The Influence of Oxidative Stress-Related Factors on Pregnancy and Neonatal Outcomes

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ABSTRACT

Background: Pregnancy is a physiological process associated with an excessive oxidative stress for both the mother and the neonate. Oxidative stress was extensively studied and is still in focus as a factor of maternal pathologies during pregnancy, with consequences on the outcome of the neonate. **Aim:** The aim of our study was to determine whether oxidative stress-related factors can influence the outcome of pregnancy, delivery, and the neonate's wellbeing. **Material and methods:** The study was conducted using a questionnaire among pregnant women with voluntary enrollment. Exclusion criteria were preeclampsia or other cardiovascular diseases, gestational diabetes, and hypothyroidism at admission. Patients were enrolled in a control group of 60 pregnant women without preexisting pathology and pregnancy with physiological course, a premature group of 21 pregnant women with premature delivery, and a cardiac group of 8 pregnant women with fetal heart disease known before birth. The study population was separated into subgroups based on dietary supplement use within the three main groups, and other subgroups for smoking/non-smoking mothers in the control group and one for smoking/non-smoking mothers in the premature and cardiac groups together. **Results:** The mean Apgar score at 1 minute was significantly higher in the control group compared to the cardiac group ($p = 0.0023$). The 1-minute Apgar score was significantly lower in infants that were born premature, from smoking mothers, compared to babies that were delivered at term ($p = 0.0191$). Although we did not obtain significant differences in birth weight corrected by gestational age between the control (mature) group and premature group, there was a good correlation in gestational age and birth weight of the preterm born infants ($r = 0.8517$, $p < 0.0001$). **Conclusions:** Smoking can aggravate oxidative stress in pregnancy, which will contribute to a difficult postnatal adaptation of newborns from smoking mothers and will increase the risk of premature delivery.

Keywords: pregnancy, smoking, Apgar score, oxidative stress, neonate

INTRODUCTION

Oxidative stress is the imbalance between the prooxidant mechanisms (protein, membrane, and DNA oxidation) and antioxidant defenses of the body. It is caused by excess of reactive oxygen, nitrogen, and chlorine species and reactive intermediates, which are causing cell, tissue, and DNA damage. This aggression can be counteracted by enzymatic or non-enzymatic antioxidants in normal, healthy pregnancy, physiological labor, and delivery without any complications.

However, with the disturbance of the defense system, an intensive oxidative aggression occurs, which can be the precursor for multiple complications in pregnant women: eclampsia, miscarriage, preterm labor, and intrauterine growth retardation. In case of fetal involvement, this aggression may lead to perinatal diseases: bronchopulmonary dysplasia/chronic lung disease, necrotizing enterocolitis, and retinopathy of prematurity, called by Saugstad the “oxygen radical diseases of neonatology”. Later, the list was completed with periventricular leukomalacia.^{1,2}

The placenta has been proven to be the major source of oxidative stress during pregnancy as it is rich in lipid peroxides. The oxidative stress effect is under the control of the placental antioxidant enzymes.³ Furthermore, both labor and delivery are accompanied by an increase of oxidative stress markers and simultaneously by a decrease in antioxidant capacity. A study conducted by Arguelles *et al.* on circulating oxidative stress biomarkers in maternal and umbilical cord blood concluded that a higher oxidative state in the maternal blood correlates with a higher oxidative state in the umbilical cord blood.⁴ Oxidative stress during embryogenesis and fetal development has an influence on the metabolism of the fetus via gene expression and/or lipid and protein peroxidation.^{5,6}

The antioxidant activity should balance the high production of free radicals in normal, uncomplicated pregnancy. However, the concentration of the components of the antioxidant defense system might not be sufficient to counteract the effects of oxidative stress.⁶ The antioxidants that protect the body against oxidative stress can be grouped into endogenous (enzymatic, non-enzymatic) and exogenous (natural, synthetic) antioxidants.⁷

Environmental factors and maternal diet can have an influence on the increased oxidative stress or decreased antioxidant status during pregnancy. Turpeinen *et al.* and Maziere C *et al.* showed in their studies that a diet rich in polyunsaturated acids could contribute to oxidative stress associated with preeclampsia. Burke noted that a deficient diet was related with greater risk of preeclampsia. Clausen

et al. confirmed that the occurrence of preeclampsia tends to rise with increasing calorie intake and polyunsaturated fats in the diet.^{8–12}

A study conducted by Arguelles *et al.* showed a higher risk of increased oxidative stress and its aggression on lipids and proteins in smoking mothers compared with non-smoking ones, and the decrease of antioxidant capacity related to smoking.⁴ Maternal smoking during pregnancy increases the risk of complications such as stillbirth, premature delivery, lower birth weight, altered cardio-respiratory response, cleft palate, and sudden infant death syndrome, as well as long-term neurobehavioral effects.^{13–16}

MATERIAL AND METHODS

Our study was conducted using a questionnaire completed by pregnant women admitted to the Obstetrics-Gynecology Clinic I of the Emergency Clinical County Hospital of Târgu Mureș between November 2019 and October 2020. The study has been approved by the Ethics Committee of the hospital and of the “George Emil Palade” University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, and participation of the included patients was voluntary. In each case, written informed consent was obtained from the participants.

The questionnaire inquired about personal information (name, age, address, phone number), biometric parameters (height, weight, blood pressure, parity, delivery data), lifestyle data (fruit and vegetable consumption, soft drinks, non-alcoholic drinks, coffee, tea, alcohol, smoking habits, dietary supplements, habitual stress, sports), health-related information (preexisting pathology, infectious diseases, pharmaceutical treatment). Exclusion criteria were preeclampsia or other cardiovascular diseases, gestational diabetes, and hypothyroidism at admission. Data was recorded at admission by highly qualified and trained hospital personnel, during labor. Information about the delivery and the newborn were obtained after delivery from the patients' medical records (delivery type, birth weight, 1- and 5-minute Apgar score).

The participants were categorized into three groups: the *control* group included 60 pregnant women with no preexisting pathology and physiological pregnancy; the *premature* group included 21 pregnant women with premature rupture of membrane and risk for premature delivery; and the *cardiac* group included 8 pregnant women with known fetal cardiac disease. The study population was further separated into subgroups based on smoking/non-smoking status for the control group and for the premature and cardiac groups together. In this group, the ex-

TABLE 1. Baseline characteristics of the study groups

Characteristics	Control group (n = 60)	Premature group (n = 21)	Cardiac group (n = 8)
Parity			
Primiparity	30 (50%)	8 (38.09%)	4 (50%)
Multiparity	30 (50%)	13 (61.90%)	4 (50%)
Delivery mode	9 (75)	7 (6.48)	0.0001
Vaginal	51 (85%)	14 (66.67%)	3 (37.50%)
Caesarian	9 (15%)	7 (33.33 %)	5 (62.50%)

clusion criterion was non-responding to the question on smoking habits. Another subgroup was created based on dietary supplement use within the three main groups.

Our study complied with the Declaration of Helsinki. Statistical analysis was performed with GraphPad InStat version 3 (GraphPad Software Inc, California, USA). We used Student's unpaired t test, with and without Welch correction, Pearson's correlation test, and Fischer's test. The level of significance was set to $p < 0.05$.

RESULTS

The average age of the mothers from the three groups did not show significant differences. Data regarding baseline characteristics, including parity and delivery mode, are shown in Table 1, while maternal ages, gestational ages, birth weights, and Apgar scores at 1 and 5 minutes are shown in Table 2.

The weight at birth was not significantly different between the control and cardiac groups, and neither be-

tween the subgroups of premature babies from smoking and non-smoking mothers.

Premature newborns had a quasi-harmonious intrauterine growth; there were no differences in weight gain adjusted to gestational age, as there was a statistically significant correlation between the two parameters ($r = 0.8517$, $p < 0.0001$), as shown in Figure 1.

Regarding the habitual use of dietary supplements there were no statistically significant differences in the mean birth weight between the subgroups of either the control or the premature group.

Postnatal adaptation was assessed by the Apgar score at 1 minute and 5 minutes of life. We obtained statistically significant differences in 1-minute Apgar scores between the control and cardiac groups with 9.08 ± 1.61 versus 7.87 ± 1.24 points, respectively ($p = 0.02$) (Figure 2).

The same statistically significant differences were observed in case of the 5-minute Apgar score, with a mean value of 9.45 ± 1.39 versus 8 ± 0.92 points, respectively ($p = 0.005$).

TABLE 2. Maternal and neonatal clinical characteristics

Characteristics	Control group (n = 60)	Premature group (n = 21)	p value
Maternal age (years, mean $\pm$ SD)	26.47 $\pm$ 6.24	23.33 $\pm$ 6.72	0.93
Gestational age (weeks, mean $\pm$ SD)	38.93 $\pm$ 0.95	32.00 $\pm$ 3.91	<0.0001*
Birth weight (g, mean $\pm$ SD)	3,409.66 $\pm$ 411.22	1,905 $\pm$ 784.73	<0.0001*
Apgar score at 1 minute	9.08 $\pm$ 1.62	7.95 $\pm$ 1.84	0.01*
Apgar score at 5 minutes	9.45 $\pm$ 1.40	8.86 $\pm$ 0.91	0.07

Characteristics	Control group (n = 60)	Cardiac group (n = 8)	p value
Maternal age (years, mean $\pm$ SD)	26.47 $\pm$ 6.24	28.88 $\pm$ 5.67	0.30
Gestational age (weeks, mean $\pm$ SD)	38.93 $\pm$ 0.95	39.00 $\pm$ 0.93	0.72
Birth weight (g, mean $\pm$ SD)	3,409.66 $\pm$ 411.22	3,328.57 $\pm$ 850.03	0.8
Apgar score at 1 minute	9.08 $\pm$ 1.62	7.95 $\pm$ 1.84	0.05
Apgar score at 5 minutes	9.45 $\pm$ 1.40	8.00 $\pm$ 0.93	0.005

FIGURE 1. Correlation between gestational age and birth weight in the premature group

There were no statistically significant differences in mean values of the 5-minute Apgar score between the control (mature) and the premature groups ($p = 0.07$). As expected, the premature infants presented a significantly lower Apgar score at 1 minute compared to controls (7.95 ± 1.83 versus 9.08 ± 1.61 , $p = 0.01$ (Table 2).

While assessing the newborns' postnatal outcomes in presence of maternal smoking, we observed that smoking influenced the Apgar score at 1 and 5 minutes in the two sub-groups of preterm infants (smoking vs. non-smoking: 7.10 ± 2.07 vs. 8.72 ± 1.19 , $p = 0.01$ at 1 minute, and 8.4 ± 0.84 vs. 9.27 ± 0.78 points, $p = 0.02$ at 5 minutes) (Figure 3).

This statistically significant difference was not observed in case of the control versus cardiac groups divided in sub-groups according to smoking habits. The incidence of ma-

ternal smoking in the control group was significantly lower than in the premature + cardiac pooled groups. Maternal smoking was significantly more frequent in case of infants who were premature or born with cardiac congenital anomalies compared to controls ($p = 0.035$, $RR = 2.29$), as shown in Figure 4.

Lifestyle- and health-related parameters, including the presence of smoking, dietary supplements, everyday stress, and maternal pathology are presented in Table 3.

Maternal pathology at admission included anemia, gestational edema, myopia, thrombophilia, thrombocytopenia, lupus erythematosus, obesity, respiratory viral infection. In the premature group, preeclampsia was present in one case and non-obstructive hypertrophic cardiomyopathy in one case.

FIGURE 2. The Apgar score at 1 minute in the newborns of the control (mature) group versus the cardiac group

FIGURE 3. The Apgar score at 1 minute in the premature newborns of smoking vs. non-smoking mothers

FIGURE 4. Incidence of maternal smoking in the control vs. pathology group (premature and cardiac)

DISCUSSIONS

Pregnancy is characterized by increased oxidative stress and lipid peroxidation compared with the non-pregnant state.¹⁷ A diet rich in fruits, vegetables, and vitamins with antioxidant capacity can counteract the oxidative damage. In their study, Mathews *et al.* conclude that dietary antioxidant intake during pregnancy is inadequate.¹⁸ Alberti-Fidanza *et al.* found that there is a transient imbalance between higher antioxidant requirements and intake during pregnancy. This is evolving gradually and progressively, regardless of changes in maternal diet.¹⁹

Preterm neonates have an immature antioxidant defense system, so we can assume that most of the complications are

related to this immature antioxidant defense mechanism in association with an immature immune system and insufficient fetal maturity. It has been found that neonates exhibit higher oxidative stress than the mother herself during labor.⁷

According to a study by Arguelles *et al.*, newborns of smoking mothers had reduced antioxidant capacity and increased risk for lipid and protein peroxidation due to increased oxidative stress. The authors did not find statistically significant differences in the concentration of oxidative stress biomarkers during different birth types. They also concluded that even if the former smoker mother did not smoke during pregnancy, her oxidative stress state was increased, and there was a 14.3-fold higher possibility for lipid peroxidation. The mother's increased oxidative state produced by smoking showed a similar increase in the neonate's oxidative state. In accordance with other authors, the consequences of smoking impact fetal development, leading to intrauterine growth retardation.⁴ Although our study did not have the power to demonstrate significant differences regarding weight at birth among different subgroups related to smoking status, it does not mean that smoking had no effect on fetal development. The exact assessment of the consequences of smoking on fetal development is to determine the parameters of oxidative stress and its effects on lipid peroxidation. However, our study showed that smoking had a negative effect on the Apgar score, which was lower in the subgroup of smoking mothers from the premature group. An association of the two risk factors (prematurity and smoking) may aggravate the postnatal outcomes of the newborn.

TABLE 3. Baseline characteristics of the study groups – maternal history

Characteristics	Control group (n = 60)	Premature group (n = 21)	Cardiac group (n = 8)
Maternal smoking*			
Yes	16 (26.66%)	9 (42.85%)	4 (50.00%)
No	37 (61.66%)	8 (38.09%)	4 (50.00%)
No response	7 (11.66%)	4 (19.04%)	0 (0.00%)
Dietary supplement*	51 (85%)	14 (66.67%)	3 (37.50%)
Yes	35 (58.33%)	10 (47.61%)	7 (87.50%)
No	16 (26.66%)	7 (33.33%)	1 (12.50%)
No response	9 (15.00%)	4 (19.04%)	0 (0.00%)
Stress*			
Yes (low/medium/high)	28/14/3 (75%)	11/4/0 (71.42%)	4/1/0 (62.50%)
No	7 (11.66%)	5 (23.80%)	2 (25.00%)
No response	8 (13.33%)	1 (4.76%)	1 (12.50%)
Maternal pathology			
Yes	35 (58.33%)	18 (85.71%)	7 (87.5%)
No	25	2	1

* Exclusion criteria to be included to statistical evaluation: no response was given

According to Karacor *et al.*, oxidative stress markers were elevated in oxytocin-induced labor, but this had no effect on the Apgar score.¹⁷ Our study also included several oxytocin-induced deliveries. According to Negi *et al.*, intrauterine growth retardation associated with oxidative stress has a negative effect on the development of fetal antioxidant defense mechanisms. They also showed that non-enzymatic antioxidant (e.g., vitamin A, E, C) levels in the cord blood of preterm low-birth-weight newborns were significantly lower compared with full-term newborns. According to their observation, these micronutrients can transform oxidative status and thus may extend the pregnancy to full term, or may prevent lipid, protein, and DNA damage.²⁰ We did not observe this protective effect of the antioxidant dietary supplements in our study group. The observation of Negi *et al.* was in accordance with the one made by Howlander *et al.*, who also concluded that there were increased levels of lipid peroxides and a decrease in the concentration of antioxidants such as vitamin C in case of preeclampsia.²¹

Knowing these facts, our exclusion criteria were based on excluding subjects from the control group with preexisting pathology that can enhance oxidative stress (hypertension, diabetes mellitus, hypothyroidism) in pregnancy, when oxidative stress is proportionally higher compared to non-pregnant status.

Study limitations

This study has the limitation of a small sample size ($n = 21$) in the premature group, and the association needs further investigation on a larger scale. Another limitation of the study is that data regarding smoking status was obtained by questionnaires filled in by the pregnant women, and there might be differences between the reported number of smoked cigarettes and their real smoking habits.

CONCLUSIONS

The Apgar score was lower in case of combined risk factors (smoking and poor antioxidant mechanisms). We also observed that smoking had a negative effect on the outcome of the newborn in case of the premature and cardiac groups. The prevention of smoking during pregnancy could be beneficial in reducing fetal exposure to increased oxidative stress.

CONFLICT OF INTEREST

Nothing to declare.

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