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Assessment of Maternal and Neonatal Oxidative Stress and Antioxidant Defence during Caesarean section

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Abstract: Background: Pregnancy is a physiological state in which there is an increased redox imbalance in utero. Foetal oxygenation is fully dependent upon integrity of uteroplacental circulation. Hypoxia is one of the main factor which induces free radical generation in the perinatal period. Studies have shown there is an increase in malondialdehyde (MDA) level, a marker of oxidative stress in vaginal delivery than elective caesarean section Hence the present study was aimed at quantifying the changes with respect to oxidative stress as well as antioxidant status for both mother and newborn during delivery by a caesarean section. **Methods:** Uncomplicated term pregnant women who delivered either by elective or emergency lower segment caesarean section (n=20) 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:** LPO was significantly higher in cord blood when compared to both before (p<0.05) & after delivery (p<0.05) samples. GSH was found to be higher in maternal blood before delivery than after delivery (p<0.001) and cord (p<0.01) blood samples. GPx & SOD did not reach any statistical significance. But GPx in cord blood sample showed significant negative correlation with maternal age in LSCS (r=-.445, p<.05). **Conclusion:** Pregnancy results in oxidative stress which is further aggravated during delivery by caesarean section and predisposes the neonate to increased oxidative stress when compared to maternal system which is reflected as an alteration in their antioxidant enzyme levels.
Keywords: free radicals, oxidative stress, lower segment caesarean section, maternal antioxidant defence.

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INTRODUCTION

Pregnancy is a state of increased oxidative stress leading to formation of susceptible oxidisable particles causing oxidative damage [1]. Maternal oxygen consumption increases significantly in normal labour [2]. Placenta is exposed to short periods of hypoxia-reperfusion during labour [3], which enhances generation of free radicals in placenta [4]. Reactive oxygen species (ROS) is a collective term used by the biologists to include not only free O_2 radicals but also some derivatives of O_2 which do not contain unpaired electrons such as hydrogen peroxide (H_2O_2), singlet oxygen (O^-), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) [5].

Birth is an oxidative challenge for the newborn. The sharp postnatal transition from the relatively low intrauterine PO_2 to the significantly higher oxygen in extrauterine environment causes formation of free radicals [6]. The oxidative aggression suffered by neonate is counterbalanced by the maturation of effective antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) against tissue damaging effects of ROS [7]. Several studies have shown that Malondialdehyde (MDA), one of the important marker of Thiobarbituric acid reacting substances (TBARS) was significantly higher in cord blood obtained from vaginal delivery than those delivered by lower segment caesarean section (LSCS) [8,9]. Also, Glutathione levels in cord blood samples were found to be higher in vaginal delivery when compared to caesarean section [10].

Since most of the above studies have been performed mainly in Western population and the incidence of child birth via caesarean section is higher in our country when

compared to the West. Therefore, the present study was aimed to evaluate and compare the oxidative stress and antioxidant defence mechanisms in maternal and the neonatal system during delivery by caesarean section in South Indian women & to study the association of various maternal and fetal 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. Singleton uncomplicated term (37-42 weeks) pregnant women (n=20) in the age group of 20-35 years who were admitted in the Obstetrics & Gynaecology Department of that institution for delivery were selected as subjects for this study. Detailed medical as well as obstetric histories regarding the present & previous pregnancies were obtained from each participant. The participants did not have a history of liver / kidney / thyroid disorder. None of them were taking any medication except vitamins, iron & calcium supplements. There was no history of smoking/alcohol/drug abuse. Pregnancy with medical complications like anaemia / jaundice / preeclampsia / heart disease / diabetes / hypertension and with multiple pregnancy were excluded. Spontaneous vaginal delivery cases were also excluded from this study. An informed written consent was obtained from all the participants after explaining the experimental protocol in detail in their native language.

Progress of labor was determined by vaginal examination every 1-2 hours and as indicated by clinical conditions. Uterine contractions and fetal heart rate were monitored continuously with a cardiotocograph. Among them 9 women delivered by emergency LSCS (EM-LSCS) due to complications arising during labour (fetal distress due to prolonged labour / post caserean with scar tenderness / meconium-stained liquor etc.) and 11 women underwent Elective LSCS (EL-LSCS) with sterilization due to previous history of operative delivery or due to bad obstetric history. None of them showed any perinatal complication.

Under strict aseptic conditions, 3 cc of whole blood was collected from antecubital vein of mother in sterile EDTA vacutainer tube. First sample was taken at the onset of active labor with minimum 4 – 5 cm dilatation of cervix, as confirmed by vaginal examination. In case of elective LSCS, first sample was taken just before going to operation theatre. Second sample was taken from umbilical cord after double clamping and removal of newborn but before placental separation. Third sample was taken again from mother immediately after delivery of the placenta. Details like maternal age, parity, duration of labour, sex of baby, birth weight, APGAR score at 1 and 5 min, colour of amniotic fluid were also noted.

Red blood cells (RBC) were separated from plasma by centrifugation at 3000 r.p.m at 4^0C 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 same speed for 5 min after each wash. Cells were lysed in 4 times volume of distilled water, then allowed 1 hour for complete hemolysis. It was centrifuged and supernatant was stored in – 80^0 c until enzyme analysis was done. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and total reduced glutathione (GSH) were estimated in hemolysate at 37^0C using ELICO SL 150 UV-VISIBLE Spectrophotometer (Elico Limited, Balanagar, Andhra Pradesh)

Estimation of lipid peroxidation (LPO): Malondialdhyde (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/100ml [11].

Estimation of Superoxide dismutase (SOD): The involvement of superoxide anion radical in the auto-oxidation of pyrogallol was used as a convenient assay of SOD. Result was expressed as superoxide dismutase for 50 % inhibition of pyrogallol autooxidation in ng/ml [12].

Estimation of Total Reduced Glulathione (GSH): Glutathione reacted with Ditrioxitrobenzoic acid (DTNB) to give a coloured compound that was absorbed maximally at 412 nm. The level of glutathione was expressed as μg/ml of hemolysate [13].

Estimation of glutathione peroxidase (GPx): Glutathione peroxidase activity was measured by its ability to utilize the standard glutathione in the presence of specific amount of hydrogen peroxide (1mM) & that was absorbed maximally at 420 nm. The activity of GPx was expressed as μg GSH utilised / min / ml of hemolysate [14].

RESULTS

Descriptive statistics were expressed as mean and standard deviation (Table – 1). Comparison of lipid peroxides both before and after delivery by LSCS (n=20) were done in maternal blood with cord blood sample using Student "t" test with Statistical Package for the Social Sciences (SPSS, 11.5 version) software. The antioxidant activities of GSH, GP_X and SOD were also compared in the same manner. Pearson's correlation was done to correlate various maternal & fetal clinical parameters with antioxidant enzymes. Difference with a p value < 0.05 was considered to be statistically significant.

Table 1: Characteristics of Maternal and Fetal Clinical Parameters (n = 20)

	Mean ± SD
Mother Age (years)	25.60 ± 3.60
Gestational Age at Delivery (weeks)	39.035 ± 0.92
Duration of Labor (mins)	113.75 ± 198.15
Parity (%)	Primi 20%
Baby Birth Weight (kg)	3.009 ± 0.39
* 1 Min Apgar Score	8
* 5 Min Apgar Score	9
Sex (%) of the baby	Male – 55% Female – 45%

* Median Value

Effect of LSCS on Antioxidant status and oxidative stress: LPO was higher in cord blood when compared to both before ($p<0.05$) & after delivery ($p<0.05$) samples which were statistically significant. GSH was found to be significantly higher before delivery when compared to after delivery ($p<0.001$) and cord ($p<0.01$) blood samples showing increased consumption due to oxidative stress. On the other hand, GPx & SOD did not show any significant difference between maternal and cord blood. (Table – 2)

While comparison with clinical parameters, antioxidant like GPx in cord blood sample showed significant negative correlation ($r=-.445$, $p<.05$) with maternal age in LSCS which was statistically significant. (Figure 1) However correlation of other clinical parameters like gestational age, parity, birth weight, APGAR score, duration of labor and sex of the baby with antioxidant enzyme levels and MDA showed very weak correlations if at all which was not statistically significant.

Table 2: Comparison between Maternal and Cord blood levels of LPO, GSH, SOD and GPX in LSCS

Variables	Comparison between	Values (Mean ± SD)	p value	significance
Lipid peroxidation (nmoles/100 ml of plasma)	B & C	0.597 ± 0.34 & 1.025 ± 0.680	.013	*** p<0.05**
	A & C	0.542 ± 0.27 & 1.025 ± 0.680	.008	*** p<0.05**
GSH (µg/ml of hemolysate)	B & C	173.72 ± 91.78 & 122.6 ±76.15	.005	*** p<0.01**
	A & C	114.2 ± 70.35 & 122.6 ±76.15	.603	p>0.05
GPx (µg GSH utilised / min / ml of hemolysate)	B & C	108.61 ± 86.0 & 102.1 ± 71.18	.372	p>0.05
	A & C	128.31 ± 82.0 & 102.1 ± 71.18	.176	p>0.05
SOD (for 50 % inhibition of pyrogallol autooxidation ng/ml)	B & C	45.87 ± 33.86 & 47.45 ± 43.63	.351	p>0.05
	A & C	42.78 ± 37.13 & 47.45 ± 43.63	.973	p>0.05

* Statistically significant, C= Cord blood, B= Before delivery, A= After delivery

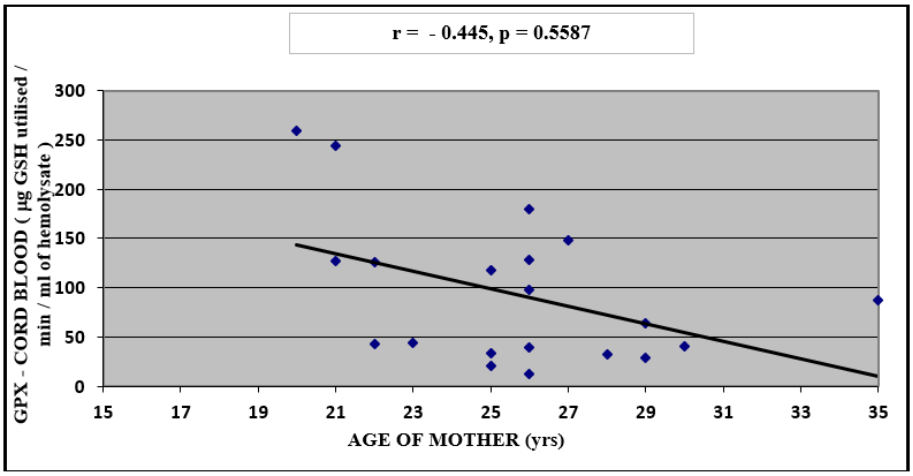

Figure 1: Correlation between maternal age and cord blood GPX level in LSCS

DISCUSSION

Pregnancy is a unique immunological state. Foetal oxygenation is fully dependent upon integrity of uteroplacental circulation which in turn depends upon proper endothelial cell function. Hypoxia is one of the main factors inducing free radical generation in the perinatal period. Plasma lipoproteins are the target of free radical induced oxidative stress during hypoxia [6,15]. Placenta is a major source of pro-oxidant agents, antioxidant enzyme systems and hormones which is able to keep the lipid peroxidation under control in normal pregnancy [1]. However the competence of antioxidant enzymes to protect the newborn against the free radicals during labour are profoundly modulated by both gestational age and by way of delivery.

In the present study LPO in cord blood was significantly higher when compared to both before and after delivery samples in LSCS which indicates that lipid peroxidation is probably induced by placenta [15] during pregnancy. The most common hypoxic stresses imposed on the fetus during labor are related to insufficiency of uterine or umbilical blood flow and occasionally to a decrease in uterine arterial oxygenation [16].

Oxidative stress is a physiologic event in fetal to neonatal transition. Glutathione is considered to be major redox buffer of mammalian cells. Glutathione is a powerful antioxidant capable of rendering adequate antioxidant protection and regenerating other antioxidants like Vitamin E and Vitamin C. It has a substantial role in maintenance of pregnancy and prevention of oxidative stress in birth process [17]. All antioxidant cellular defenses in glutathione redox cycle are modulated by oxidative stress as an adaptive response in newborn infants [18]. Term labor triggers a compensatory up regulation of antioxidant reserve in fetal RBC in contrast after preterm delivery which enhances vulnerability to free radical damage [19]. GSH was found to be significantly higher before delivery ($p<0.001$) when compared to both after delivery and cord blood samples which could be due to either maternal hyperoxygenation during surgery [20], also pain and anxiety during spinal anaesthesia before LSCS which could have affected maternal and fetal oxygenation and LPO levels [21].

It has been reported that gestational age has more influence in governing the degree of oxidative stress than the mode of delivery itself [22]. The increase of lipid peroxidation is greater as gestational age decreases [23]. Preterm babies born via caesarean section are predisposed to more oxidative stress due to poor antioxidant defence than full term babies [22]. In our study only full-term neonates were taken into consideration. Therefore, in the present study GPx & SOD level compared between maternal and cord blood did not reach any statistical significance.

The process of labour can be influenced by certain maternal and fetal variables. Maternal and fetal parameters like maternal age, parity, weight of the baby, sex of the baby, duration of labour were studied with respect to oxidative status. While comparison with clinical parameters, antioxidants like GPx in cord blood sample showed significant negative correlation ($r=-.445$, $p<.05$) with maternal age in LSCS which implies that maternal age was an important determinant of oxidative stress. Previous study has demonstrated that the capability of glutathione peroxidase activity enhancement / reversal to near normal values with simultaneous suppression in ROS decreases appreciably after 14 days postpartum in elderly primigravidas. Antioxidant network combating ROS was stronger in younger primigravidas. This may act as one of the important parameters leading to a variety of complications encountered by elderly primigravidas [24]. However, an in-depth investigation is mandatory to convincingly conclude the above.

CONCLUSION

Pregnancy is a unique immunological state. The basic pathophysiology in producing oxidative stress during pregnancy lies in the integrity of uteroplacental circulation. Though considered as physiological state, still pregnancy is responsible for the high susceptibility of not only serum lipids but also from the cells to peroxidation. It is further aggravated but not initiated as such by labor itself. Infants delivered by elective caesarean section though mother is not in labor are still prone to develop oxidative stress similar to those who are delivered by emergency caesarean section. So, the possibility of elective caesarean section being advantageous to avoid oxidative stress has to be further studied with a larger and also representative bigger sample. In the present study all neonates were born healthy with good Apgar score irrespective of oxidative parameters. Nonetheless in view of all above observations linking the role of oxidative stress during pregnancy as well as in delivery with or without labor, it may be reasonable to assess whether antioxidant supplementation during pregnancy may reduce the incidence of maternal and fetal complications.

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