RESEARCH ARTICLE

Oxidative status of maternal blood in pregnancies burdened by inherited thrombophilias

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Abstract

Oxidative status of maternal blood represents an important parameter of pregnancy that is involved in both, regulation of physiological processes and (if significantly altered) development of different pregnancy complications. Inherited thrombophilias represent genetic disorders that increase the risk of thromboembolism in pregnancy. Little is known about the impact of thrombophilia on the oxidative status of maternal blood. In this study, we analyzed oxidative status of blood of 56 women with pregnancies burdened by inherited thrombophilias. The status was established at three different trimesters using biochemical assays and electrochemical measurements, and it was compared to 10 age- and trimester-matching controls. Activities of superoxide dismutase, catalase, and glutathione reductase in the 1st and the 2nd trimester of thrombophilic pregnancy were lower than controls. Also, there was less oxidation in the plasma, according to higher concentration of reduced thiols and lower oxidation-reduction potential. Therefore, it appears that thrombophilic mothers do not experience oxidative stress in the circulation in the first two trimesters. However, the rise in GPx, GR and SOD activities in the 3rd trimester of thrombophilic pregnancy implies that the risk of oxidative stress is increased during the late pregnancy. These results are important for developing antioxidative treatment that could tackle thrombophilia-related pregnancy complications.

Introduction

Oxidative status of maternal blood is an important parameter of pregnancy [1]. It has a regulatory role in the processes of placental angiogenesis and development, gestation maintenance, and the recognition of fetus-cells and immune maternal responses [2–4]. A positive correlation has been established between oxidative status of the mother and the neonate [5]. It is important to note that several studies have found that normal pregnancy is characterized by mild

pro-oxidative changes in the status of maternal blood when compared to non-pregnant or postpartum women [6–8]. However, more pronounced pro-oxidative changes (*i.e.* oxidative stress) have been related to the development of different pregnancy complications, such as pre-eclampsia, fetal growth restriction, miscarriage, and others [1, 9, 10]. Further, the development of oxidative stress in the blood is known to induce thrombogenesis via mechanisms that have been extensively studied [11–13], and which appear to take place in pregnancy complications that are related to oxidative stress [14, 15]. However, whether pro-thrombic conditions that are induced by some other factors, such as inherited thrombophilias, may lead to oxidative stress, is not clear.

Inherited thrombophilias represent genetic disorders that increase the risk of different complications, fetal loss, and thromboembolism in pregnancy [16–18]. It has been suggested that more than half of vascular complications of pregnancy can be attributed to genetic thrombophilias [19]. Pro-thrombic state results in haemostatic response and microthrombi formation [17]. This may further lead to increased production of reactive oxygen species in the circulation in relation to ischemia, mechanical stress/endothelial injury, and/or inflammation [20, 21]. Pertinent to this, we have found previously that oxidative status of thrombophilic mothers shows significant pro-oxidative changes postpartum, immediately following the delivery [22, 23]. Besides this, little is known about the impact of thrombophilia on the oxidative status.

The aim of the present study was to determine oxidative status of maternal blood during pregnancy burdened by inherited thrombophilias, and to compare it to women with normal pregnancies. It is important to note that pregnancy represents a dynamic period with maternal circulation undergoing significant physiological changes to meet the demands of the fetus and the mother. For example, cardiac output rises by increasing heart rate and stroke volume, reaching ~50% above the pre-pregnancy baseline near the end of the pregnancy [24, 25]. Also, it appears that oxidative status of maternal blood shows a timeline of changes during normal pregnancy [26, 27]. Therefore, it was important to measure parameters of oxidative status at different timepoints–trimesters. Knowing oxidative status at different trimesters may be particularly important for improving the performance of antioxidant supplements in pregnancy by matching the time of application with the periods of high risk of oxidative stress development. The best approach in assessing oxidative status of circulation is to analyze: (i) antioxidative enzymes in erythrocytes: superoxide dismutase (SOD; converts intracellular superoxide radical anion to hydrogen peroxide (H_2O_2)), and catalase (CAT), and glutathione peroxidase (GPx) and glutathione reductase (GR) couple, which remove H_2O_2 [28–31]; and (ii) redox parameters of plasma: the level of reduced thiols (R-SH; highly susceptible to oxidation) [32], and static oxidation-reduction potential (ORP; an integrated comprehensive metabolomic analyte that measures the balance between stable oxidative and reductive species in biological fluids) [33, 34].

Materials and methods

Patients

Study cohorts included 56 pregnant women with inherited thrombophilias, and three groups of 10 age- and parity-matching women with normal pregnancy–controls (population details are available in Table 1).

Inherited thrombophilias were detected by evaluating the presence of specific mutations/deficiencies: protein S deficiency, prothrombin *i.e.* coagulation factor II (*F2*) gene mutation, Factor V Leiden mutation, plasminogen activator inhibitor-1 (*PAI*-1) gene polymorphism, and methylenetetrahydrofolate reductase (*MTHFR*) gene mutation [35, 36]. All patients were diagnosed before the index pregnancy. Other complications involved: chronic hypertension—

Table 1. Population characteristics.

	Controls			Thrombophilia	Types of thrombophilia	n	Zygosity[#]
	1st trimester	2nd trimester	3rd trimester				
n	30			56	Protein S deficiency	4	-
	10	10	10				
Age (years)*	35.4 ± 1.0			33.0 ± 0.7	*F2* gene mutation	6	1/5
	35.4 ± 1.5	33.7 ± 1.7	37.0 ± 1.9				
Parity*	0.96 ± 0.13			0.58 ± 0.10	Factor V Leiden mutation	13	1/12
	0.90 ± 0.23	1.20 ± 0.20	0.80 ± 0.20				
Chronic hypertens. (n)	0			6	*PAI-1* gene polymorphism	31	20/11
Gestational hypertens. (n)	0			14	*MTHFR* gene mutation	2	1/1
Pre-eclampsia (n)	0			8			

* Values are presented as mean ± standard error. Values for controls are presented for all 30 subjects, and for 10 subjects in each trimester.

Presented as homozygotes (n) /heterozygotes (n)

blood pressure exceeding 140/90 mm Hg before pregnancy or before gestation week 20, gestational hypertension–hypertension that developed after gestation week 20, and pre-eclampsia. All women with thrombophilia received therapeutic doses of low molecular weight heparin, from the beginning of the pregnancy to the postpartum period. Normal pregnancies were eligible for the control group if they had an uncomplicated pregnancy that resulted in the birth of a healthy newborn and no history of thromboembolism. No smokers were involved in the study. All patients (controls and thrombophilic) received 1000 mg of vitamin C and 5 mg of folate each day until gestational week 16; after that all patients received multivitamin supplement that contained 100 mg of vitamin C each day. Institutional approval for the study was granted by The Ethics Committee of University Clinic for Gynecology and Obstetrics „Narodni front", in accordance with internationally accepted ethical standards (The Helsinki Declaration of 1964, as revised in 1975, 1983 and 1989), and each patient had signed the informed consent form.

Samples

Blood samples (3 mL) were taken from all 56 pregnant women with thrombophilia in the 1st (gestational week: 10–12), the 2nd (gestational weeks: 22–26), and the 3rd trimester (gestational week: 34–38). Control samples were collected from 10 women with normal pregnancy for each trimester in the same periods of gestation. The blood extraction was performed at the same time to routine clinical measurements. Venous blood was collected after overnight fasting using tubes containing 0.072 mL of 7.5% K_3EDTA as the anticoagulant (Vacuette EDTA, Greiner Bio-One, Austria), and centrifuged at 2000 g/15 min/4˚C to separate erythrocytes and plasma. Erythrocytes were washed three times with 0.9% NaCl at 4˚C. Plasma and washed erythrocytes were immediately placed in tubes, snap-frozen in liquid nitrogen, and stored at -80˚C for further analysis.

Biochemical and electrochemical analysis

All chemicals were purchased from Merck (Darmstadt, Germany). Erythrocytes (0.5 mL) were lysed by adding 3 mL of ice-cold distilled water. Hemoglobin (Hb) concentration was measured by Drabkin method. SOD activity was determined by the adrenaline method [37]. One unit of activity is defined as the amount of enzyme that decreases the rate of adrenaline auto-oxidation at pH 10.2 by 50%. Interference with Hb was eliminated by precipitation prior to the

assay using ethanol/chloroform (1:1, v/v), that was followed by centrifugation at 5000 g/5 min/ 4˚C. The activity of CAT was determined as described previously [38]. One unit is defined as the amount of enzyme that reduces 1 mM of H_2O_2 per min. SOD and CAT activities were normalized to Hb. The activity of GPx was determined using *t*-butylhydroperoxide as substrate [39], and expressed in μmol NADPH/min/g Hb. GR activity was assayed as reported by Glatzle *et al*. [40], and expressed in μmol NADPH/min/g Hb. The content of R-SH in plasma was determined according to Ellman [41]. Static ORP (in mV) was measured at room temperature using RedoxSYS System (Aytu BioScience, Inc., Englewood, CO, USA) [34]. Plasma (40 μL) was applied to a pre-inserted sensor, and the readings were initiated automatically.

Statistical analysis

Statistical analyses were performed using STATISTICA 8.0 (StatSoft Inc., Tulsa, OK, USA). Results are presented as box. Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range. Outliers and extremes are defined as data point values that are more than 1.5× and 3× interquartile range outside the box, respectively. We analyzed differences between controls and thrombophilic pregnancies, controls and 4 different inherited thrombophilias taken separately (data for *MTHFR* gene mutation thrombophilia (n = 2) were an exception), controls and thrombophilic women with hypertension (n = 20), and thrombophilic women with and without hypertension using nonparametric two-tailed Mann–Whitney *U* test. The differences between different trimesters in thrombophilic pregnancies and between different types of inherited thrombophilia were established using Kruskal-Wallis ANOVA by ranks with Dunn's *post hoc* test. Results were considered to be statistically significant if $p < 0.05$.

Results

The activity of SOD in erythrocytes was about two-fold lower in thrombophilias than controls during the entire pregnancy (Fig 1A). Similarly, CAT activity was lower in inherited thrombophilias during the first two trimesters (Fig 1B). It is important to note that SOD activity in the 3rd trimester of thrombophilic pregnancy was significantly higher than in the first two trimesters.

Further, GPx activities in healthy and thrombophilic pregnancies were not significantly different in the first two trimesters (Fig 2A). In the 3rd trimester, GPx activity showed a drastic increase in thrombophilic pregnancy and it was three-fold higher than in controls. GR activity was lower in inherited thrombophilias than controls only in the 1st trimester (Fig 2B). Both, GPx and GR activities showed a significant increase in the 3rd trimester of thrombophilic pregnancies compared to the first two trimesters.

Finally, R-SH concentrations were higher in the 1st and the 2nd trimester, whereas ORP was lower in the 1st trimester in the plasma of pregnant women with thrombophilia than controls (Fig 3). It is important to note here that lower ORP values stand for lower level of oxidizing and/or higher level of reducing species. So, the results implicate that there is less oxidation in thrombophilic pregnancies than controls. It is noteworthy that there were no changes in R-SH concentration and ORP in different trimesters of thrombophilic pregnancy. Further, there was no significant difference in any of the measured parameters between different types of inherited thrombophilia. Also, thrombophilias with hypertension showed similar trends as all thrombophilias in comparison to control values. There were no significant differences in any of the parameters between thrombophilic pregnancies with or without hypertension (not shown).
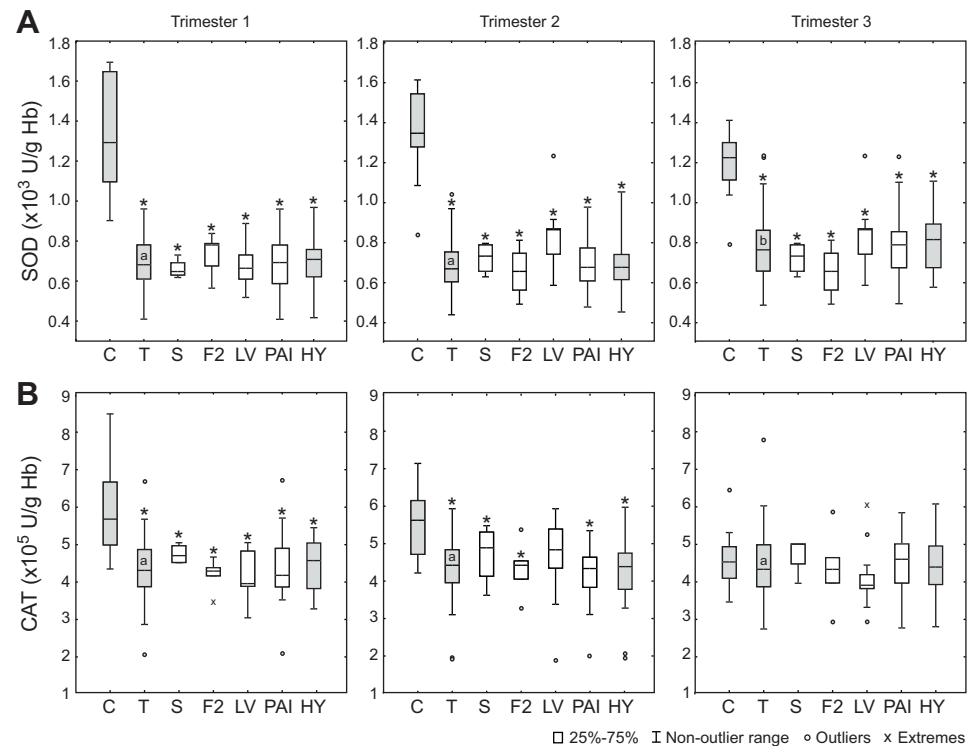
Fig 1. The activities of superoxide dismutase (SOD) and catalase (CAT) in erythrocytes in different trimesters. (**A**) SOD activity. (**B**) CAT activity. C–control (n = 10 for each trimester); T–thrombophilias (all; n = 56); S–protein S deficiency (n = 4); F2 –*F2* gene mutation (n = 6); LV–Factor V Leiden mutation (n = 13); PAI–*PAI*-1 polymorphism (n = 31); HY–thrombophilic pregnancies burdened with hypertension (n = 20). Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range; circles–outliers; x—extremes. *—Statistically different compared to control (p < 0.05). Different trimesters in thrombophilic pregnancy not sharing a common letter in the box are significantly different.

Discussion

Surprisingly, the blood of pregnant women with inherited thrombophilias showed less oxidation than controls during the first two trimesters. Pro-reductive oxidative status is implicated by higher R-SH levels and by lower ORP in plasma, as well as by lower activities of SOD, CAT and GR in erythrocytes. Therefore, it can be concluded that thrombophilic mothers do not experience oxidative stress in the circulation in the first two trimesters. However, the rise in GPx, GR and SOD activities in the 3rd trimester of thrombophilic pregnancy implies that the risk of development of oxidative stress is increased during the late pregnancy. This is in accord with our previous findings that placental tissue of thrombophilic mothers shows several-fold higher activity of H_2O_2-removing enzymes (CAT, GPx and GR) than controls [22], and that pro-oxidative changes of oxidative status take place in the blood of thrombophilic mothers immediately after the delivery [23]. It is possible that an increase in the cardiovascular load during pregnancy [24, 25], in combination with pro-thrombic conditions progressively leads to pro-oxidative pressure that exceeds the capacities of baseline antioxidative defense and provokes upregulation of antioxidative enzymes. Pertinent to this, GPx showed particularly pronounced increase near the term of thrombophilic pregnancy. Within erythrocytes, GPx appears to be mainly located close to the membrane. It has been proposed that GPx is in charge of preventing lipid peroxidation and removing H_2O_2 that enters the erythrocytes from plasma [42, 43]. Therefore, erythrocytes, in addition to placental antioxidative system [22], may play
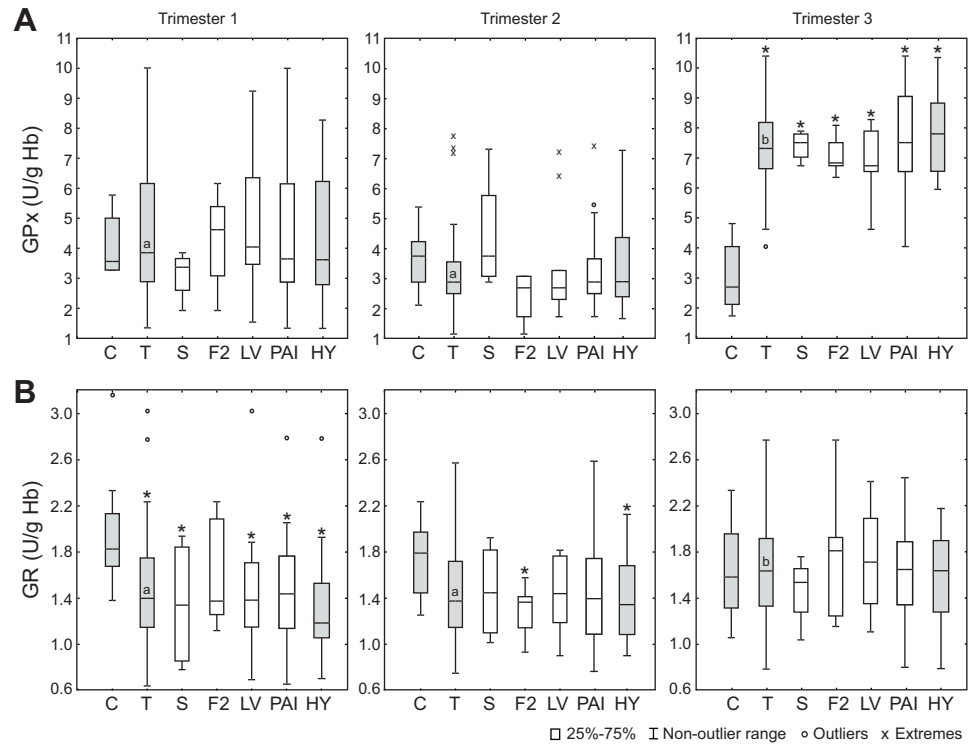
Fig 2. The activities of glutathione peroxidase (GPx) and reductase (GR) in erythrocytes in different trimesters.
(**A**) GPx activity. (**B**) GR activity. C–control (n = 10 for each trimester); T–thrombophilias (all; n = 56); S–protein S deficiency (n = 4); F2 –*F2* gene mutation (n = 6); LV–Factor V Leiden mutation (n = 13); PAI–*PAI*-1 polymorphism (n = 31); HY–thrombophilic pregnancies burdened with hypertension (n = 20). Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range; circles–outliers; x—extremes. *—Statistically different compared to control (p < 0.05). Different trimesters in thrombophilic pregnancy not sharing a common letter in the box are significantly different.

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an important role in the protection of maternal circulation from excessive amounts of H_2O_2 in the last trimester of thrombophilic pregnancy. In close, it appears that near the term, pro-thrombic conditions lead to increased production of H_2O_2 in the circulation (in relation to ischemia/re-perfusion, endothelial injury, or inflammation [20, 21]). This is compensated by upregulated enzymatic antioxidative systems in erythrocytes and placenta. Erythrocytes have been proposed to act as a sink for extracellular H_2O_2 [44], whereas placental blood flow shows a large volume of 600–700 mL/min [45]. It is important to stress out here that H_2O_2 diffusion across cell membranes is facilitated by specific aquaporins (AQP) [46], which are present in placenta (AQP3, AQP8, AQP9) [47], and on erythrocytes membrane (AQP3) [48]. At the delivery, placenta is discarded and the protection of maternal circulation is weakened. So a pro-oxidative shift in oxidative status of blood takes place [22], which may be the cause of the increased risk of thrombosis and thromboembolism postpartum [49]. It is worth mentioning that no significant differences were observed between different thrombophilia types or between thrombophilic mothers with and without hypertension for any of the investigated parameters. This implies that the established trimestral profile of oxidative status is not related to a specific mutation or polymorphism or to the development of hypertension and that it represents a feature of thrombophilia *per se*. Finally, our results imply that the application of anti-oxidative supplements/therapy is most appropriate in the 3rd trimester of thrombophilic pregnancies.
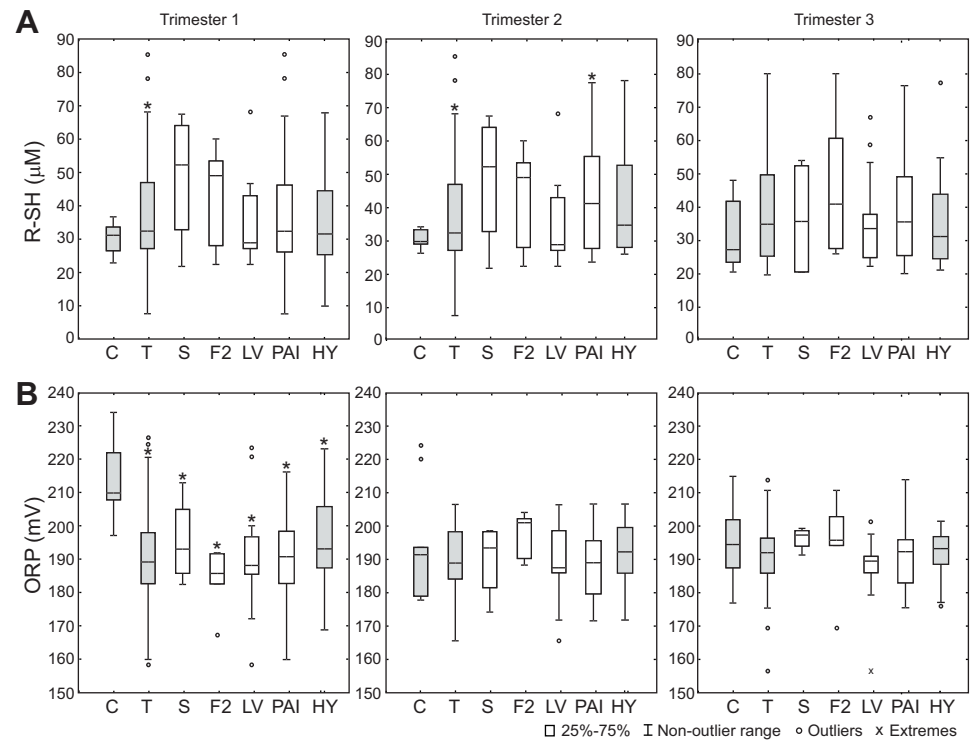
Fig 3. Redox settings in the plasma in different trimesters. (**A**) Concentration of reduced thiols. (**B**) Static ORP of plasma. C–control (n = 10 for each trimester); T–thrombophilias (all; n = 56); S–protein S deficiency (n = 4); F2 –*F2* gene mutation (n = 6); LV–Factor V Leiden mutation (n = 13); PAI–*PAI*-1 polymorphism (n = 31); HY–thrombophilic pregnancies burdened with hypertension (n = 20). Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range; circles–outliers; x—extremes. *—Statistically different compared to control (p < 0.05). There was no statistical difference between values obtained at different trimesters of thrombophilic pregnancy.

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Supporting information

S1 Dataset. Data underlying the findings described are available in S1 Dataset.
(XLSX)

Author Contributions

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References

1. Ramiro-Cortijo D, Herrera T, Rodríguez-Rodríguez P, López De Pablo ÁL, De La Calle M, López-Giménez MR, et al. Maternal plasma antioxidant status in the first trimester of pregnancy and development of obstetric complications. Placenta. 2016; 47:37–45. https://doi.org/10.1016/j.placenta.2016.08.090 PMID: 27780538

2. Pereira RD, De Long NE, Wang RC, Yazdi FT, Holloway AC, Raha S. Angiogenesis in the placenta: the role of reactive oxygen species signaling. Biomed Res Int. 2015; 2015:814543. https://doi.org/10.1155/2015/814543 PMID: 25705690

3. Yang F, Zheng Q, Jin L. Dynamic function and composition changes of immune cells during normal and pathological pregnancy at the maternal-fetal interface. Front Immunol. 2019; 10:2317. https://doi.org/10.3389/fimmu.2019.02317 PMID: 31681264

4. Hu XQ, Song R, Zhang L. Effect of oxidative stress on the estrogen-NOS-NO-KCa channel pathway in uteroplacental dysfunction: Its implication in pregnancy complications. Oxid Med Cell Longev. 2019; 2019:9194269. https://doi.org/10.1155/2019/9194269 PMID: 30881600

5. Argüelles S, Machado MJ, Ayala A, Machado A, Hervías B. Correlation between circulating biomarkers of oxidative stress of maternal and umbilical cord blood at birth. Free Radic Res. 2006; 40:565–70. https://doi.org/10.1080/10715760500519834 PMID: 16753833

6. Mannaerts D, Faes E, Cos P, Briedé JJ, Gyselaers W, Cornette J, et al. Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function. PLoS One. 2018; 13:e0202919. https://doi.org/10.1371/journal.pone.0202919 PMID: 30204759

7. Ishihara O, Hayashi M, Osawa H, Kobayashi K, Takeda S, Vessby B, et al. Isoprostanes, prostaglandins and tocopherols in pre-eclampsia, normal pregnancy and non-pregnancy. Free Radic Res. 2004; 38:913–8. https://doi.org/10.1080/10715760412331273421 PMID: 15621708

8. Raijmakers MT, Zusterzeel PL, Steegers EA, Hectors MP, Demacker PN, Peters WH. Plasma thiol status in preeclampsia. Obstet Gynecol. 2000; 95:180–4. https://doi.org/10.1016/s0029-7844(99)00514-1 PMID: 10674576

9. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. Reprod Biol Endocrinol. 2012; 10:49. https://doi.org/10.1186/1477-7827-10-49 PMID: 22748101

10. Duhig K, Chappell LC, Shennan AH. Oxidative stress in pregnancy and reproduction. Obstet Med. 2016; 9:113–6. https://doi.org/10.1177/1753495X16648495 PMID: 27630746

11. Freedman JE. Oxidative stress and platelets. Arterioscler Thromb Vasc Biol. 2008; 28:s11–6. https://doi.org/10.1161/ATVBAHA.107.159178 PMID: 18174453

12. Gu SX, Stevens JW, Lentz SR. Regulation of thrombosis and vascular function by protein methionine oxidation. Blood. 2015; 125:3851–9. https://doi.org/10.1182/blood-2015-01-544676 PMID: 25900980

13. Violi F, Pignatelli P. Platelet oxidative stress and thrombosis. Thromb Res. 2012; 129:378–81. https://doi.org/10.1016/j.thromres.2011.12.002 PMID: 22209450

14. Egan K, Kevane B, Ní Áinle F. Elevated venous thromboembolism risk in preeclampsia: molecular mechanisms and clinical impact. Biochem Soc Trans. 2015; 43:696–701. https://doi.org/10.1042/BST20140310 PMID: 26551715

15. Pabinger I, Grafenhofer H, Kaider A, Ilic A, Eichinger S, Quehenberger P, et al. Preeclampsia and fetal loss in women with a history of venous thromboembolism. Arterioscler Thromb Vasc Biol. 2001; 21:874–9. https://doi.org/10.1161/01.atv.21.5.874 PMID: 11348890

16. Sarig G, Brenner B. Coagulation, inflammation and pregnancy complications. Lancet. 2004; 363:96–7. https://doi.org/10.1016/S0140-6736(03)15309-3 PMID: 14726160

17. Kupferminc MJ. Thrombophilia and pregnancy. Reprod Biol Endocrinol. 2003; 1:111. https://doi.org/10.1186/1477-7827-1-111 PMID: 14617365

18. Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, et al. The association of factor V leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies. PLoS Med. 2010; 7:e1000292. https://doi.org/10.1371/journal.pmed.1000292 PMID: 20563311

19. Walker ID. Thrombophilia in pregnancy. J Clin Pathol. 2000; 53:573–80. https://doi.org/10.1136/jcp.53.8.573 PMID: 11002758

20. Crimi E, Ignarro LJ, Napoli C. Microcirculation and oxidative stress. Free Rad Res. 2007; 41:1364–75.

21. Laude I, Rongières-Bertrand C, Boyer-Neumann C, Wolf M, Mairovitz V, Hugel B, et al. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. Thromb Haemost. 2001; 85:18–21. PMID: 11204573

22. Bogdanović Pristov J, Spasojević I, Miković Ž, Mandić V, Cerović N, Spasić M. Antioxidative defense enzymes in placenta protect placenta and fetus in inherited thrombophilia from hydrogen peroxide. Oxid Med Cell Longev. 2009; 2:14–8. https://doi.org/10.4161/oxim.2.1.7705 PMID: 20046640

23. Bogdanović Pristov J, Maglić D, Opačić M, Mandić V, Miković Ž, Spasić M, et al. Ante- and postpartum redox status of blood in women with inherited thrombophilia treated with heparin. Thromb Res. 2012; 130:826–9. https://doi.org/10.1016/j.thromres.2012.08.310 PMID: 22981678

24. Duvekot JJ, Peeters LL. Maternal cardiovascular hemodynamic adaptation to pregnancy. Obstet Gynecol Surv. 1994; 49:S1–14. https://doi.org/10.1097/00006254-199412011-00001 PMID: 7877788

25. Napso T, Yong HEJ, Lopez-Tello J, Sferruzzi-Perri AN. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. Front Physiol. 2018; 9:1091. https://doi.org/10.3389/fphys.2018.01091 PMID: 30174608

26. Yüksel S, Yiğit AA. Malondialdehyde and nitric oxide levels and catalase, superoxide dismutase, and glutathione peroxidase levels in maternal blood during different trimesters of pregnancy and in the cord blood of newborns. Turk J Med Sci. 2015; 45:454–9. PMID: 26084141

27. Moore TA, Ahmad IM, Schmid KK, Berger AM, Ruiz RJ, Pickler RH, et al. Oxidative stress levels throughout pregnancy, at birth, and in the neonate. Biol Res Nurs. 2019; 21:485–94. https://doi.org/10.1177/1099800419858670 PMID: 31284724

28. Ghaffari S. Oxidative stress in the regulation of normal and neoplastic hematopoiesis. Antioxid Redox Signal. 2008; 10:1923–40. https://doi.org/10.1089/ars.2008.2142 PMID: 18707226

29. Nikolić-Kokić A, Blagojević DP, Spasić MB. Complexity of free radical metabolism in human erythrocytes. J Med Biochem. 2010; 29:189–95.

30. Tsantes AE, Bonovas S, Travlou A, Sitaras NM. Redox imbalance, macrocytosis, and RBC homeostasis. Antioxid Redox Signal. 2006; 8:1205–16.

31. Chilelli NC, Cosma C, Burlina S, Plebani M, Lapolla A. Antioxidant capacity in patients with type 2 diabetes: a preliminary investigation on gender-specific differences in an Italian population. Clin Chem Lab Med. 2018; 56:101–4. https://doi.org/10.1515/cclm-2017-0721 PMID: 29140790

32. Oliveira PVS, Laurindo FRM. Implications of plasma thiol redox in disease. Clin Sci (Lond). 2018; 132:1257–80.

33. Opačić M, Stević Z, Baščarević V, Živić M, Spasić M, Spasojević I. Can oxidation-reduction potential of cerebrospinal fluid be a monitoring biomarker in amyotrophic lateral sclerosis? Antioxid Redox Signal. 2018; 28:1570–5. https://doi.org/10.1089/ars.2017.7433 PMID: 29113448

34. Rael LT, Bar-Or R, Aumann RM, Slone DS, Mains CW, Bar-Or D. Oxidation-reduction potential and paraoxonasearylesterase activity in trauma patients. Biochem Biophys Res Commun. 2007; 361:561–5. https://doi.org/10.1016/j.bbrc.2007.07.078 PMID: 17662690

35. ACOG Practice Bulletin No. 197: Inherited thrombophilias in pregnancy. Obstet Gynecol. 2018; 132: e18–34. https://doi.org/10.1097/AOG.0000000000002703 PMID: 29939939

36. Ormesher L, Simcox L, Tower C, Greer IA. Management of inherited thrombophilia in pregnancy. Womens Health (Lond). 2016; 12:433–41. https://doi.org/10.1177/1745505716653702 PMID: 27638899

37. Misra HIF. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170–5. PMID: 4623845

38. Claiborne A. Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press Inc.; 1984.

39. Paglia DE, Valentine NW. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70:74–7.

40. Glatzle D, Vuilleumier JP, Weber F, Decker K. Glutathione reductase test with whole blood: a convenient procedure for the assessment of the riboflavin status in humans. Experientia. 1974; 30:665–8. https://doi.org/10.1007/BF01921531 PMID: 4151937

41. Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82:70–7. https://doi.org/10.1016/0003-9861(59)90090-6 PMID: 13650640

42. Nagababu E, Chrest FJ, Rifkind JM. Hydrogen peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. Biochim Biophys Acta. 2003; 1620:211–7. https://doi.org/10.1016/s0304-4165(02)00537-8 PMID: 12595091

43. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol. 2014; 5:84. https://doi.org/10.3389/fphys.2014.00084 PMID: 24616707

44. Winterbourn CC, Stern A. Human red cells scavenge extracellular hydrogen peroxide and inhibit formation of hypochlorous acid and hydroxyl radical. J Clin Invest. 1987; 80:1486–91. https://doi.org/10.1172/JCI113230 PMID: 2824562

45. Wang Y, Zhao S. Vascular Biology of the Placenta. San Rafael (CA): Morgan & Claypool Life Sciences; 2010.

46. Bienert GP, Chaumont F. Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. Biochim Biophys Acta. 2014; 1840:1596–604. https://doi.org/10.1016/j.bbagen.2013.09.017 PMID: 24060746

47. Sha XY, Xiong ZF, Liu HS, Di XD, Ma TH. Maternal-fetal fluid balance and aquaporins: from molecule to physiology. Acta Pharmacol Sin. 2011; 32:716–20. https://doi.org/10.1038/aps.2011.59 PMID: 21602839

48. Roudier N, Verbavatz JM, Maurel C, Ripoche P, Tacnet F. Evidence for the presence of aquaporin-3 in human red blood cells. J Biol Chem. 1998; 273:8407–12. https://doi.org/10.1074/jbc.273.14.8407 PMID: 9525951

49. Calderwood CJ, Thanoon OI. Thromboembolism and thrombophilia in pregnancy. Obstet Gynecol Reprod Med. 2009; 19:339–43.