic debris shed from the syncytial surface of the placenta acts as the inflammatory stimulus in all pregnancies, so that a maternal systemic inflammatory response is also expressed in normal trimester but less intensively that in preeclampsia. It is probable that preeclampsia is only an excessive inflammatory response of maternal organism to pregnancy [25].

Defects in placental development and function may reflect the changes in circulating levels of various markers of oxidative stress and inflammation. Normal late pregnancy was associated with formation of susceptible oxidisable particles measured by significantly increased lipid hydroperoxid [26]. Plasma levels of the lipid peroxidation products – malondialdehyde and free 8-iso-prostane have been observed to be elevated in preeclampsia [3, 8]. Another sensitive marker of reactive oxygen species-mediated damage – carbonyl derivatives of proteins are also significantly elevated in preeclamptic women in comparison with healthy pregnant ones [27]. Several studies have described increased levels of some cytokines and complement, neutrophil and macrophage activation in women with preeclampsia [28–30].

In our study, we determined AOPP as a marker of oxidative stress and CRP as a non-specific but a precise and sensitive parameter of systemic inflammation in patients with preeclampsia. AOPP are able to trigger the oxidative burst and the synthesis of inflammatory cytokines in neutrophils and monocytes [31]. Witko-Sarsat et al. [32] hypothese that AOPP formed by the reaction between chlorinated oxidants and plasma proteins, may constitute new uremic toxins with pro-inflammatory effects. Similarly as in uremia, AOPP could be considered to be mediators of the proinflammatory effects of oxidative stress in preeclampsia. AOPP levels in preeclampsia were significantly higher than in non-pregnant women, but the increase was not statistically significant in comparison with pregnant women in the same gestational age.

Proinflammatory state in the normal pregnancy and particulary in preeclampsia is expressed by significantly high levels of CRP observed recently by Teran et al. [30]. On the contrary to AOPP, the difference in CRP levels between normal and preeclamptic pregnancy was statistically significant.

Plasma levels of CRP as an acute-phase reactant are increased in acute infection, inflammatory diseases as well as in malignant neoplasia. CRP can bind damaged cell membranes, different phospholipids, small nuclear ribonucleoprotein particles and apoptotic cells. Because placentas of preeclamptic pregnancies are characterised by a high rate of apoptosis, it is possible that increased biosynthesis of CRP is stimulated by an elevated formation of apoptotic cells [33]. The maternal inflammatory response assessed by CRP measurement precedes the manifestation of preeclampsia. Tjoa et al. [34] even reported that CRP levels have been already elevated in the first trimester in women who will subsequent develop preeclampsia.

Antiphospholipid antibodies [APL] may serve as an indirect marker of lipid peroxidation, because they recognise epitopes of oxidized phospholipids and cross-react with oxidatively modified low-density lipoproteins [35]. Pregnant women with high APL levels usually have an adverse pregnancy outcome including recurrent spontaneous abortions and foetal death and an early onset of preeclampsia [36]. The reports dealing with preeclampsia in connection with antiphospholipid antibodies are controversial. Some studies found elevated APL in pregnancies complicated with preeclampsia, others did not observe an association between APL and preeclampsia [37, 38]. In our patients, ACA IgG were decreased in comparison with normal pregnancy outcome, but the levels were significantly higher than in non-pregnant subjects. A proteinuria in preeclamptic patients, which may be accompanied by the loss of IgG, might participate in the decrease of ACA IgG.

The increased AOPP and CRP levels in preeclampsia support the assumption that preeclampsia may be an excessive inflammatory response of maternal organism to pregnancy [20]. The difference in inflammatory reactions between the normal and preeclamptic pregnancy may be less pronounced than those between non-pregnant subjects and uncomplicated pregnancy. Inflammation and oxidative stress may participate in the pathogenesis of preeclampsia, but it is possible that additional factors in comparison with uncomplicated pregnancy regulate its development. Nevertheless, the significance of increased AOPP levels in preeclamptic patients will require further investigation. It will especially concern in the use of AOPP determination at the evaluation of severity disease.

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