

Maternal Oxidative Stress and Antioxidant Defence during Labour

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Abstract: Pregnancy induces maternal adaptations physiologically to meet the growing demands of the developing fetus. As pregnancy advances, due to increased oxygen demand, there is increased production of free radicals. Oxidative stress is induced in pregnancy due to more production of free radicals and less efficient antioxidant system. This process intensifies during labour and severely affects the maternal antioxidant reserve. The present study was aimed at quantifying the distinguishing changes that accompanies during labour with respect to oxidative stress. 20 healthy pregnant women without any complications were taken as study group and 13 age matched non-pregnant subjects were included as control group. Plasma malondialdehyde (MDA) and antioxidants such as reduced Glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPX) were estimated before and after delivery in plasma and hemolysate respectively using Spectrophotometric method. The results showed significantly elevated plasma MDA levels in study group before and after delivery when compared to the control group ($p < 0.05$). SOD and GSH levels were also significantly elevated before and after delivery when compared to that of controls ($P < 0.001$, $P < 0.05$). However, GPX level was significantly lower than in controls both before ($P < 0.01$) and after delivery ($P < 0.05$). There was also a significant correlation between GPX and maternal age ($r = 0.016$, $P < 0.05$). Other parameters like parity and duration of labour did not show any correlation with MDA and antioxidant enzymes. We conclude that oxidative stress occurs during labour and it can produce alterations in the maternal antioxidant system which can greatly influence the pregnancy outcome.

Keywords: Free radicals, oxidative stress, uncomplicated pregnancy, maternal antioxidant defense.

I. Introduction

Pregnancy is a physiological stress due to hormonal, neuronal and metabolic changes occurring at various levels to meet the growing demands of the fetus. More commonly, the pregnancy-stress gets overwhelmed in the form of oxidative stress at the time of labour. Oxidative stress occurs whenever the balance between the production of reactive oxidizing species (ROS) and the antioxidant defense is disrupted [1, 2]. Normally also transitional and mild oxidative stress occurs in non-pregnant woman. In pregnancy, as a part of respiratory adaptation, there is triggering of aerobic environment that favours oxidative stress [3,4]. An imbalance due to increased lipid peroxidation and decreased antioxidant levels leads to complications of pregnancy [5,6]. During labour, oxidative stress increases several fold because of the repeated uterine contractions leading to ischemia; this is followed by reperfusion, resulting in increased production of ROS [7]. The resultant stress, during spontaneous vaginal delivery is, in turn, influenced by neural and hormonal factors in addition to pain, anxiety, fear and duration of labor [8].

ROS interacts with polyunsaturated fatty acids in membranes or lipoprotein and initiates the process of lipid peroxidation and production of chemicals like peroxidases and free radicals paving way for oxidative stress. Oxidative stress of higher intensity may disrupt the normal functioning of the body starting from the simple cell membrane damage up to the danger of cell death by triggering apoptosis or necrosis [9]. To counteract, body is provided with anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) which can remove the ROS efficiently and protect the body from the adverse effects of oxidative stress [1]. Earlier studies also reported significantly elevated levels of lipid peroxides in pre-eclampsia with decreased antioxidant levels [10, 11, 12]. In dairy cows, susceptibility to periparturient disorders were observed due to increased oxidative stress and reduced availability of antioxidant defense during parturition [13]. The imbalance between the antioxidant defense and oxidative stress in complications of pregnancy and during labour was reported in some of the earlier studies also [14,15].

However, the data regarding the effect of altered antioxidant mechanisms in uncomplicated pregnancy with spontaneous vaginal delivery is scanty and hence an attempt had been made in the present study to assess the impact of oxidative stress on maternal antioxidant defense during labour and correlate it with maternal age, parity and duration of labour.

II. Materials And Methods

This study was conducted on singleton term pregnant women in labour attending the obstetric department for delivery in Sri Ramachandra Medical College and Research Institute (SRMC & RI). Ethical clearance was obtained from the Institutional Ethics Committee. Informed consent and detailed obstetric history were collected from all the participants. 20 pregnant women at term, in the age group of 21-35 years were included in the present study. All of them were categorized for spontaneous vaginal delivery. Pregnancy with any type of maternal complications such as diabetes, hypertension, pregnancy-induced hypertension (PIH), anemia, infections and premature rupture of membranes and fetal complications like fetal distress, abnormal presentation, multiple gestations etc., were excluded. 13 age-matched non pregnant parous women were included as control subjects. When subjects were admitted in the hospital during labour, two samples of 3 ml blood were collected under strict aseptic conditions in EDTA containing sterile vacutainer tubes. Assessment of progress of labour was done every one to two hours. First sample was taken from the mother at the onset of active labour, with at least 4-5 cm dilatation of cervix. Second sample was taken at the end of the labour. EDTA blood sample was also collected once from non-pregnant women who acted as controls. Red blood cells (RBC) were separated from plasma by centrifugation at 3000 rpm at 4°C for 30 min. Supernatant plasma was used for estimation of malondialdehyde (MDA). MDA, a metabolite of lipid peroxidation detectable in plasma, was used as the indicator. Plasma MDA concentrations were estimated as thiobarbituric acid reacting substances (TBARS) by the method of Cynamon et al [16]. The lipid peroxidation activity was expressed as nmol/100 ml of plasma.

RBC were washed 3 times with 0.9% ice cold sterile saline and centrifuged at same speed for 5 min after each wash. Cells were lysed in 4 times volume of distilled water and allowed to stand for one hour for complete hemolysis. It was centrifuged and supernatant fluid (hemolysate) was collected and stored in -80°C until analysis was done. Antioxidant enzymes such as SOD, GPX and total reduced Glutathione (GSH) were estimated in hemolysate at 37°C using ELICO SL 150 UV-VIS spectrophotometer.

The involvement of superoxide anion radical in the autooxidation of pyrogallol was used as a convenient assay of SOD [17]. GSH reacts with 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) to give a coloured compound that is absorbed maximally at 412nm. Total Reduced GSH estimation was done by the method given by Moron et al [18]. GPX activity was measured by its ability to utilize the standard glutathione in the presence of specific amount of hydrogen peroxide (H₂O₂) (1mM) [19]. Antioxidant activity was expressed as U/L. Estimation of lipid peroxides before delivery (B) and after delivery (A) was done in the study group and compared with those of controls. The antioxidant activities of GSH, GPX and SOD were also estimated in B and A and compared with control.

Statistical Analysis: The data were tabulated and analyzed in SPSS (11.5 versions). The results were expressed as mean ± standard error of mean (SEM). The level of significance was kept at p<0.05. Independent 't' tests and ANOVA (Analysis of variance) was used to compare the values between the groups. Pearson's correlation was done to correlate maternal parameters with antioxidant enzymes.

III. Results

Description of the clinical characteristics of the study groups is shown in (Table-1). There was no statistically significant difference in the age between study group (23.95 ± 3.83) and control group (24.23 ± 4.48). Plasma MDA: Compared to the control group, there was a significant increase in the concentration of plasma MDA in both the study groups (B and A) (Table 2). However, there was no significant difference between the two samples of the study group (B and A). Reduced Glutathione (GSH): GSH activity was elevated significantly in Group B, when compared to that of control group. Group A showed non-significant reduction in GSH activity when compared with group B (Table 2).

Table 1: Descriptive statistics of the study groups (n= 20)

Parameters	Mean ± SD
Age (years)	23.95 ±3.83
Parity	1.95 ± 0.94
Gestational age (weeks)	38.1± 1.27
Duration of labour (minutes)	289.6 ± 240.09

GPX activity was found to be reduced significantly in study groups (B and A) compared to that of control group. SOD levels of study groups(from the hemolysate) showed significant increase when compared with controls. However, between the study groups, there was no significant change in SOD levels.

Table 2. Comparison of maternal levels of MDA, GSH, SOD and GPX with control

Variable	Comparison between	Values	Significance
MDA(n Mol/ 100 ml of Plasma)	Control and B	0.361±0.03 & 0.572 ±0.07	P <0.05*
	Control and A	0.361±0.03 & 0.588 ± 0.09	P <0.001*
	B and A	0.572 ± 0.07 & 0.588 ± 0.09	P <0.459
GSH (U/L)	Control and B	12.40±1.17 & 50.04± 7.85	P <0.001*
	Control and A	12.04± 1.17 & 45.67 ±6.26	P <0.001*
	B and A	50.04± 7.85 & 45.67 ±6.26	P <0.804
SOD (U/L)	Control and B	8.058± 1.15 & 35.45±2.26	P <0.05*
	Control and A	8.058± 1.15 & 35.03±2.4	P <0.05*
	B and A	35.45±2.26 & 35.03±2.4	P <0.397
GPX (U/L)	Control and B	84.89±9.54 & 65.51±11.16	P <0.01*
	Control and A	84.89±9.54 & 77.78 ± 8.94	P <0.05*
	B and A	65.51±11.16 & 77.78 ± 8.94	P <0.396

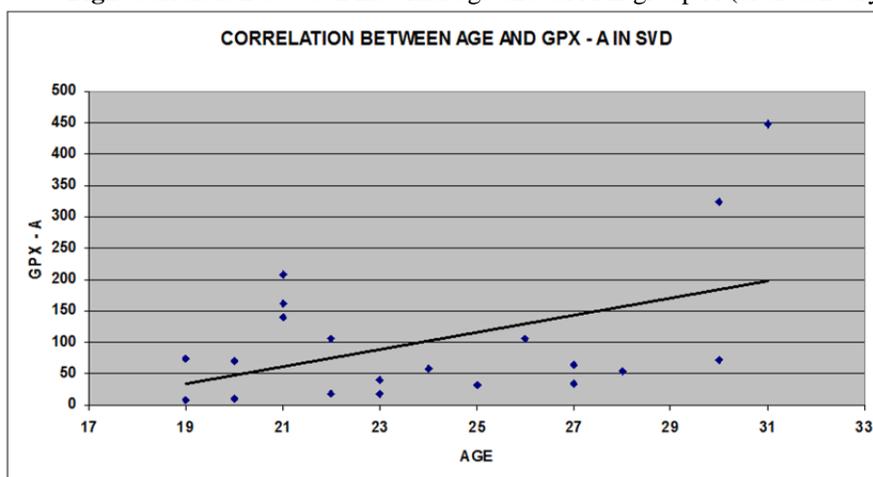
Values are expressed as mean±SEM, * indicates Significance
 B-Before delivery A- After delivery

Maternal age, duration of labour and parity were the parameters correlated using Pearson Correlation with LPO, GSH, GPx and SOD. There was strong correlation between GPx and maternal age in Group A (r= 0.016)

IV. Discussion

Labour is a stressful process for mother and the newborn. The enhanced production of free radicals during labour and their removal by the existing antioxidants imposes a challenge to the mother. Oxidative stress occurs during pregnancy due to lipid peroxidation that is induced in the placenta [11]. Oxidative stress increases during pregnancy because of the increased oxygen requirements of the fetus and the placenta is well equipped with a large number of mitochondria to meet this demand [20]. And also, the antioxidant system was reported to be stronger than peroxidation during pregnancy [12,27]. In normal pregnancy, placental lipid production is controlled by placental antioxidant systems[10]. An imbalance develops in peroxidative and antioxidative status during delivery which may affect the fetus[21]. Oxidative stress is induced by neural and hormonal factors during labour and this may induce the production of high MDA in the newborn if it is not counteracted by maternal antioxidant defense system[22]. Monitoring of lipid peroxidation level is important as it helps in understanding the relationship between oxidative stress and pregnancy outcome. Thus, the antioxidant system of the mother plays a key role in combating the stress induced during labour.

Fig1. Correlation between maternal age and GPX in group A (after delivery)



The results of our present study showed that there was significantly elevated plasma MDA levels in both the study groups (B and A) when compared to controls. Similar observations were reported in previous studies also [23, 24] indicating that labour was associated with increased lipid peroxidation. In dairy cows also it was found that the LPO (Lipid Peroxides) activity was higher after delivery when compared to late pregnancy and increased metabolic demands associated with parturition and initiation of lactation were speculated as the reason for it [25]. Increased production of pro-inflammatory mediators such as prostaglandins and thromboxanes at the onset of labour also was suggested for the increased production of free radicals [26]. The oscillation of oxygen levels during labour contractions followed by tissue reoxygenation was another reason speculated for the increased production of free radicals [27]. Labour pain with physical and psychological stress of the mother was yet another probability for the increased production of MDA [28]. However, plasma MDA levels in group B and group A did not show any significant difference in our present study. This is in agreement with the observation made by Nakai et al [29] and it implies that oxidative stress continues to be present even at post-partum [30]. These biochemical modifications may affect the long term cardiovascular health of the mother with high parity [31].

Glutathione is another potent non-enzymatic antioxidant. It is present in abundance intracellularly and it protects the cells from free radical injury. It also helps to regenerate the stores of other antioxidants like Vitamin C and E. In the blood 99.5% of glutathione is in the RBC to maintain the hemoglobin in reduced form. It plays an important role in maintenance of pregnancy and prevents the oxidative stress that occurs during labour and birth process [32]. The process of labour at term induces up regulation of glutathione in both the maternal and fetal compartment which is highly beneficial [33]. Our study also clearly demonstrated an elevated level of reduced glutathione in groups B and A when compared to control.

The results of our present study also show that SOD activity was elevated significantly before and after delivery, when compared to control. Nakai et al also reported a similar result [29]. Increase in oxidative stress induces antioxidant enzyme activity like SOD in RBC which plays an important role in protecting the growing embryo from the danger of free radical damages [30, 34]. However, GPx activity during labour was significantly depressed in B and A in our study when compared to control which is in accordance with the earlier observations. [34,35,36]. Previous studies have suggested that high MDA levels possibly inhibit the activity of GPx with advancement of pregnancy with a significant decrease of Se and SeGSH-Px activities in both blood and plasma with a drop in total antioxidant status [37].

The process of labour also shows wide variation as it is influenced by maternal as well as fetal factors. The maternal factors are age, parity, gravidity and nutritional status, which can modify the process of labour. When correlated with maternal age it was positively correlated with GPX levels ($r = 0.016$). This implies that maternal age is an important determinant of oxidative stress. Elderly primigravida experienced greater degree of oxidative stress evidenced by lesser GPX activity in the postpartum period [36]. However, our results did not show any correlation between duration of labour and oxidative stress markers in maternal blood which coincides with the report of Simkoet al [38]. According to Guruprasad Rao et al, a positive correlation exists between MDA level and duration of labour [39]. This could be due to the inclusion of only uncomplicated deliveries as study group and further study is needed to confirm this fact.

The nutritional status of the mother is yet another important factor to be considered with respect to oxidative stress. The non-enzymatic antioxidants such as Vitamin C, Vitamin E and micronutrients such as copper, zinc, manganese and selenium which are co-factors for enzymatic antioxidants can also modulate the oxidative stress. The importance of antioxidant micronutrient supplementations of antenatal mothers have well been explored [40]. The stress during labour and the efficiency of maternal antioxidant system should not be overlooked as various other factors may induce oxidative stress through external sources like chemicals in drinking water and diet, tobacco usage, air pollution, deficit of antioxidants etc. The level of antioxidants may also be modulated by genetic polymorphism of metabolizing and oxidative stress related enzymes [41]. Enhancing the antioxidant potential by antenatal supplementation of micronutrients to reduce the deleterious effects of free radicals should be considered and explored by further probes.

V. Conclusion

Spontaneous vaginal delivery evokes changes in the redox environment of the mother due to fluctuations in the oxygen concentration as a result of hypoxia followed by reperfusion. The markers of oxidative stress, determined by the quantification of MDA were present to a greater degree, indicating that labour ending in childbirth is a stressful condition. This is counteracted to a certain extent by alterations in the levels of antioxidants.

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SOD (U/L)	Control and B	8.058 ± 1.15 & 35.45 ± 2.26	P < 0.05*
	Control and A	8.058 ± 1.15 & 35.03 ± 2.4	P < 0.05*
	B and A	35.45 ± 2.26 & 35.03 ± 2.4	P < 0.397
GPX (U/L)	Control and B	84.89 ± 9.54 & 65.51 ± 11.16	P < 0.01*
	Control and A	84.89 ± 9.54 & 77.78 ± 8.94	P < 0.05*
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Values are expressed as mean ± SEM, *Significant difference from control group
 B-Before delivery, A- After delivery