ORIGINAL ARTICLE

UDC: 577.1::618.3-06
https://doi.org/10.2298/VSP150517251B

Preeclampsia and level of oxidative stress in the first trimester of pregnancy

Preeklampsija i nivo oksidativnog stresa u prvom trimestru trudnoće

Mirjana Bogavac*[†], Ana Jakovljević[†‡], Zoran Stajić[§], Aleksandra Nikolić[†‖], Mirjana Milošević-Tošić[†¶], Jadranka Dejanović[†], Zagorka Lozanov-Crvenković[†¶]**

Clinical Centre of Vojvodina, *Department of Obstetrics and Gynecology, [‡]Centre for Laboratory Medicine, [‖]Emergency Centre, Novi Sad, Serbia; University of Novi Sad, [†]Faculty of Medicine, [¶]Faculty of Science, Novi Sad, Serbia; Institute for Health Protection of the Ministry of Interior, Department of Internal Medicine, [§]Division of Cardiology, Belgrade, Serbia; Institute for Cardiovascular Diseases, **Clinic of Cardiology, Sremska Kamenica, Serbia

Abstract

Background/Aim. Preeclampsia (PE) is a multisystemic syndrome that complicates 5–8% of all pregnancies. The aim of this study was to evaluate the biochemical parameters of oxidative stress in the first trimester of pregnancy in patients with preeclampsia, with the purpose of comparing the level of oxidative stress with normal pregnancy. **Methods.** The study was conducted as a prospective study. It included totally 107 pregnant women divided into two groups. In the study group (n = 33) there were women who developed preeclampsia in the current pregnancy. The control group (n = 74) included healthy pregnant women. Blood samples were taken between 11th and 14th weeks of gestation, and the values of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and total antioxidant status (TAS) were determined in serum by enzymatic colorimetric methods. **Results.** The values of SOD and GHS-Px were statistically higher in the study group, while the values of TAS were statistically higher in the control group. The level of TAS inversely correlated with GSH-Px and SOD, but there is no statistically significant correlation between GSH-Px and SOD in the study group. **Conclusion.** The results of this study suggest a higher level of oxidative stress in the first trimester of pregnancy with preeclampsia, which may indicate that the initiation and development of pathophysiological processes underlying preeclampsia start much earlier than the clinical syndrome exhibit.

Keywords:
pregnancy complications; pre-eclampsia; oxidative stress; superoxide dismutase; glutathione peroxidase; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Preeklampsija (PE) je multisistemski sindrom koji komplikuje 5–8% svih trudnoća. Cilj ove studije bio je procena biohemijskih pokazatelja oksidativnog stresa u prvom trimestru trudnoće kod bolesnice koje su u toku aktuelne trudnoće razvile preeklampsiju u poređenju sa stepenom oksidativnog stresa kod trudnica sa fiziološkom trudnoćom. **Metode.** Istraživanje je sprovedeno kao prospektivna studija i uključila je ukupno 107 trudnica podeljenih u ispitivanu i kontrolnu grupu. Ispitivanu grupu (n = 33) činile su trudnice koje su u toku aktuelne trudnoće razvile preeklampsiju, dok su kontrolnu grupu (n = 74) činile trudnice sa fiziološkom trudnoćom. Uzorci krvi uzimani su svim trudnicama između 11. i 14. nedelje gestacije i vrednosti superoksid dismutaze (SOD), glutation peroksidaze (GSH-Px) i totalnog antioksidativnog statusa (TAS) određivani su enzimskim kolorimetrijskim metodama. **Rezultati.** Vrednosti SOD i GSH-Px bile su statistički značajno više u ispitivanoj grupi dok su vrednosti TAS bili statistički značajno više u kontrolnoj grupi. Utvrđena je statistički značajna inverzna korelacija TAS sa SOD i GSH-Px, dok između SOD i GSH-Px nije bilo statistički značajne povezanosti. **Zaključak.** Rezultati ove studije ukazuju na viši stepen oksidativnog stresa u prvom trimestru trudnoće kod trudnica sa preeklampsijom, što sugeriše da patofiziološki mehanizmi koji čine osnovu preeklampsije započinju znatno pre, nego se klinički sindrom ispolji.

Ključne reči:
trudnoća, komplikacije; preeklampsija; stres, oksidativni; peroksid dismutaza; glutation peroksidaza; osetljivost i specifičnost.

Correspondence to: Mirjana Bogavac, Clinical Centre of Vojvodina, Department of Obstetrics and Gynecology, 21 000 Novi Sad, Serbia. E-mail: mbogavac@yahoo.com

Introduction

The integrity and functionality of all cells and tissues depend on the precisely regulated balance between the production of reactive oxygen species (ROS) and components activity of the antioxidant protection [1]. Many physiological processes are sources of ROS but in limited and controlled amounts. Pregnancy is a physiological state, which modulates the processes of metabolism, hormonal status, coagulation and immune mechanisms, all of which affect the redox balance [2, 3]. The levels of circulating markers of lipid peroxidation in maternal circulation are considerably increased compared to the situation before pregnancy, which indicates a certain degree of physiological oxidative stress in normal pregnancy [4].

The primary antioxidant system protection consists of many enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which are the first line of defense of the organism and catalyze the removal of toxic forms of oxygen in cells [5, 6]. SOD catalyzes the dismutation reaction of superoxide anion radicals (O_2^-), with the production of hydrogen peroxide (H_2O_2) and molecular oxygen, while GSH-Px reduces H_2O_2 and hydroperoxides of fatty acids with the involvement of glutathione as an electron donor [7, 8]. However, no single antioxidant can reflect the overall defense activity of the organism, as the total antioxidant status (TAS) can, which is a measure of antioxidant capacity, and joint action of all antioxidants, such as enzymatic and non-enzymatic ones in blood and biological fluids [9, 10].

Changes in the concentrations of some of the oxidative stress markers are preceded by the development of the clinical symptoms, which indicates a phenomenon of chronic oxidative stress during pregnancy [11]. According to the literature, oxidative stress during pregnancy significantly affects placental and systemic pathophysiological processes that lead to disorders of placental vascularization, causing endothelial and immune dysfunction [12, 13]. It is believed that oxidative stress could be a central process in the pathogenesis of placental disorders. For this reason, the oxidative imbalance is considered to be a significant factor in the development of pathological conditions in pregnancy, such as miscarriage, preeclampsia (PE), premature birth, hydatid mole, etc [14–16].

Preeclampsia is characterized by new-onset hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg at least on two occasions) and proteinuria (urinary excretion of ≥ 300 mg of protein in 24h) after 20 weeks of gestation. Insufficient remodeling of the spiral arteries and reduced uteroplacental perfusion might be one of the trigger factors responsible for maternal endothelial cell dysfunction, inflammation and oxidative stress [17, 18].

Intention to understand etiopathogenesis of preeclampsia at the molecular level is the topic of numerous studies but most data provides only a partial explanation of the problem. For this reason, it is very important to find biomarkers which are sensitive and specific enough to detect high-risk pregnancy early, long before the onset of clinical symptoms and signs of the disease [19, 20].

The aim of this study was to evaluate the markers of oxidative stress in the first trimester of pregnancy in patients with preeclampsia, comparing the level of oxidative stress with normal pregnancy.

Methods

The research was conducted at the Department of Obstetrics and Gynecology, Clinical Center of Vojvodina in Novi Sad as a prospective study between 2010 and 2014. The study included a total of 107 pregnant women who were concordant with participation in the study, which was confirmed by their written consent in accordance with the criteria of the Helsinki Declaration.The protocol was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad.

Criteria for inclusion in the study were pregnancy age between 11–14 weeks and singleton pregnancy.

The criteria for exclusion were: fetal chromosomal abnormalities, infectious diseases in current pregnancy, maternal diseases (anemia, chronic and gestational hypertension, diabetes mellitus) and local factors: anatomical malformations of the uterus and vagina, cervical insufficiency, and malignancies. Chromosomal and genetic fetal disorders were excluded, by controlling all included pregnancies in the study, until the delivery. Only pregnancies with genetically healthy newborn babies were included. All pregnant women with obesity [body mass index (BMI)≥ 30] and hypertriglyceridemia were excluded from the study [21–23]. None of the pregnant women were smokers and none of them received supplementation with antioxidant vitamins.

The study involved two groups of pregnant women: the study group (n = 33) women who developed preeclampsia in the current pregnancy, and the control group (n = 74), which consisted of healthy pregnant women. After taking anamnesis about place of living (rural/urban) in addition to the impact of environmental toxins on oxidative stress and clinical examination, the blood samples were taken – whole blood and serum in which certain basic hematological and biochemical parameters were determined as well as markers of oxidative stress: SOD, TAS, and GSH-Px. All parameters were determined in the first trimester before clinical signs of preeclampsia.

Body height (BH, cm) was measured with Martin anthropometer. Body mass (BM, kg) was measured on the medical decimal scale. A BMI was calculated based on the formula: BMI (kg/m^2) = BM (kg)/BH (m^2). Blood pressure (mmHg) was measured by the Riva-Rocci method.

Blood counts (complete blood cells – CBC) and C-reactive protein (CRP) were determined on an automated hematology analyzer ABX Micros CRP 200 (HoribaABX Diagnostics). Fibrinogen concentration was determined by the BFT II Fibrintimer Siemens Health Care Diagnostics (modified method by Klaus).

GSH-Px activity was determined by a modified method of Paglia and Valentine with cumene hydroperoxide using RanSel (Randox, Ireland) tests [24]. The activity of SOD was measured in EDTA hemolysates with Xanthine oxidase (XOD) method using RanSOD tests (Randox, Ireland) [25].

The total antioxidant status was determined in samples of sera by monitoring the inhibition of ABTS + colors using sets TAS BIOREX (BIOREX Diagnostic Limited, Antrim, United Kingdom) [26].

Data were analyzed using the statistical package Statistica 12 (StatSoft Inc., Tulsa, OK, USA), University license for Novi Sad University; *p* values less than 0.05 were considered statistically significant.

Results

Table 1 shows demographic, anthropometrical, clinical and biochemical characteristics of pregnant women. There were no statistically significant differences in age of patients, dwelling place, BMI, blood pressure, hematological parameters and markers of infections between two groups of pregnant women.

In the Figures 1–3 the values of parameters of oxidative stress in pregnant women who developed preeclampsia and in healthy pregnant women in the first trimester are displayed.

The values of SOD in EDTA hemolysate of women in the control and the study group are displayed in Figure 1. The mean value of SOD activity (IU/L) in the serum of pregnant women in the study group was 45.6 (13.6–77.5) whereas the mean value in the control group was 29.733 (9–70.5). Patients with preeclampsia had significantly higher mean values of SOD compared to healthy controls ($p < 0.0001$).

The values of GSH-Px in the serum of women in the study group and the control group are displayed in Figure 2. The mean value of GSH-Px activity (IU/L) in the serum of pregnant women of the study group was 634.712 (35–995.30) while the average value of the control group was 519.46 (253.6–827.1). The results showed significantly higher mean values of GSH-Px in pregnant women with preeclampsia compared to healthy control group ($p = 0.0058$).

The values of TAS in the serum of women in the study and the control group are displayed in Figure 3. The mean value of TAS (mEq/L) in the serum of pregnant women in the study group was 0.97 (0.2–5.3), whereas the mean value in the control group was 1.9 (0.35–5.03).Values of TAS were significantly lower in the study group compared to the values in the control group ($p = 0.0075$).

Correlation analysis of oxidative stress parameters in the study group showed that level of TAS inversely corre-

Table 1

Demographic, anthropometric, clinical and biochemical characteristics of pregnant women

Characteristics of pregnant women	Study group (n = 33)	Control group (n = 74)	*p*-values
Age of patients (years), $\bar{x} \pm SD$	30.61 ± 6.52	29.26 ± 5.05	ns
Dwelling place, n (%)			
village	15 (45.46)	41 (55.41)	ns
city	18 (54.54)	33 (44.59)	ns
BMI (kg/m^2), $\bar{x} \pm SD$	23.96 ± 3.98	23.52 ± 3.99	ns
Systolic arterial blood pressure (mmHg), $\bar{x} \pm SD$	116 ± 9.5	114 ± 7.67	ns
Diastolic arterial blood pressure (mmHg), $\bar{x} \pm SD$	75 ± 6.5	77 ± 3.45	ns
CRP (mg/L), $\bar{x} \pm SD$	3.08 ± 0.35	3.57 ± 0.39	ns
Fibrinogen (g/L), $\bar{x} \pm SD$	3.91 ± 0.56	3.45 ± 0.4	ns
Total number of leukocytes ($\times 10^9$/L), $\bar{x} \pm SD$	9.31 ± 2.23	9.84 ± 1.22	ns
Erythrocytes ($\times 10^{12}$/L), $\bar{x} \pm SD$	4.14 ± 0.38	4.17 ± 0.34	ns
Platelets ($\times 10^9$/L), $\bar{x} \pm SD$	235.54 ± 54.93	214.33 ± 43.21	ns
Hemoglobin (g/L), $\bar{x} \pm SD$	119.0 ± 8.05	121 ± 9.79	ns

$\bar{x}$ – mean; SD – standard deviation; *ns – no significance.
BMI – body mass index; CRP – C-reactive protein.

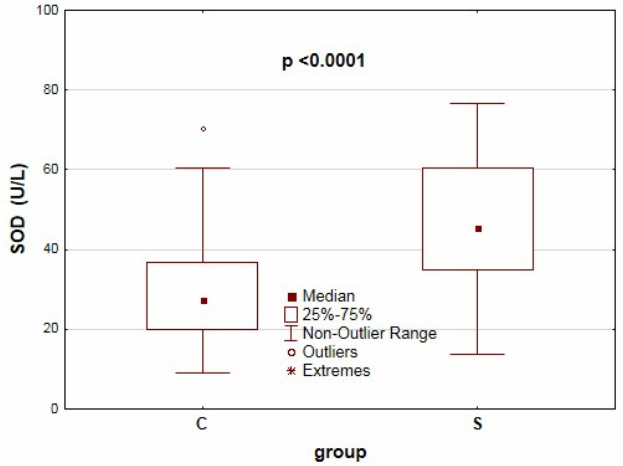

Fig. 1 – Values of superoxide dismutase (SOD) activity in the serum of pregnant women in the control group (C) and the study group (S).

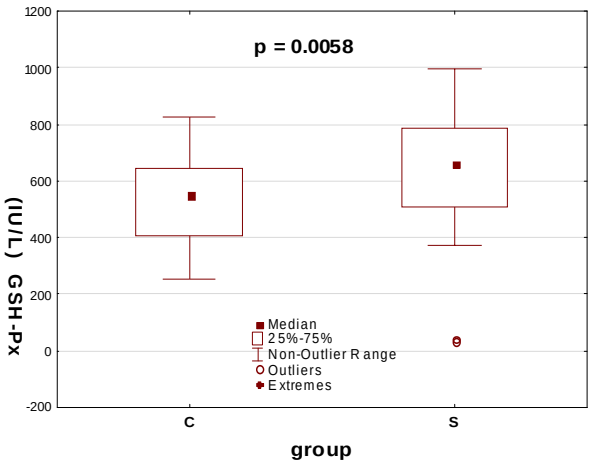

Fig. 2 – Values of glutathione peroxidase (GSH-Px) activity in the serum of pregnant women in the control group (C) and the study group (S).

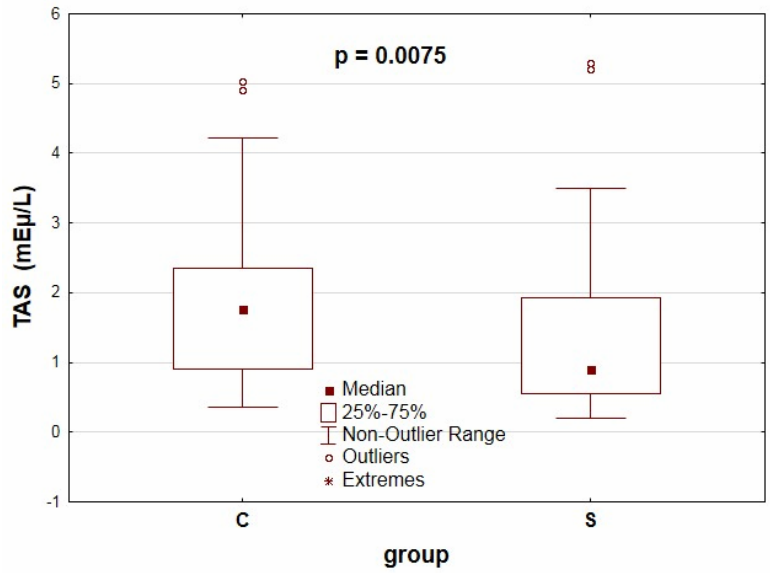

Fig. 3 – Values of total antioxidant status (TAS) in the serum of pregnant women in the control group (C) and the study group (S).

lated with serum activity of GSH-Px (r = -0.43; $p = 0.025$) and SOD (r = -0.37; $p = 0.03$). There is no statistically significant correlation between GSH-Px and SOD serum activity ($p > 0.05$) in the study group.

Discussion

ROS production is increased in normal pregnancy and it is necessary for proper development of the placenta. It is assumed that growth and evolvement of the placenta are associated with a trophoblastic necrosis and apoptosis, which leads to the physiologically enhanced production of ROS [24–27]. Many studies indicate that poor placental implantation may represent the initial event in the development of preeclampsia [28–30]. Placental and systemic oxidative stress with an imbalance in the oxidant/antioxidant activity seems to play a central role in the pathogenesis of preeclampsia [28].

This study evaluates oxidative status in the first trimester of pregnancy by determination most important enzymatic antioxidants, SOD, GSH-Px, and TAS in pregnant women with preeclampsia and healthy pregnant woman. Parameters of oxidative stress in our study were measured in early pregnancy before the clinical signs of preeclampsia developed.

In our study, the activities of SOD and GSH-Px were significantly higher in the PE group (the study group) than in the healthy pregnancy group (the control group). These results are consistent with other studies [7, 13, 31–36] showing the significant increase of enzymes activities in preeclamptic patients compared to healthy pregnant women. Increased values of activity two important antioxidative enzymes – SOD, that catalysed dismutation of superoxide radical, and GSH-Px, that removed H_2O_2 from tissues, indicate some level of preserved antioxidative mechanisms in the PE group in our study. On the other hand, induction of antioxidant defence mechanisms in the first trimester may indicate a higher level of oxidative stress in the preeclamptic group of pregnant women. Significantly lower values of TAS in the study group may propose a greater consumption of antioxidants in early pregnancy in the group that will later develop preeclampsia. In addition to SOD and GSH-Px, TAS involves different parameters of antioxidant status, such as catalase activity, cellular antioxidants (*acidum uricum*, bilirubin) and non-enzymatic antioxidants (vitamins C, vitamin E, coenzyme Q). As a complex antioxidative parameter, TAS can provide better information on the current state of antioxidative protection than enzymes alone and point to a reduced ability of complex antioxidant defence mechanisms in early pregnancy which will later develop into preeclampsia [7, 8, 13, 32]. Correlation analysis demonstrates the mild inverse association between elevated enzymes and lower TAS (r = -0.37 for SOD and r = -0.43 for GSH-Px) in the group of women with preeclampsia. Results of correlation analysis could indicate that in the first trimester, there is a decrease of antioxidant capacity (perhaps because of inadequate production of antioxidants) and increase in prooxidants that could interfere with normal trophoblastic development, causing early placental development disorders, impairment of angiogenesis and vasculogenesis. Placental injury in PE, with ischemia and reperfusion, is a trigger factor for releasing many cytokines, inflammatory proteins, and ROS into the circulation, initiating pathophysiological processes that precede the development of preeclampsia. Higher oxidative stress in these pregnancies could be also explained by over-consumption of antioxidants in early pregnancy, demonstrating that oxidative imbalance not only could be the cause but also complication of previous placental impairment [33, 34].

Llurba at al. [35] also showed the increase in antioxidant concentrations (SOD; GSH-Px) but their cumulative data suggested no clear systemic generalised increase in oxidative stress in PE. The study would rather reflect a low oxidative stress level in blood of preeclamptic women

which does not represent a pathogenetically relevant process contributing to preeclampsia. Results of other investigators show the strong association between oxidative stress and preeclampsia, by significantly reducing the incidence of preeclampsia with multivitamin supplementation in early pregnancy [36, 37].

However, a limited number of samples in our study, the heterogeneity of disease-induced preeclampsia and the fact that we have only studied some antioxidative parameters in early pregnancy and not a wide spectrum of oxidation products were likely the reasons why the complete pathophysiologic role of oxidative stress cannot be elucidated in our study. But the results of our study indicate that SOD, GSH-Px, and TAS could be included in the diagnostic algorithm for early detection of preeclampsia.

Conclusion

The results of this study suggest a higher level of oxidative stress in the first trimester of pregnancy with preeclampsia, which may indicate that the initiation and development of pathophysiological processes underlying preeclampsia start much earlier than the clinical syndrome exhibit.

Acknowledgment

The work of Z. Lozanov-Crvenković was supported by the grant No.174019 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

R E F E R E N C E S

1. *Kambayashi Y, Tero-Kubota S, Yamamoto Y, Kato M, Nakano M, Yagi K*, et al. Formation of superoxide anion during ferrous ion-induced decomposition of linoleic acid hydroperoxide under aerobic conditions. J Biochem 2003; 134(6): 903−9.
2. *Gupta S, Malhotra N, Sharma D, Chandra A, Agarwal A.* Oxidative stress and role in female infertility and assisted reproduction: clinical implications. Int J Fertil Steril 2009; 2: 147−64.
3. *Kashinakunti SV, Sunitha H, Gurupadappa K, Shankarprasad DS, Suryaprakash G, Ingin JB.* Lipid Peroxidation and Antioxidant Status in Preeclampsia. Al Ameen J Med Sci 2010; 3(1): 38−41.
4. *Djukic MM.* Oxidative stress-free radicals, prooxidants and antioxidants. Belgrade: Mono i Manjana; 2008. (Serbian)
5. *Halliwell B, Gutteridge JM.* Free radicals in biology and medicine. 3rd ed. New York: Oxford University Press Inc; 1999.
6. *Bogavac M, Lakic N, Simin N, Nikolic A, Sudji J, Bozin B.* Bacterial vaginosis and biomarkers of oxidative stress in amniotic fluid. J Matern Fetal Neonatal Med 2012; 25(7): 1050−4.
7. *Ruder EH, Hartman TJ, Goldman MB.* Impact of oxidative stress on female fertility. Curr Opin Obstet Gynecol 2009; 21(3): 219−22.
8. *Al-Gubory KH, Fowler PA, Garrel C.* The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell Biol 2010; 42(10): 1634−50.
9. *Koruk M, Taysi S, Savas CM, Yilmaz O, Akcay F, Karakok M.* Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis. Ann Clin Lab Sci 2004; 34(1): 57−62.
10. *Suresh DR, Annam V, Pratibha K, Prasad BV.* Total antioxidant capacity: A novel early biochemical marker of oxidative stress in HIV infected individuals. J Biomed Sci 2009; 16(1): 61.
11. *Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ.* Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol 2000; 157(6): 2111−22.
12. *Perrone S, Longini M, Bellieni CV, Centini G, Kenanidis A, De Marco L*, et al. Early oxidative stress in amniotic fluid of pregnancies with Down syndrome. Clin Biochem 2007; 40(3−4): 177−80.
13. *Chamy VM, Lepe J, Catalán A, Retamal D, Escobar JA, Madrid EM.* Oxidative stress is closely related to clinical severity of pre-eclampsia. Biol Res 2006; 39(2): 229−36.
14. *Palmieri B, Sblendorio V.* Oxidative stress tests: Overview on reliability and use. Part II. Eur Rev Med Pharmacol Sci 2007; 11(6): 383−99.
15. *Gupta S, Agarwal A, Banerjee J, Alvarez JG.* The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: A systematic review. Obstet Gynecol Surv 2007; 62(5): 335−47; quiz 353−4.
16. *Jakovljevic B, Novakov-Mikic A, Brkic S, Bogavac M, Tomic S, Miler V.* Lipid peroxidation in the first trimester of pregnancy J Matern Fetal Neonatal Med 2012; 25(8): 1316−8.
17. *Hutcheon JA, Lisonkova S, Joseph KS.* Epidemiology of preeclampsia and the other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol 2011; 25(4): 391−403.
18. *Ferrazzani S, Luciano R, Garofalo S, Andrea VD, Carolis SD, Carolis MP*, et al. Neonatal outcome in hypertensive disorders of pregnancy. Early Hum Dev 2011; 87(6) 445−9.
19. *Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW.* Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: The Generation R Study. Am J Epidemiol 2013; 177(8): 743−54.
20. *Wallis AB, Saftlas AF, Hsia J, Atrash HK.* Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. Am J Hypertens 2008; 21(5): 521−6.
21. *Lei Q, Lv LJ, Zhang BY, Wen JY, Liu GC, Lin XH*, et al. Antepartum and post-partum markers of metabolic syndrome in pre-eclampsia. J Hum Hypertens 2011; 25(1): 11−7.
22. Ministry of Health of the Republic Serbia. Guideline for Diagnostics and Treatment of lipid Disorders. Available from: http://www.azus.gov.rs/wp-content/uploads/2011/04/ (Serbian)
23. Ministry of Health of the Republic of Serbia. National Guideline of good Clinical Practice – Diabetes Mellius. Available from: http//www.minzdravlja.info/downloads/2008/Sa%20Zdravlja/dokumenta/Vodici/diabetes.pdf (Serbian)
24. *Paglia DE, Valentine WN.* Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70(1): 158−69.
25. *Arthur JR, Boyne R.* Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci 1985; 36(16): 1569−75.
26. *Erel O.* A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37(4): 277−85.
27. *Yang X, Guo L, Li H, Chen X, Tong X.* Analysis of the original causes of placental oxidative stress in normal pregnancy and pre-eclampsia: a hypothesis. J Matern Fetal Neonatal Med 2012; 25(7): 884−8.

28. *George EM, Granger JP*. Endothelin: key mediator of hypertension in preeclampsia. Am J Hypertens 2011; 24(9): 964−9.
29. *Rosta K, Molvarec A, Enzsöly A, Nagy B, Rónai Z, Fekete A,* et al. Association of extracellular superoxide dismutase (SOD3) Ala40Thr gene polymorphism with pre-eclampsia complicated by severe fetal growth restriction. Eur J Obstet Gynecol Reprod Biol 2009; 142(2): 134−8.
30. *Sharma JB, Sharma A, Bahadur A, Vimala N, Satyam A, Mittal S*. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. Int J Gynaecol Obstet 2006; 94(1): 23−7.
31. *Wruck CJ, Huppertz B, Bose P, Brandenburg L, Pufe T, Kadyrov M*. Role of a fetal defence mechanism against oxidative stress in the aetiology of preeclampsia. Histopathology 2009; 55(1): 102−6.
32. *Genc H, Uzun H, Benian A, Simsek G, Gelisgen R, Madazli R,* et al. Evaluation of oxidative stress markers in first trimester for assessment of preeclampsia risk. Arch Gynecol Obstet 2011; 284(6): 1367−73.
33. *Hansson SR, Nääv Å, Erlandsson L*. Oxidative stress in preeclampsia and the role of free fetal hemoglobin. Front Physiol 2015; 5: 516.
34. *Lissette C, Prada CE, Medina C, Lopez M*. Endothelial dysfunction and preeclampsia: Role of oxidative stress. Front Physiol 2014; 5: 372.
35. *Llurba E, Gratacós E, Martín-Gallán P, Cabero L, Dominguez C*. A comprehensive study of oxidative stress and antioxidant status in preeclampsia and normal pregnancy. Free Radic Biol Med 2004; 37(4): 557−70.
36. *Cunninghm FG, Lenevo KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY*. Pregnancy hypertension (Chapter 34). In: *Cunninghm FG, Lenevo KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY*, editors. Williams Obstretics. 23rd ed. New York: McGraw-Hill; 2010. p. 706−56.
37. *Vanderlelie J, Scott R, Shibl R, Lewkowicz J, Perkins A, Scuffham PA*. First trimester multivitamin/mineral use is associated with reduced risk of pre-eclampsia among overweight and obese women. Matern Child Nutr 2014; doi: 10.1111/mcn.12133. (In Press).

Received on May 17, 2015.
Revised on December 20, 2015.
Accepted on December 24, 2015.
Online First September, 2016.