

Maternal Parity and Blood Oxidative Stress in Mother and Neonate

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Abstract

Background: Parturition has been associated with free radicals, itself linked with poor pregnancy outcome.

Objectives: This study aimed to investigate the relationship between oxidative stress biomarkers levels of maternal and cord blood samples at the second stage of labor with the maternal parity number.

Materials and Methods: In this analytical cross-sectional study, subjects were selected from Fatemeh teaching hospital, Hamadan, Iran, and allocated into the two groups according to their number of parity: the primiparous group (n = 33), and multiparous group (n = 35). Maternal and umbilical cord blood samples were taken from all subjects and then assessed for catalase activity (CAT), total thiol molecules (TTM) and total antioxidant capacity (TAC).

Results: Total antioxidant capacity levels were significantly higher in newborns of primiparous women compared to multiparous women (P = 0.006). The CAT levels were significantly lower (P = 0.04) and TAC levels significantly higher (P = 0.03) in maternal plasma of primiparous women compared to those of multiparous women.

Conclusions: Increment in the number of parity can lead to decrease antioxidant defense mechanisms in multiparous women and their newborns. So, control of oxidative stress is considered to be beneficial in multiparous women.

Keywords: Oxidative Stress, Antioxidants, Parity

1. Background

Oxidative stress defined as a disbalance between the production of free radicals and antioxidant defenses resulting in an oxidative stress (1). Parturition is associated with physiological changes that increased production of free radicals. The increase on the oxygen intake and use of it during pregnancy and parturition results in the formation of oxygen free radicals and changes in the oxidants levels and antioxidant defense system, which eventually causes oxidative stress (1). Studies show that oxidative stress is involved in normal pregnancies, even in the absence of complications (2). The serum levels of oxidative stress have been found higher in pregnant women than in nonpregnant women (3).

There is growing evidence that parturition may be involved in strong oxidative stress for both mother and neonate. During the progression of labor, powerful contractions of the uterine myometrium resulting in increased intrauterine pressure periodically and increase in suppress uteroplacental blood flow (4) and lead to appearance of cycles of ischemia and reperfusion (5). Hypoxic intrauterine and other physiologic processes involved in the termination of pregnancy and delivery are other reasons for this situation of oxidative stress (6).

Fetuses exposed to oxidative stress in the intrauterine life has been associated with increment intrauterine fetal death (7), poor perinatal outcome (8), increased risk of birth asphyxia (2). oxidative stress lead to vascular dysfunction in the placenta (9).

Though, it is well-known that oxidative stress biomarkers affect gestation, fetus and newborn negatively but the effect of maternal number of birth on these stress biomarkers is not obvious. With regard to stress due to pregnancy and childbirth, it can be assumed that free radicals may be generated more in primiparous women than multiparous women. In multiparous women, increasing parity may be complicated the child bearing (10). We hypothesize that increasing the number of maternal parity likely lead to increased levels of oxidative stress biomarkers. This increment may affect the outcome of the pregnancy and delivery in multiparous women.

2. Objectives

The aim of this study was to measure catalase activity (CAT), total thiol molecules (TTM) and total antioxidant capacity (TAC), in the blood of mothers (primiparous and

multiparous) and cord blood of their newborns at the second stage of labor, in order to evaluate the relationship between blood oxidative stress in mothers and their newborns with maternal parity number.

3. Materials and Methods

This analytical cross-sectional study was carried out from January 2015 to June 2015, in Fatemeh teaching hospital, Hamadan, Iran. Sixty-eight healthy mothers aged 17 - 39 years and their newborns were recruited into the study.

Sample size formula:

$$(1) \quad n = \frac{Z^2_{1-\frac{\alpha}{2}} \times P(1-p)}{d^2}$$

Where $n=51=68$, $P=0.05$, $\alpha=0.05$, $d=0.06$.

Subjects were divided into two groups according to their parity number: the primiparous group ($n=33$) (primiparous women), and multiparous group ($n=35$) (multiparous were the women whose having borne more than one child). Inclusion criteria included mothers who had singleton pregnancies, had gestational age between 37 - 42 weeks, normal-weight of fetus and finally Apgar scores of ≥ 9 at the first minute. Exclusion criteria included mothers who delivered via emergency cesarean section, who had a prolonged labor (total duration of the first and second phases of delivery exceeded 18 hours or whose second stage of labor exceeded 2 hours), had gestational complications such as oligohydramnios, preeclampsia, infection, hypertensive, gestational diabetes or chronic diseases active or passive smokers, had taken any medication during pregnancy. Informed consent was obtained from the mothers after the nature and objectives of the study were explained to them and were fully understood before blood samples were taken. Women could leave the study at any time.

The study was performed according to the Helsinki declaration protocol and Good clinical practice guidelines. Ethical clearance was gotten from ethical and research committee of Hamadan University of Medical Sciences, Hamadan, Iran. Trial registration code of project was IRCT201502266888N7.

3.1. Blood Sampling

At the start of expulsion when the fetus was at station +2, maternal vein blood sample was taken from the ante-cubital vein into 5-cc plastic syringes. Immediately after delivery a segment of umbilical cord was double clamped and blood was drawn from umbilical vein into 5 cc plastic syringes. Venous and umbilical cord blood were poured into lithium heparin containing specimen bottles. The samples were centrifuged at 2,000 g for 15 minutes and extracted plasma was poured into plain specimen tube. The plasma was immediately kept frozen and stored at

-80°C until laboratory analysis.

This plasma was used for the laboratory measurement of total antioxidant capacity (TAC), catalase activity (CAT), and total thiol molecules (TTM).

3.2. Reagents and Chemicals

The 2-thionitrobenzoic acid (DTNB), ethylenediamine tetraacetic acid (EDTA), 2, 4, 6-tripyridyl-s-triazine (TPTZ) and peroxide hydrogen (H_2O_2) were used in this study. All other chemicals were obtained from the Sigma.

3.3. Oxidative Stress Biomarkers

3.3.1. Measurement of Catalase Activity

Catalase activity is defined as the number of micro-moles of hydrogen peroxide decomposed in 5 minutes at 0°C per mL. Catalase activity was assayed in the samples by measuring the absorbance decrease at 240 nm in a reaction medium containing H_2O_2 (10 mM), sodium phosphate buffer (50 mM, pH = 7.0). One unit of the enzyme is defined as 1mol H_2O_2 as substrate consumed/min, and the specific activity is reported as Unit/mL plasma. CAT activity methods assay is the best method in biological samples such as urine, plasma, serum, blood, homogenate tissues, and its scale is unit/mL or U/mL (12). One mL reaction mixture contains 0.05 M potassium phosphate buffer (pH 7), 11.6 mM H_2O_2 and 50 mL of enzyme.

3.3.2. Assay of Total Antioxidant Capacity

It was measured by the ferric reducing ability of plasma (FRAP) method. The FRAP assay was carried out as described by Benzie and Strain. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of three pyridyl triazin (TPTZ). The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (11). Twenty-five mL of 300.0 mmol/L acetate buffer, 2.5 mL of 10 mmol/L TPTZ solution, and 2.5 mL of 20 mmol/L $FeCl_3$ solution, in a 10:1:1 ratio. 10 μ L of sample was mixed with 200 μ L of FRAP reagent; the contents were mixed vigorously. Ferric tripyridyltriazine (Fe^{III} -TPTZ) complex is reduced to ferrous tripyridyltriazine (Fe^{II} -TPTZ) form in the presence of antioxidants and develops an intense blue color, with maximum absorption at 593 nm.

3.3.3. Assay of Total Thiol Molecules

To evaluate the plasma total thiol molecules, DTNB was used as a reagent. The 2-thionitrobenzoic acid reacts with thiol molecules and creates a yellow complex, which has good absorbance at 412 nm in a spectrophotometer (12).

We added one mL of Tris the buffer to the 50 mL of plasma into a test tube and then read its optical absorption in the wavelength 412 nm in front of the blank (1 mL of Tris buffer) was read (A1). Then added to the tubes 20 mL of the DTNB reagent and was kept for 15 minutes at room

temperature and then its optical absorption was read (A2). The control tube containing Tris buffer and DTNB (without sample) that its optical absorption in the wavelength 412 nm was read (B). Obtained values of A1, A2, B was inserted in the formula to compute the thiol groups.

3.4. Statistical Analysis

All data analyses were performed using SPSS software version 16.0, $P < 0.05$ was accepted as significant. In order to evaluate the normality of distributions, one-sample Kolmogorov-Smirnov test was used. Continuous and categorical variables were displayed as means \pm SD and percentages separately. The chi-square test was used to compare the two groups in terms of demographic characteristics. The comparisons of TAC, CAT, and TTG, which showed normal distribution, were performed using an independent t-test.

4. Results

Kolmogorov-Smirnov test showed that the distribution of data for each variable is normal ($P > 0.05$). Table 1 show the mean maternal age, mean birth weight in the two

study groups. The chi-square test showed that the two parturient groups were comparable in terms of baseline characteristics such as age ($P = 0.26$), newborn weight ($P = 0.62$), Gestational age ($P = 0.7$), first stage of labor duration ($P = 0.1$), second stage of labor duration ($P = 0.3$), third stage of labor duration ($P = 0.8$), Apgar score 1st minute ($P = 0.6$), Apgar score 5th minute ($P = 0.5$).

Table 2 demonstrates the mean \pm SD (95% confidence interval) of oxidative stress biomarkers in plasma and umbilical cord samples of the two groups. The P value obtained from the independent t-test showed that there was a significant difference between TAC levels of newborns cord of the two groups ($P = 0.006$). According to the mean of the two groups, the TAC level was significantly higher in newborns' cord blood of primiparous women compared to that in multiparous ones.

The P value obtained from the independent t-test showed that there was a significant difference between TAC levels of maternal blood of the two groups ($P = 0.03$). According to the mean of the two groups, the TAC level was significantly higher in maternal plasma of primiparous women compared to that in multiparous (Table 2 and Figure 1).

Table 1. Baseline Characteristics and Clinical Data of Study Population^a

	Primiparous (n = 33)	Multiparous (n = 35)	P Value
Age, y	16 - 33	20 - 39	0.26
Mean \pm SD	22.7 \pm 4.34	29.86 \pm 5.49	
Birth weight, g	3380 \pm 0.504	3296 \pm 0.464	0.62
Gestational age, wk	38.2 \pm 0.6	39 \pm 0.1	0.7
Labor duration			
First stage of labor, min	147.48 \pm 80.54	115.37 \pm 42.11	0.1
Second stage of labor, min	58.42 \pm 40.42	46.54 \pm 22.21	0.3
Third stage of labor, min	8.15 \pm 4.83	7.91 \pm 4.69	0.8
Apgar score			
Apgar score, 1st min	8.82 \pm 0.5	8.55 \pm 0.7	0.6
Apgar score, 5th min	9.56 \pm 0.6	9.28 \pm 0.8	0.5

^aValues are expressed as mean \pm SD.

Table 2. Oxidative Stress Biomarkers in Maternal Plasma and Umbilical Cord Samples of Two Groups

Variables	Primiparous (n = 33)	Multiparous (n = 35)	P Value
TAC, mmol/mL			
Umbilical cord	0.61 \pm 0.13	0.45 \pm 0.21	0.006
Maternal plasma	0.68 \pm 0.24	0.54 \pm 0.13	0.03
Catalas activity, mmol/mL			
Umbilical cord	0.33 \pm 0.25	0.28 \pm 0.15	0.26
Maternal plasma	0.15 \pm 0.16	0.39 \pm 0.31	0.04
Thiol groups, mmol/mL			
Umbilical cord	0.38 \pm 0.16	0.28 \pm 0.44	0.9
Maternal plasma	0.48 \pm 0.17	0.59 \pm 0.49	0.4

The P value obtained from the independent t-test showed that there was a significant difference between the CAT levels of maternal plasma of the two groups ($P = 0.04$). According to the mean of the two groups, the CAT level was significantly higher in maternal plasma of multiparous women compared to that in primiparous (Table 2 and Figure 2).

The P value obtained from the independent t-test showed that there was no significant difference between the TTG ($P = 0.9$) and CAT ($P = 0.26$) levels of newborns' cord blood between the two groups. The P value obtained from the independent t-test showed that there was no significant difference between the TTG of maternal plasma with maternal number of parity ($P = 0.4$). Results of the current study revealed that the TTG was decreased in maternal plasma of primiparous women compared to that of multiparous; however, it was not significant ($P = 0.4$) (Table 2 and Figure 3).

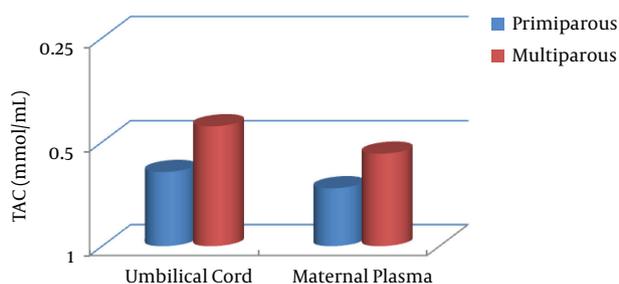


Figure 1. Total Antioxidant Capacity (mmol/mL) in Maternal Plasma and Umbilical Cord Samples of the Two Groups

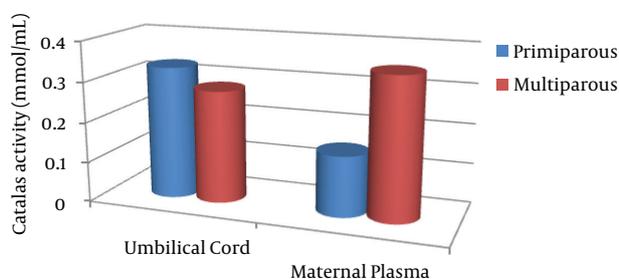


Figure 2. Catalase Activity (mmol/mL) in Maternal Plasma and Umbilical Cord Samples of the Two Groups

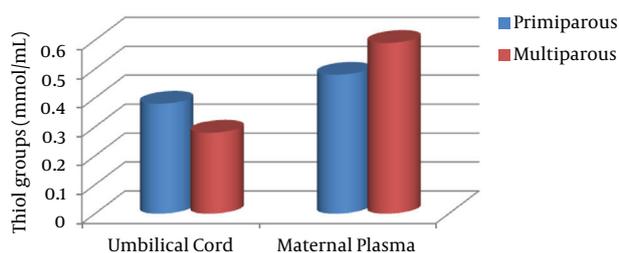


Figure 3. Thiol Groups (mmol/mL) in Maternal Plasma and Umbilical Cord Samples of the Two Groups

5. Discussion

Oxidative stress in biology and medicine occurs when production of oxidants overcome to the existent antioxidants or when antioxidants in the system are low (13). Dominance of antioxidants can cause fetal and maternal complications during pregnancy and parturition. So, in order to prevent tissue damage that caused by oxidative stress, maintaining balance between oxidant and antioxidant biomarker is very important (14). According to studies, several factors contribute to oxidative stress during delivery. Myometrium contractions during delivery increase oxygen and energy consumption and lead to increased production of free radicals (15). Some studies have shown the relationship between the number of pregnancies and problems such as diabetes mellitus, preeclampsia, anemia, intrauterine fetal death and risk of premature birth (16, 17). These problems may be caused by the increment of the exposure of the multiparous women and their newborn to oxidative stress and the defense system consisting of antioxidants. This study observed significant differences in the maternal plasma levels of TAC and TTG and CAT among the two studied groups. It has been reported that antioxidant capacity is lower in newborns of multiparous women than those of primiparous women, and oxidative stress indicators are decreased in the newborns of primiparous women (18). So, we compared the antioxidant capacity and oxidative stress biomarkers in maternal plasma and cord blood of the newborns of primiparous women and multiparous women. Review of the literature revealed few studies about relationship between multiparity and oxidant and antioxidant systems. In This study, we compared the antioxidant and oxidative stress biomarker levels in maternal plasma and umbilical cord blood of the newborns among multiparous women and primiparous women. One study showed that TAC levels are significantly higher in newborns of primiparous women compared to multiparous (18). Our finding of the increased TAC in cord blood of the newborns of primiparous women supports these findings.

According to the results of current study, TAC levels of maternal plasma in multiparous women were lower than those of primiparous women and also the CAT levels of maternal serum in multiparous women were higher than those of primiparous women. This indicates that the antioxidants are depleted and oxidative stress indicators are increased after the first pregnancy. A literature search revealed no research concerning the effects of maternal parity on oxidative stress. However, some studies have shown that complications such as hypertension, diabetes mellitus, preeclampsia, anemia, intrauterine fetal death and risk of premature birth were increased with increasing the number of parities (19), which can be caused by the diminishing of the antioxidant defense system.

It has been reported that oxidative stress indicators levels (total oxidant status (TOS) and oxidative stress in-

dex (OSI) were higher in the newborns of multiparous women compared to those of primiparous women (18). We found no significant differences in oxidative stress indicators levels (TTG and CAT) of newborns' cord blood between the two groups. This difference may be due to differences in oxidative stress biomarkers investigated in two studies. So, more studies with larger sample sizes are recommended.

Many studies have shown no significant difference in birth weight among multiparous and primiparous women; however, some studies have shown that increasing the number of parity and pregnancy associated with birth weight gain (20) complications during pregnancy can lead to low birth weight and stress in newborn (21). In order to exclude the effects of complications during pregnancy on birth weight, we excluded pregnant women that had complications during pregnancy. The neonatal weights were similar in the two groups.

In this study, there was no significant difference in age between the two groups. Some studies have shown that aging after 40 years increases oxidative stress (22). In order to exclude the effects of aging on increasing the oxidative stress (21), we excluded mothers older than 40 years old in our study. Levels of oxidative stress biomarkers in premature infants is higher than term infants (23). In order to exclude the effects of gestational age on increasing the oxidative stress (23), we included pregnant women with gestational age of 37 to 42 weeks in our study. Total labor duration could be effective on antioxidant and oxidant system; so, total stages of labor duration were recorded. There was no significant difference in total duration of stages of labor between the two groups. In conclusion, increasing the number of parity may lead to decrease antioxidant defense mechanisms in multiparous women and their newborns. So, management of oxidative stress is considered to be beneficial in multiparous women. One of the limitations of this study includes the small samples of participants in each group. It is recommended that future studies with larger sample sizes be performed.

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Footnotes

Authors' Contribution: Fatemeh Shobeiri, Faegheh Golalizadeh and Akram Ranjbar carried out the study design, participated in data collection and drafted the manuscript. Mansour Nazari participated in the design of the study and performed the statistical analysis. Fatemeh Shobeiri, Faegheh Golalizadeh, Akram Ranjbar and

Mansour Nazari conceived the study, and participated in its design and coordination.

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