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Assessment of Maternal Oxidative Stress and Antioxidant Defence during Caesarean section

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Introduction. Pregnancy is a physiological stressful condition accompanied by an increased requirement for tissue oxygen because of a rapidly developing embryo and a subsequent fetal growth. Pregnant women exhibit consistently elevated levels of lipid peroxides when compared to nonpregnant women leading to oxidative damage [23, 10]. The onset of labour is associated with pain, fear, anxiety and above all hypoxia which induces the production of free radicals [6]. Uterine contractions are capable of a significant reduction in the blood flow leading to tissue ischemia followed by reperfusion which increases the production of free radicals [1, 7, 11, 28]. During labour there are periods of apnea and/or shallow respiration in between uterine contractions which leads to frequent oscillation of maternal and fetal oxygenation [15, 22, 24].

Reactive oxygen species (ROS) are partially reduced forms of O₂ which cause lipid peroxidation, cross linking and fragmentation of protein, degradation of DNA ultimately leading to the irreversible cell damage [26]. Antioxidants system are there for scavenging the free radicals such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) which can offer defence against tissue damaging effects of ROS [4].

In a healthy body there is a balance between oxygen derived from a free radical production and their removal by antioxidants. Stipek et al found that maternal antioxidant systems are overloaded during a vaginal delivery than a caesarean section particularly in an early postpartum due to an increase in malondialdehyde (MDA) level [21]. The antioxidant system works more efficiently to overcome oxidative stress in a newborn delivered via a caesarean section than a vaginal delivery [8]. Most of the above studies have been performed mainly in the Western population. The incidence of a child birth via a caesarean section is higher in our country when compared to the West. Therefore, the present study was aimed at quantifying the degree of oxidative stress as well as an antioxidant enzyme status during a delivery by a caesarean section in South Indian women and to study the association of various maternal clinical parameters along with it.

Material and Methods. This observational study was conducted in the Department of Physiology of a tertiary care hospital at Chennai. The study protocol was approved by the institutional ethical committee. The study group consisted of singleton uncomplicated term (37-42 weeks) pregnant women (n=20) in the age group of 20-35 years who were categorized for a delivery by

a caesarean section (LSCS) in the Obstetrics and Gynecology Department of the above mentioned institution. While age matched, healthy, nonpregnant, parous women ($n=13$) were taken for a control group. Detailed medical as well as obstetric histories regarding the present and previous pregnancies were obtained from each participant. The participants did not have the history of any liver /kidney / thyroid disorder. None of them were taking any medication except vitamins, iron and calcium supplements. There was no history of smoking/alcohol/drug abuse. Pregnancy with medical complications like anemia / jaundice / preeclampsia / heart disease / diabetes / hypertension and with a multiple pregnancy were excluded. Spontaneous vaginal delivery cases were excluded from this study as well. An informed written consent was obtained from all the participants after explaining the experimental protocol in detail in their native language.

Under strict aseptic conditions, 3 cc of whole blood was collected from pregnant women in the sterile EDTA vacutainer tube. The first sample was taken at the onset of an active labour with minimum 4-5 cm dilatation of cervix, as confirmed by a vaginal examination or before going to an operation theatre whichever is earlier. The second sample was taken immediately after a delivery of the placenta. Details like a maternal age, parity, duration of labour, colour of amniotic fluid were also noted. Similarly blood samples were also collected once from non-pregnant controls.

Red blood cells (RBC) were separated from plasma by centrifugation at 3000 r.p.m at 4°C for 15 mins. Supernatant plasma was used for lipid peroxidation estimation immediately. Erythrocytes were washed 3 times with 0.9% ice cold sterile saline and centrifuged at the same speed for 5 min after each wash. Cells were lysed in 4 times volume of distilled water, allowed 1 hour for complete hemolysis. It was centrifuged and supernatant was stored in – 80°C until the enzyme analysis was done. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and total reduced glutathione (GSH) were estimated in hemolysate at 37°C using ELICO SL 150 UV-VISIBLE spectrophotometer (Elico Limited, Balanagar, Andhra Pradesh, India).

Estimation of lipid peroxidation (LPO): Malondialdehyde (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) which was expressed as nmoles/100 ml [5].

Estimation of superoxide dismutase (SOD): The involvement of a superoxide anion radical in the auto-oxidation of pyrogallol was used as a convenient assay of SOD [12].

Estimation of total reduced glutathione (GSH): Glutathione reacted with Ditrioxitrobenzoic acid (DTNB) to give a coloured compound that was absorbed maximally at 412 nm [16].

Estimation of glutathione peroxidase (GPx): A glutathione peroxidase activity was measured by its ability to utilize the standard glutathione in the presence of a specific amount of hydrogen peroxide (1 mM) and that was absorbed maximally at 420 nm [20].

Results. Descriptive statistics was expressed as the mean and standard deviation. The comparison of lipid peroxides both before and after delivery by LSCS ($n=20$) was done in the study and the control groups ($n=13$) using Student 't' test with Statistical Package for the Social Sciences (SPSS) (11.5 version software). The antioxidant activities of GSH, GPx and SOD were also compared in the same manner with the control group. Pearson's correlation was done to correlate various maternal parameters with antioxidant enzymes. The difference with p value < 0.05 was considered to be statistically significant.

The effect of LSCS on Antioxidant status and oxidative stress. LPO was higher both before and after delivery by LSCS ($p < 0.05$) when compared to the control group which was statistically significant. But no difference was obtained between before and after delivery samples. There was statistically

significant increase in a GSH level in LSCS ($p < 0.001$) than in the control group. GSH was also found to be significantly higher before delivery ($p < 0.001$) than compared to after delivery showing the increased consumption due to oxidative stress. Changes in a GPx level was quantitatively higher in LSCS than in the control group, but did not reach significance. On the other hand, SOD was significantly higher ($p < 0.01$) in LSCS than in the control group though no difference was obtained between before and after delivery samples (Table 2).

Table 1

Characteristics of Maternal Clinical Parameters (n = 20)

	Mean ± SD	Range	Control
Mother Age (years)	25.6 ± 3.6	20-35	24.23 ± 4.48
Gestational Age at Delivery (Weeks)	39.04 ± 0.92	37-40	–
Duration of Labour (mins)	113.75 ± 198.15	0-807	–
Parity (%)	Primi 20%	1-5	–

* Median Value

Table 2

Maternal levels of LPO, GSH, SOD and GPx in LSCS compared with non pregnant women

Variables	Comparison between	Values Mean ± SD	p value	Significance
Lipid peroxidation nmoles/100 ml of plasma	Control & B	0.361± 0.10 & 0.597 ± 0.34	.007	p<0.05
	Control & A	0.361± 0.10 & 0.542 ± 0.27	.012	p<0.05
	B & A	0.597 ± 0.34 & 0.542 ± 0.2	.165	p>0.05
GSH U/ml	Control & B	12.4 ± 4.23 & 173.72 ± 91.78	.000	p<0.001
	Control & A	12.4 ± 4.23 & 114.2 ± 70.35	.000	p<0.001
	B & A	173.72 ± 91.78 & 114.2 ± 70.35	.000	p<0.001
GPx U/ml	Control & B	84.89 ± 34.39 & 108.61 ± 86.0	.303	p>0.05
	Control & A	84.897 ± 34.39 & 128.31 ± 82.0	.154	p>0.05
	B & A	108.61 ± 86.0 & 128.31 ± 82.0	.975	p>0.05
SOD U/ml	Control & B	8.06 ± 4.16 & 45.87 ± 33.86	.000	p<0.001
	Control & A	8.06 ± 4.16 & 42.78 ± 37.13	.001	p<0.001
	B & A	45.87 ± 33.86 & 42.78 ± 37.13	.293	p>0.05

Control (Non pregnant women), B - Before delivery, A - After delivery

While comparing with clinical parameters, antioxidants like GPx before a delivery showed a significant positive correlation (Pearson) ($P < 0.01$, $r = 0.597$) with the maternal age in LSCS (Figure). However other clinical parameters like parity, duration of labour and colour of the amniotic fluid showed very weak correlations if at all which were not statistically significant.

Discussion. Free radicals are produced primarily by univalent, biochemical redox reactions involving oxygen. Because of their high activity it is often difficult to detect free radicals in vivo, particularly in biologic systems. The production of reactive oxygen species (ROS) is increasing during pregnancy due to the increased cellular metabolism or the decreased antioxidant enzyme activity. The basic pathophysiology in producing oxidative stress during pregnancy lies in the integrity of uteroplacental circulation. Repetitive ischemia reperfusion in the uterus results in the increased production of free radicals all ultimately leading to endothelial dysfunction [9].

In the present study LPO was found to be higher in LSCS than in the control group. This was consistent with previous reports showing that a lipid peroxides level in the third trimester increased significantly as compared to the nonpregnant control [10, 27]. Plasma lipoproteins are the target of free radical induced oxidative stress during hypoxia [3]. There is an increase in serum lipid during pregnancy which autoxidize spontaneously to form lipid peroxide, the source of which is unknown [13].

But no significant difference was found between before and after delivery samples which was on the contrary with the previous study where a higher MDA level was found in a postpartum sample than before delivery [17]. This may be due to the difference in timing of collecting the postpartum sample. Here, the before delivery sample was taken at the onset of active labour and the postpartum sample immediately after separation of placenta. But in that study, the before delivery collection was done at 36th weeks of gestation and 3 postpartum samples were taken at intervals of 1, 24 and 48 hours respectively. This might be the probable reason for insignificant difference.

Antioxidants are substances that protect the body by reacting with free radicals and other reactive oxygen species hence hindering the process of oxidation. Glutathione, a tripeptide is the most abundant intracellular thiol and potent antioxidant that protects cells from free radicals that are generated during oxidative metabolism. In blood, 99.5% of glutathione is in the RBC, where it maintains hemoglobin in a reduced form [2].

In the present study GSH was found to be higher in LSCS than in the control group, which could be due to the fact that in anticipation of oxidative stress the maternal blood has raised its GSH level to combat the forthcoming oxidative stress during labour. Among antioxidants, glutathione plays an important role in quenching ROS, resulting in oxidation of glutathione which is excreted from RBC during prolonged oxidative stress. There was also a significant difference between before and after delivery samples showing the increased consumption of GSH during the stressful moments or due to low levels of production from the membranes [19]. All antioxidant cellular defenses in the glutathione redox cycle are modulated by oxidative stress as an adaptive response in newborn infants [25].

However, the competence of antioxidant enzymes to protect a newborn against free radicals during labour are profoundly modulated by both the gestational age and by the way of delivery. Specifically the GPx and SOD are more affected by the gestational age rather than by a way of delivery [6]. The increase of lipid peroxidation is greater as the gestational age decreases [19]. In the present study though SOD showed significantly higher values in LSCS than controls but the activity of GPX was not statistically significant although quantitatively it was higher in LSCS than in the control group. This might

be explained by the fact that the present study involved a small number of women ($n=20$) and all of them were in term pregnancy, only therefore it did not reach any statistical significance.

Among clinical parameters, antioxidants like GPx before delivery showed the significant positive correlation ($P < 0.01$, $r = 0.597$) with the maternal age which implied that it was an important determinant of oxidative stress. The previous study has demonstrated that the capability of a GPx activity enhancement to suppress ROS decreases in elderly primigravida in the postpartum period [18]. It was proven that the placental peroxidation activity increases with gestation. Therefore higher GPx levels towards the term in the maternal system is reported to be a protective mechanism against harmful effects of oxidizing agents like hydrogen peroxide [14].

Conclusion. Pregnancy is responsible for high susceptibility of serum as well as cellular lipids to undergo peroxidation. The process of labour evokes changes in the redox environment of the mother due to fluctuations in the oxygen concentration as a result of hypoxia followed by reperfusion. Therefore antioxidant enzyme activities are modulated during delivery by caesarean section to counteract the oxidative stress. Though the process is still unknown but detailed and prospective studies will help us to understand the mechanism underlying it. Moreover, it will be reasonable to correlate the association of maternal nutritional status governing the oxidative stress during pregnancy.

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RESEARCH ARTICLE

Assessment of Maternal Oxidative Stress and Antioxidant Defence during Caesarean section*PARAMITA BHATTACHARYYA¹, MATHANGI DC², PRICILLA JOHNSON³*¹ *Associate Professor of Physiology, Pondicherry Institute of Medical Sciences, Pondicherry, India*² *Professor of Physiology, Chettinad Hospital and Research Centre, Kelambakkam, Chennai, India*³ *Professor of Physiology, Sri Ramachandra University, Chennai, India
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Introduction. Pregnancy is a physiological state which causes the increased redox imbalance in utero. During labour, the production of damaging free radicals exceeds the body's antioxidant defense mechanism resulting in oxidative stress. In case of uncomplicated term pregnancy, a vaginal delivery has been proved to be more stressful condition than caesarean section. Hence it was proposed to estimate the degree of oxidative stress as well as antioxidant status during delivery by a caesarean section.

Material and Methods. Uncomplicated term pregnant women ($n=20$) who delivered by caesarean sections along with healthy parous of nonpregnant age matching a control group ($n=13$) were included in the study. Plasma malondialdehyde (MDA) for lipid peroxidation (LPO) and other antioxidant enzymes such as Superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and total reduced Glutathione (GSH) were estimated in plasma and hemolysate respectively using the spectrophotometric method. Results were analysed by Student 't' test taking $p<0.05$ as statistically significant.

Results. Plasma LPO increased significantly in a caesarean section as compared to the control group ($p<0.05$). Similar findings were observed for both GSH and SOD levels ($P<0.001$, $P<0.01$). But GPx did not show any statistical significance. But a significant positive correlation was obtained between GPx before delivery and maternal age ($r=0.597$, $P<0.01$).

Conclusion. Pregnancy results in oxidative stress which is further aggravated during delivery by a caesarean section to produce alterations in the maternal antioxidant system. Further research is required in this field to understand the basic mechanism of underlying it.

Key words: free radicals, oxidative stress, lower segment caesarean section, maternal antioxidant defence.