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Maternal oxidative imbalance in symptomatic SARS-COV-2 during pregnancy

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The coronavirus infection (Covid-19) leads to overactive immune responses, resulting in oxidative stress (OS), excessive reactive oxygen/ nitrogen (ROS/RNS) production, reduced antioxidant enzymes, and disrupted total redox balance. Pregnant women are more susceptible to respiratory pathogens, i.e. to Covid-19. The aim of the study was to investigate the effects of symptomatic Covid-19 during pregnancy on oxidative/antioxidant status in blood during pregnancy and the consequences for the mother. The patients were divided: 1) Controls (n = 100); singleton pregnancy, without Covid-19; and 2) Covid-19 group (n = 65), singleton pregnancy, with symptomatic Covid-19 (>30 gestational weeks). Serum superoxide dismutase ($p < 0.01$), catalase ($p < 0.01$), protein carbonylation ($p < 0.001$), ROS production ($p < 0.01$) and albumin oxidation ($p < 0.05$) were increased in symptomatic Covid-19 pregnancies *vs*. controls. The increased maternal OS/ redox imbalance in pregnant mothers with symptomatic Covid-19 could be associated with preterm labour.

Keywords: symptomatic CoV-19 pregnancies, health pregnancies, OS, ROS/RNS

INTRODUCTION

The acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (Covid-19). Advanced age, increased body mass index (BMI), type 2 diabetes (T2D), hypertension, and ineffective immune responses are significant risk factors that contribute to SARS-CoV-2 cytokine hyper-inflammation [1, 2]. SARS-CoV-2 infection leads to overactive immune responses, resulting in oxidative stress (OS), excessive production of reactive oxygen/nitrogen species (ROS/RNS), reduced antioxidant enzymes, and disrupted total redox balance [2, 3]. OS damages tissues and blood components through oxidation of nucleic acids, proteins, and lipids, mainly macromolecules involved in the SARS-CoV-2 pathogenesis [2, 4]. Viral infections alter the cellular structures and function through lipid peroxidation, resulting in increased ROS/RNS production and acute metabolic dysfunction [2, 5, 6].

Pregnant women are more susceptible to respiratory pathogens and severe pneumonia [7]. This is due to the physiological changes in the respiratory system during pregnancy and increased oxygen consumption. Physiological diaphragm elevation leads to restrictions in the lung expansion, hormonal inducement of edema of upper respiratory tract mucosa. As the pregnancy progresses, other changes like closure of small airways and subsequent reduction of functional residual capacity (FRC) and expiratory reserve volume (ERV). This makes pregnant women more sensitive to hypoxia and generally more susceptible to respiratory pathogens [8, 9]. Pregnancy is associated with changes in the functioning of the immune system. All these facts increase the risk of Covid-19 [8, 9]. Pro-inflammatory cytokines release is inhibited by hormonal cues in pregnancy, mainly due to high progesterone level. A Th2-polarization phenomenon allows for the suppression of dominant cell-mediated pro-inflammatory Th1 immunity in favour of a physiological shift to humoral Th2-dominant immunity. As a result, there is an increase in the intercellular susceptibility to infection weather viruses, bacteria and parasites and this can explain why pregnant women are more vulnerable to viral infection in comparison to non-pregnant women. Pregnant women with pneumonia are at high risk for miscarriage, preterm delivery, premature rupture of membranes, preeclampsia (PE), foetal distress, low birth weight (<2500 g), small-for-gestational-age (SGA) and neonates with low Apgar score [8, 9]. However, increased systemic inflammatory response, hyper-coagulation, decreased arterial oxygen saturation, and increased OS are considered risk factors directly related to poor obstetric outcomes [8, 9]. Despite the importance of OS for the proper macromolecule metabolism and for foetal development, there are only a few studies on the role

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of OS changes in symptomatic Covid-19 in pregnant women, leading to preterm labour [10, 11].

According to the above, the aim of the present study was to investigate the effects of CoV-19 during pregnancy on the oxidative/antioxidant status and to clarify the crucial role of both factors during pregnancy and their consequences for the mother and foetus.

EXPERIMENTAL

The 165 pregnant women were examined after admission to the Obstetrics and Gynaecology Clinic, Stara Zagora, Bulgaria, between June 2021- October 2023. The inclusion criteria were: pregnant patients without previous chronic pathologies, singleton pregnancy; body mass index 18–30 kg/m^2; experienced symptomatic Covid-19 infection with (PCR (+)) test, from the >30th gestational week onwards and the control group with Covid-19 and PCR (−) test, again with singleton and body mass index 18–30 kg/m^2. Exclusion criteria were: chronic disease with long-term treatment; previous infectious diseases; body mass index <18 or >30 kg/m^2; malnutrition; previous foetal demise. All symptomatic Covid-19 pregnant patients enrolled in the study reported severe symptoms (cough (31%), fever ≥ 38.5 °C (50.6 %); respiratory rate ≥ 20 (9%); heart rate ≥ 100 bpm (13%); oxygen saturation SpO_2 less than 92% (7%)) requiring hospital stay and antiviral therapy with Ritonavir and Lopinavir.

The study included: 1) Controls (n = 100); singleton pregnant women, who have not suffered from Covid-19 and gave birth at term; 2) Covid-19 group (n = 65), singleton pregnant women who suffered from symptomatic Covid-19 during pregnancy (>30 gestational weeks). The study was approved by the Ethics Committee of the University Multidisciplinary Hospital for Active Treatment "St. Kirkovich" (referenced 10/816 -12 Oct. 2019). Serum samples (5 mL) taken from *v. cubitalis* were collected during birth and examined up to 2 h, after 3500 rpm centrifugation at 4°C, 15 min.

Serum superoxide dismutase (SOD) and catalase (CAT) activities were assayed according to the spectrophotometric methods described earlier [12, 13], at 550 nm and at 240 nm. Protein carbonylation (PCC; nmol/mg) was evaluated by an immune-enzyme method using commercially available ELISA kits (126287/BioVision Inc., USA). ROS production was examined by a mixture of 900µl phenyl N-test-butyl nitrone (PBN) (50 mM) in dimethyl sulfoxide (DMSO) to 100 mL plasma, centrifuged at 4000 rpm (10 min), at 4°C [14].

The albumin oxidation was evaluated using spin-conjugation with 10 mM 5-MSL dissolved in saline (pH=7.4). The mixture was added to 70 mL serum, and centrifuged at 4000 rpm/10 min, at 4°C [15]. The ROS formation and albumin oxidation were read in EPR-Emxmicro spectrometer (Bruker) in triplicate, in arbitrary units with characteristics: 3505 G centerfield, 6.42 MW power, 5 G amplitude, 1-5 scans.

Statistical Analysis

The clinical data were analysed using Statistica (Version 10.0 software, StaSoft, Inc., USA), and performed using one-way ANOVA. Student's t-test and Mann–Whitney U test were used to compare the baseline characteristics between groups. EPR spectral processing was performed using Win-EPR and Simfonia software after consecutive replicates and $p \leq 0.05$ was considered statistically significant. The primary analysis was performed on GraphPad Prism 9 including log-odds (log2) data of the study components and comparison with birth outcomes. Only a p-value ≤ 0.05 and absolute changes ≥ 1.5 were considered significant.

RESULTS AND DISCUSSION

SARS-CoV-2 infection causes OS disorder and metabolic dysfunctions during pregnancy, which is followed by changes in the condition and development of the fetus. Respiratory infections, including Covid-19, are associated with increased ROS/RNS production, increased antioxidant consumption, and a long-term impaired immune response [7, 8, 16]. Physiologically, Delgado-Roche and Mesta [16] comment that long-term accompanied OS plays a critical role in Covid-19 viral infection. The clinically measured haematological and biochemical characteristics of the symptomatic SARS-CoV-2 pregnant mothers *versus* controls are shown in Table 1.

No statistically significant differences were observed between both groups in age, weight, BMI, parity, and delivery. Statistically, significant changes were found in serum Fe ($p < 0.01$), ferritin ($p < 0.001$), and CRP ($p < 0.001$) concentrations with lower/higher values in the Covid-19 mothers compared to controls. It should be emphasized that the serum samples were collected from groups with no statistical differences between vaginal delivery and Caesarean section. Therefore, differences from endogenous OS due to birth outcome are minimized [8].

The antioxidant SOD and CAT activities showed a decrease in serum capacity in mothers suffering of Covid-19 compared to controls; 1.5-fold ($p < 0.01$) and 1.7-fold ($p < 0.01$), respectively (Table 2).

Table 1. Obstetric and clinical characteristics of healthy controls (n = 100) and pregnant mothers suffering from symptomatic CoV-19 (n = 65). Significantly different *vs.* controls, *p < 0.01, Student t-test; *p < 0.001, Student t-test.

Obstetric and clinical characteristics	Controls (n=100)	Symptomatic CoV-19 (n=65)
Age (years)	31.22 ± 1.4	29.91 ± 1.88
Body mass index (BMI), kg/m^2	28.6 ± 0.84	27.4 ± 2.91
Infectious diseases	None	None
Vaginal delivery (%)	50.2	60.7
Cesarean delivery (%)	50.1	49.9
Hemoglobin (g/L)	12.98 ± 0.51	12.21 ± 0.6
Hematocrit (%)	36.2 ± 0.77	32.11± 0.07*
Serum Fe (µg/dL)	96.13 ± 11.35	62.33 ± 10.11 *
Ferritin (ng/mL)	273 ± 11.54	1307 ± 451.5 **
CRP (mg/dL)	4.34 ± 0.12	183 ± 13.55 **

Table 2. Antioxidant and oxidative parameters in serum of healthy controls (n = 100) and pregnant mothers suffering from symptomatic CoV-19 (n = 65). Significantly different *vs*. controls, *p < 0.01, Student t-test; *p < 0.001, Student t-test. The primary analysis was performed on log-odds (log2) data of the study components and birth outcomes.

Antioxidant / Oxidative parameters	Controls (100)	Symptomatic CoV-19 (n=65)	log2 Analysis
SOD (mU/mg)	5.73 ± 0.91	2.91 ± 0.37 *	≥ 1.6 fold
CAT (mU/mg)	81.01 ± 6.12	54.86 ± 3.966*	≥ 1.5 fold
Protein carbonyl groups (nmol/mg)	6,711 ± 0.22	14.53 ± 0.54**	≥ 2.1 fold
ROS production (arb. units)	2.371 ± 0.51	8.14 ± 0.81**	≥ 3.2 fold
5-MSL (-SH/ albumin changes) (arb. units)	4.27 ± 0.012	1.033 ± 0.05**	≥ 3.1 fold

As a result of Covid-19 viral infection, an interplay between mitochondrial ROS/RNS and endothelial damage leads to vasoconstriction, OS-enhanced inflammation, and redox imbalance [17].

Placental ROS coupled with OS directly damage macromolecules, inhibit their versatility and compromise cellular antioxidant defences. This exacerbates OS values and OS impairments. Therefore, in the blood of pregnant females with symptomatic Covid-19, oxygen desaturation and improper placental perfusion will activate a redox imbalance [16, 18-19]. Different studies have reported increased OS and gestational disorders such as intrauterine growth retardation, gestational diabetes, maternal obesity, and preeclampsia [20, 21]. Mandò *et al*., [21] hypothesized that increased inflammation and OS in the Covid-19-altered intrauterine environment may alter antioxidant dynamics, depending on the severity of Covid-19. In addition, SARS-CoV-2 provokes enzymatic and redox imbalance even in non-pregnant patients [21].

Berktas *et al.,* [9] comment that lipid peroxides from the placenta are secreted into the maternal blood circulation. Placental antioxidant defence systems (SOD, CAT, GST, and glutathione reductase (GR)) maintain lipid peroxide levels in normal pregnancy, while in symptomatic CoV-19 pregnancy, ROS production increases [9, 21]. ROS production in blood serum is shown in Table 2. The results show that the ROS production/ residual lipid peroxidation concentration increased almost 3.1-fold ($p < 0.001$) in the pregnant woman with symptomatic Covid-19, compared to controls.

Blood serum and placenta are extremely susceptible to OS, especially when SOD and CAT metabolism is impaired. As a result, if the unique immunological response of the pregnant woman with SARS-CoV-2 does not minimize the ROS generation and lipid peroxides, this may lead to improper implantation and restriction of embryo growth especially when the infection occurs during the first trimester [21, 22].

Solis-Paredes *et al.,* [23] commented that oxidation of structural proteins in pregnant women who developed severe Covid-19 occurs in red blood cells and activates coagulopathic and thromboembolic events, due to increased ROS production. Pregnant women with severe Covid-19 had a 2.1-fold (52%) increase in the levels of carbonylated proteins ($p < 0.001$, Table 2). In this regard, a statistically significant increase in protein oxidative damage and ROS production in SARS-CoV-2 pregnant women has been reported in other studies [23, 24].

Blood albumin is a negative acute-phase reactant, multifunctional transporter of scavenged ROS, and promoted vascular permeability in normal labour [25]; used as a biomarker with significant prognostic value in severe Covid-19 infection during pregnancy. We used 5-MSL and by conjugating it to the weakened SH-albumin regions we used it to assess the conformational changes (*Patent:*

BG/U/2022/5487) occurring upon oxidation in amyloid albumin aggregates [26]. We observed a 3.1-fold decrease in albumin, i.e. > 69% SH-disrupted sites, and RS•/ RSOO• overproduction, additional initiators of lipid peroxidation, and characteristic hypoalbuminemia in Covid-19 pregnant women. In agreement with our results, a significant association was observed between OS-decreased albumins during severe Covid-19 infection [25] during pregnancy.

CONCLUSIONS

In the present study, we reported for the first time that maternal OS imbalance could be associated with preterm labour, in Bulgarian population. In this report, maternal OS imbalance was significantly elevated in pregnant mothers with symptomatic Covid-19 compared to control group. To our knowledge, this is the first evidence of the OS biomarkers expression in symptomatic Covid-19 pregnancy in the Bulgarian population.

PATENTS

The patents resulting from the work reported in this manuscript are with the incoming number of the patent application: BG/U/2022/5487

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REFERENCES

1. X. Li, S. Xu, M. Yu, K. Wang, Y. Tao, Y. Zhou, J. Shi, M. Zhou, B. Wu, Z. Yang, C. Zhang, *J. Clin. Immunol.*, **146**, 1, 110 (2020).
2. A. Avila-Nava, A. Geovanny Pech-Aguilar, R. Lugo, I. Medina-Vera, M. Guevara-Cruz, A. L.Gutiérrez-Solis, *Oxid Med Cell Long.*, **2022**, ID 1058813 (2022).
3. G. E. Forcados, A. Muhammad, O. O. Oladipo, S. Makama, C. A. Meseko, *Front. Cell. Infect. Microbiol.*, **11**, 654813 (2021).
4. R. Cecchini, A. L. Cecchini, *Med. Hypot.*, **143**, 110102 (2020).
5. M. A. Beck, J. Handy, and O. A. Levander, *Annals New York Acad. Sci.*, **917**(1), 906 (2000).
6. N. Žarković, B. Orehovec, L. Milković, B. Baršić, F. Tatzber, W. Wonisch, M. Tarle, M. Kmet, A. Mataić, A. Jakovčević, T. Vuković, *Antiox.*, **10** (9),1341 (2021).
7. M. Behram, S. C.Oğlak, Y. Başkıran, S. S. Çaypınar, S. Akgöl, Ş. Tunç, Z. G. Özköse, E. Akay, I. Dağ, *Ginekol. Pol.,* **92** (9), 631 (2021).
8. J. Moreno-Fernandez, J. J. Ochoa, C. De Paco Matallana, A. Caño, E. Martín-Alvarez, J. Sanchez-Romero, J. M. Toledano, M. Puche-Juarez, S. Prados, S. Ruiz-Duran, L. Diaz-Meca, *Antiox*., **11** (2), 184 (2022).
9. O. A. Berktas, S. O. Tutar, E. G. G. Peker, C. Kaya, *Aeg. J. Obst. Gynecol.*, **4** (2), 26 (2022).
10. M. Mirbeyk, A. Saghazadeh, N. A. Rezaei, *Arch. Gynecol. Obstet*., **304**, 5 (2021).
11. G. Capobianco, L. Saderi, S. Aliberti, M. Mondoni, A. Piana, F. Dessole, M. Dessole, P. L. Cherchi, S. Dessole, G. Sotgiu, *Eur. J. Obstet. Gynecol. Reprod. Biol*., **252**, 543 (2020).
12. J. D. Crapo, J.M. McCord, I. Fridovich, *Meth. Enzymol*., **53**, 382 (1978).
13. H. Aebi, *Meth Enzymol*., **105**, 121 (1984).
14. H. Shi, Y. Sui, X. Wang, Y. Luo, L. Ji, *Comp. Biochem. Physiol. Part C: Toxicol. & Pharmacol.*, **140** (1), 115 (2005).
15. K. Takeshita, K. Saito, J.I. Ueda, K. Anzai, T. Ozawa, *Biochim. Et. Biophys. Acta (BBA)-Gen. Subj*., **1573**, 156 (2002).
16. L. Delgado-Roche, F. Mesta, *Arch. Med. Res.,* **51**, 384 (2020).
17. O. Holland, M. Dekker Nitert, L. A. Gallo, M. Vejzovic, J. J. Fisher, A. V. Perkins, *Placenta*, **54**, 2 (2017).
18. H. C. Lee, Y. H. Wei, *Int. J. Biochem. Cell Biol.* **37**, 822 (2005).
19. L. Loffredo, F. Violi, *Int. J. Cardiol.*, **312**, 136 (2020).
20. M. Zambon, C. Mandò, A. Lissoni, G. M. Anelli, C. Novielli, M. Cardellicchio, R. Leone, M. N. Monari, M. Massari, I. Cetin, S. Abati, *Reprod. Sci*., **25**, 1474 (2018).
21. C. Mandò, V. M. Savasi, G. M. Anelli, S. Corti, A. Serati, F. Lisso, C. Tasca, C. Novielli, I. Cetin, *Antiox*., **24**(10),1517 (2021).
22. K. Toboła-Wróbel, M. Pietryga, P. Dydowicz, M. Napierała, J. Brązert, E. Florek, *Oxidative Med. Cell. Longev,*. **2020**, 6398520 (2020).
23. J. M. Solis-Paredes, A. Montoya-Estrada, A. Cruz-Rico, E. Reyes-Muñoz, J. Perez-Duran, S. Espino y Sosa, V. R. Garcia-Salgado, R. Sevilla-Montoya, R. J. Martinez-Portilla, G. Estrada-Gutierrez, J. A. Gomez-Ruiz, *Viruses*, **14** (4), 723 (2022).
24. S. Espino-Y-Sosa, R. J. Martinez-Portilla, J. Torres-Torres, J. M. Solis-Paredes, G. Estrada-Gutierrez, J. A. Hernandez-Pacheco, A. Espejel-Nuñez, P. Mateu-Rogell, A. Juarez-Reyes, F. E. Lopez-Ceh, *Viruses*, **13,** 1906 (2021).
25. T. Okamoto, K. Watanabe, T. Banno, T. Saitou, K. Sugiura, A. Iwasaki, A.Wakatsuki, *PIH*, (2022) https://doi.org/10.1016/j.preghy.2022.05.009
26. E. Georgieva, Y. Karamalakova, G. Arabadzhiev, V. Atanasov, R. Kostandieva, M. Mitev, V. Tsoneva, Y. Yovchev, G. Nikolova, *Antioxidants*, **11** (12), 2311 (2022).