# Comparing maternal and neonatal prooxidant-antioxidant balance during delivery

Maryam Zakerihamidi[1], Gholamali Maamouri[2], Sedigheh Ayati[3], Abbas Boskabadi[2], Fatemeh Bagheri[4], Farnaz Kalani[2], Hassan Boskabadi[2], Nafiseh Pourbadakhshan[2], Ali Moradi[5,6]

[1]Department of Midwifery, School of Medical Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
[2]Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Department of Obstetrics and Gynecology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Nursing, School of Medical Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[5]Orthopedic Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran
[6]Clinical Research Development Unit, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

## *Abstract*

**Objective:** Oxidative stress (OS) is due to a disturbance in the balance between the production of free radicals and antioxidant defense, resulting in a predominance of free radicals over endogenous anti-oxidant defenses. OS may have many causes. Pregnancy, and especially delivery, are associated with increased OS. The relationship between maternal and infant prooxidant-antioxidant balance (PAB) is unclear. Therefore, the aim of the present study was to compare PAB in mother and baby pairs.

**Material and Methods:** This cross-sectional study was conducted in 104 mothers and normal term infants during 2017-2020. PAB was measured in healthy mothers before delivery and in umbilical cord samples after delivery. Data on the infant characteristics including age, gestational age, birth weight, Apgar score, and maternal history including the duration of mother's education, weight of the last month, and gravidity were collected using a researcher-made questionnaire. The cord and maternal PAB were compared by statistical methods.

**Results:** In this study, the mean PAB of the neonates and mothers was 30.76 and 214.87 HK, respectively. The results revealed a moderate association between the PAB neonate and maternal PAB before delivery but it was not significant.

**Conclusion:** Overall, the level of oxidants and antioxidants reduced during pregnancy and before delivery, and it was found that the relative incidence of neonatal PAB increases by increasing maternal PAB. (J Turk Ger Gynecol Assoc 2023; 24: 92-6)

**Keywords:** Neonatal PAB, prooxidant-antioxidant balance, mother, delivery, umbilical cord

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## Introduction

Free radicals are atoms or molecules that have one or more unpaired electrons and are thus highly reactive. Oxidation is a chemical reaction in which an electron or hydrogen ion is transferred from one molecule to another, leading to unstable products called free radicals that can trigger chain reactions at a cellular level and cause death or cell damage (1,2).

The human body has developed protective systems to ameliorate free radical damage through the "antioxidant defense system". This system includes enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (3,4).

Oxidative stress (OS) is defined as a disturbance in the balance between the production of free radicals and antioxidant defenses, and cellular damage occurs when these defenses

***Address for Correspondence:*** *Hassan Boskabadi*
*e.mail: boskabadih@mums.ac.ir ORCID: orcid.org/0000-0003-4022-5426*

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cannot neutralize active oxygen species. OS may damage any biological molecule, including proteins, lipids, and DNA, and may also disrupt cellular signaling systems (5,6).

Pregnancy is associated with biochemical changes in the body and leads to the absorption of oxygen by increasing energy demands and thus tends to increase OS through the production of oxygen-free species (2,7). During the labor phase, severe maternal pain, muscle contractions, and high oxygen uptake can increase the production of free radicals (2,8). In addition, the release of inflammatory mediators, such as arachidonic acid metabolites and interleukin 6, which are involved in the onset of natural delivery, can increase the production of free radicals (9). It should be noted that deficiency of antioxidant vitamins (vitamins C and E, and beta-carotene) may increase the level of oxygen-free radicals (10). Increased OS during pregnancy can endanger the health of the fetus and the mother (1,11). In certain conditions, the production of free radicals exceeds the antioxidant capacity and may damage fetal tissues and organs, including lungs, brain, and retina, and thus threaten the life or affect quality of life of the baby (5,12).

It has been suggested that the antioxidant capacity of the umbilical cord blood and a placental barrier is sufficient for protecting the fetus from oxidative damage caused by the increased OS in pregnant women with preeclampsia (13). OS impairs placental vascular function and adverse outcomes in prenatal periods (14). It has been reported that increased OS may occur following the contraction of skeletal and smooth uterine muscles during normal vaginal delivery compared to cesarean delivery (15). However, there is no relationship between the prooxidant-antioxidant balance (PAB) and the type of delivery (16). It is critical to maintain a balance between the production of oxygen-free radicals and the functional failure of the fetal and neonatal antioxidant system during, before, and after delivery (17).

Although the antioxidant activity during natural pregnancy is determined by many factors including genetics, levels of antioxidant vitamins (maternal diet), maternal body habitus and co-existent pathologies, antioxidant concentrations may be insufficient to respond to the increased production of free radicals. Many pregnancy-related complications, such as fetal death, intrauterine growth retardation, and preeclampsia, occur due to insufficient maternal antioxidant protection to balance the high production of oxygen-free radicals and fat peroxidation in the placenta (18). All tissues, especially the placenta and fetus, require greater oxygenation during pregnancy. Although free oxygen species produced by the mother and the fetus promote the growth and development of fetal cells, its increase and imbalance with the creation of OS causes changes in the structure of cells, and thus causes damage with harmful effects on fetal and maternal cells (1,19). Neonatal antioxidant status indicates a decrease in the concentration of glutathione peroxidase, superoxide dismutase, beta-carotene, riboflavin, alpha proteinase, vitamin E, copper, zinc, transferrin, and other plasma factors (20).

To the best of our knowledge, no previous study has compared maternal and neonatal PAB. Given the importance of this balance in maternal, infant, and delivery health, the current study compared PAB in the mother and the infant.

## Material and Methods

This cross-sectional study was performed in 104 healthy mothers and neonates during 2017-2020 after receiving informed consent from participants. In this study, PAB in healthy mothers and newborns, via cord blood sampling was evaluated. Exclusion criteria were mothers with diabetes, preeclampsia, hypertension, and other complication during pregnancy and newborns with congenital anomalies.

A checklist was compiled to obtain the required data, including newborn characteristics (birth weight, length, age, head circumference, gestational age, and first minute Apgar score) and maternal history (maternal duration of education, maternal weight in the last month and gravidity). PAB was measured in umbilical cord blood samples from neonates and mothers before delivery. At least 0.2 mL of serum was taken from the umbilical cord blood and was sent to Bu-Ali Research Institute to measure PAB through a cold chain.

The amount of antioxidants was investigated by 3.3-3.5 tetramethylbenzidine (TMB) staining and oxidation of colored cations. The standard solution consisted of 250 $\mu$mol of hydrogen peroxide and 3 millimoles of uric acid per 10 millimoles of NaOH.

To make the TMB cation, 60 mg of TMB powder was dissolved in a ratio of 10 mL to 20 mL of the solution and mixed well. Then, it was placed in a dark and dry container for two hours. Afterward, 25 units of peroxidase enzyme were added to 20 mL of the solution which is distributed in each milliliter of the solution and kept at 20 °C.

Subsequently for reagent preparation, 200 mL of TMB was added to 10 mL of acetate (0.05 mol, pH=5.8). The prepared solution was mixed with 1 mL of TMB cation and 10 mL of TMB solution, respectively. Next, it was kept in a dark and dry place for two minutes. In addition, 10 $\mu$L of each sample was mixed as the standard with 200 $\mu$L of the working solution and placed in 96 plates in a dark environment at 37 °C for 12 minutes. Then, 100 $\mu$L of 2N hydrochloric acid was added to each well. Color development was measured at 450 and 620 nm wavelengths.

From standard samples, a standard curve was created representing the value of PAB in HK units and the role of hydrogen peroxide in the standard solution.

This work was approved by the ethical and scientific committees of the School of Medicine, Mashhad University of Medical Sciences (approval number: IR.MUMS.MEDICAL.REC.1398.375).

**Statistical analysis**

PAB values of samples were read from the standard curve created for each assay. The data were analyzed using SPSS, version 22 (IBM Inc., Armonk, NY, USA). Further, parametric and non-parametric variables were measured by the correlation method and the correlation coefficient with statistical tests appropriate for the type of the variable. Finally, the central index and the paired samples test and Pearson paired samples correlation test were used. A p-value less than 0.05 was considered to indicate significance.

## Results

In the present study, 16 neonates were excluded from the evaluation due to lysis of the laboratory sample. The mean gestational age and 1-minute Apgar score were 39.65±0.8 weeks and 9.4±0.7, respectively. The other characteristics of neonates and mothers are presented in Table 1.

Neonatal PAB increased as maternal PAB increased (Figure 1). In this study, the mean PAB of neonates was 30/76 HK while that of mothers was 214/87 HK. According to Pearson's correlation test between neonatal and maternal PAB before delivery, there was a moderate association although it was not significant (Figure 1, r=0.135, p>0.05).

## Discussion

In the present study, the mean maternal PAB during delivery was 215 HK while in another study, the means of maternal PAB in non-pregnant and pregnant women in the first trimester and the labor phase were reported 129, 168, and 221 HK, respectively (2). In the first trimester of pregnancy, placental tissue contains low concentrations and activities of antioxidant enzymes. Therefore, trophoblastic cells are poorly protected from oxygen-related injuries (21). In the second trimester, when the oxygen pressure in the intervillous space increases three-fold, the OS in the placenta increases significantly. Thus, oxidative damage changes the function of the placenta, affecting the later stages of pregnancy (22). Abnormalities of the placenta may lead to OS, and endothelial dysfunction plays a key role in the development of pregnancy complications such as recurrent miscarriage and preeclampsia (21). The consumption of antioxidant supplements by at-risk women for preeclampsia is associated with improved antioxidant activity (23). The results of previous research demonstrated a significant reduction in vitamin E levels during labor compared with the postpartum period in mothers and neonates, indicating an increase in OS during delivery (24). Therefore, there appears to be an increase in PAB in pregnancy and a peak before delivery. The PAB balance is easily disrupted during labor and practitioners should ensure that mothers maintain a proper balance during pregnancy, especially during labor. In the present study, the mean PAB of neonates was equal to 31 HK. The findings of a study comparing PAB levels in neonates with and without asphyxia indicated mean PAB levels were 20 and 40 HK in healthy neonates and neonates with asphyxia, respectively (5). In two other studies, the mean PAB of neonates was reported to be about 40 HK after jaundice recovery (25,26). A study investigated the effects of selenium consumption, critical to the function of some anti-oxidant enzymes, during pregnancy on the PAB levels of neonates. The mean PAB level in the neonates of the control group was 31 HK (27). Based on the results of the present study, the mean

**Table 1. Mean comparison of the characteristics of mothers and neonates**

| Variable | Mean ± SD |
|---|---|
| Birth weight (gram) | 3239.06±417.245 |
| Height (cm) | 50.83±1.822 |
| Head circumference (cm) | 34.67±0.944 |
| Gestational age (week) | 39.65±0.813 |
| First minute Apgar score | 9.43±0.720 |
| Maternal length of education (year) | 9.3±1.542 |
| Maternal weight of the last month (kg) | 68.78±4.785 |
| Gravidity | 2.35±1.147 |
| Neonatal PAB (HK) | 31.276±52.975 |
| Maternal PAB before delivery (HK) | 214.87±39.96 |
| SD: Standard deviation, PAB: Prooxidant-antioxidant balance | |

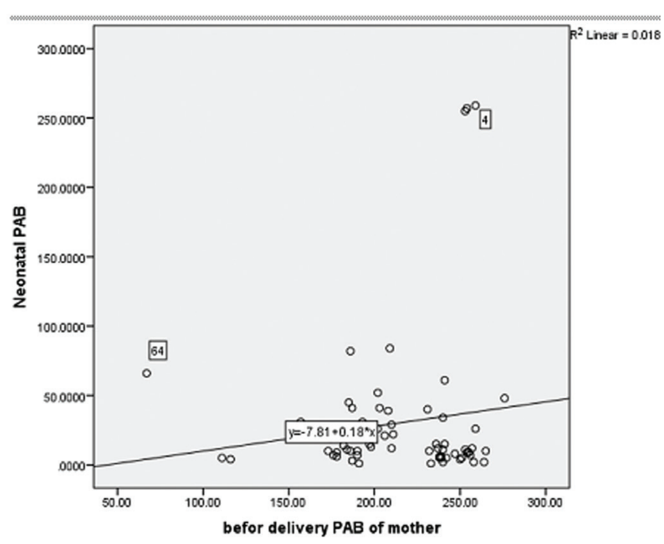


**Figure 1. The correlation between maternal PAB before delivery and neonatal PAB**
***PAB: Prooxidant-antioxidant balance***

neonatal PAB was one-sixth of maternal PAB and there was a moderate association between neonatal and maternal PAB before delivery although it was not significant.

Levels of vitamins A, E, and beta-carotene have been reported to be lower in the umbilical cord plasma of neonates compared to maternal plasma. This is due to low levels of lipophilic molecules in the umbilical cord which limits the transmission capacity of the umbilical cord plasma for tocopherols and carotenoids (28). OS is found to play a role in the pathogenesis of many neonatal conditions, including asphyxia, retinopathy of prematurity (ROP), and bronchopulmonary dysplasia. However, PAB may be disturbed and OS increased due to the immaturity of the fetal antioxidant system. Premature neonates frequently need extra oxygen, and rapid changes in tissue oxygen levels from low intrauterine to high levels, especially if sudden, can lead to the decreased vascular endothelial growth factor. This results in impaired vascularization of the retinal vessels and may lead to ROP (29).

There is a critical and sensitive balance in the cell between free radical formation and antioxidant defense and repair systems, as there is a normal physiological balance between antioxidants and peroxides in healthy cells, when free radicals are produced by normal cellular function but neutralized by the antioxidant system. This system can pass through the placenta, and the highest amounts of antioxidants are transferred in the third trimester of pregnancy (2,5).

In the present study, the mean value of PAB (measured in HK units) in neonates was about one-sixth of the level in the mothers. Thus this system appears to be inadequate in neonates. However, neonates are exposed to increased OS in high-risk pregnancies and deliveries including premature rupture of membrane and preeclampsia, and in these cases, the lower levels of natural PAB may be inadequate to protect fetal tissues.

## Conclusion

Pregnancy and labor change the PAB by increasing oxygen consumption and energy demands. We suggest that more care should be provided for mothers in order to maintain a proper balance during pregnancy, especially during delivery when PAB increases and reaches its peak before labor. The mean PAB of neonates was found to be one-sixth of its level in mothers. The low levels of PAB in neonates predispose them to greater vulnerability compared to their mothers.

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***Informed Consent:*** *It was obtained.*

***Peer-review:*** *Externally peer-reviewed.*

***Author Contributions:***

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