Original Article

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Paediatrics Section

Comparison of Oxidative Stress in Neonates Born by Normal Vaginal and Elective Caesarean Delivery

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ABSTRACT

Introduction: Oxidative stress occurs when generation of free radicals (oxidants) exceeds the available antioxidants. In pregnancy and parturition, there is an increased formation of reactive oxygen species. This study aims to evaluate and compare oxidative stress in newborns delivered by two different modes i.e., normal Vaginal Delivery (VD) and elective Caesarean Section (CS).

Aim: To evaluate and compare oxidative stress in term new born via two different modes of delivery i.e. Normal VD and elective CS by estimating the following serum biochemical markers in a cord blood sample.

1) Superoxide Dismutase (SOD)

2) Uric Acid (UA)

3) Malondialdehyde (MDA)

4) Ischemia Modified Albumin (IMA).

Materials and Methods: A hospital based cross-sectional study was conducted wherein a total 92 neonates, 46 in each group of normal VD and elective CS were included in the study. Cord blood levels of oxidative stress parameters i.e., MDA, IMA and antioxidants - SOD and UA were estimated. Statistical analysis of the outcome variables was done by Chi square, Fischer Exact, unpaired 't'-tests and ANOVA. Correlation between parameters by Pearson's correlation and significant parameters were subjected to ROC curve analysis to see their efficacy.

Results: The IMA levels in cord blood were significantly higher in VD ($p<0.05$) when compared to CS, while MDA levels were not significantly different between the groups ($p>0.05$). The levels of antioxidant parameters SOD and UA was significantly lower in neonates born by VD than CS ($p<0.05$). IMA showed a significant weak positive correlation with the period of gestation. The area under ROC of IMA was 0.985 showed significant difference between both groups.

Conclusion: There is increased oxidative stress and decreased antioxidant status in neonates born via normal VD as compared to CS. There is decreased oxidative stress during caesarean delivery as depicted by decreased IMA and higher antioxidant reserve (SOD and UA) in the cord blood these babies which in turn is a protective mechanism. The negative correlation between the oxidative parameter (IMA) and antioxidant parameters (SOD, UA) can be explained by the free radical scavenging effect of antioxidants which is reflected with comparatively lower levels.

Keywords: Ischemia modified albumin, Malondialdehyde, Oxidative stress, Superoxide dismutase

CASE REPORT

In human body, normal metabolism generates Reactive Oxygen Species (ROS i.e., free radicals) and there is an effective antioxidant system that minimizes their detrimental effect and the disruption in the balance is defined as oxidative stress [1]. This stress occurs even in uncomplicated normal pregnancies because of the high mitochondrial metabolism in placenta and foetus [2]. Oxidative stress in utero results in intrauterine growth retardation, foetal death and poor perinatal outcomes. Intrauterine oxidative stress may be further aggravated by stress of delivery and labour making the foetus at higher risk of birth asphyxia [3].

After birth, the newborn is exposed to an oxygen rich environment (PO2 100 TORR) in comparison to the intrauterine environment (PO2 20-25 TORR). Following delivery there is a drastic four-fold increase in oxygen exposure as compared to intrauterine environment which results in surge of free radicals. Normally, this triggers various cell process viz. coagulation, fibrinolysis, cell cycle and signal transduction required for cellular metabolism but when over produced it becomes toxic as all biological molecular components of foetal structure are affected [4].

There is a paucity of data regarding blood levels of oxidative stress indicators such as MDA, IMA and the antioxidants status i.e., SOD and UA during delivery. Therefore, this study was designed to compare oxidative stress in newborns delivered by two different modes and understand this process better.

MATERIALS AND METHODS

This hospital based cross-sectional study was conducted in the period of October 2013 to September 2015, for the duration of 23 months in a tertiary care referral maternity hospital (Government Lady Goshen hospital), Mangalore, India, with a level three NICU.

A total of 92 normal neonates at birth born by vaginal route and elective CS of birth weight ≥2.5 kg were included. Babies with congenital anomalies, intra-uterine growth retardation, preterm babies, neonates with birth asphyxia, presence of maternal diseases like hypertension, diabetes mellitus, thyroid diseases, hypercholesterolemia or any other chronic maternal illness, multiple pregnancies were excluded from the study.

The sample size for the study was calculated in accordance with a study done by using the formula:

$n=2(Z\alpha+Z\beta)^2\times\sigma^2$,

where, $Z\alpha=1.96$ at 95% confidence level; $Z\beta=1.28$ at 90% power; σ is combined standard deviation=21.4; and d is difference between means=11.The minimum sample size calculated was 92 with 46 in each group [5].

Neonates were recruited after obtaining informed consent from parents/relatives. Necessary approvals including Institutional Ethics Committee approval was obtained prior to the study. A semi structured proforma was prepared to record the demographic, neonatal and delivery details. 10 mL cord blood sample was collected, serum was separated and stored in Aliquots at -20^{0}C. At a later date estimation of MDA, IMA, uric acid and SOD were done. Following methods were applied i.e., SOD by NBT reduction method [6,7]. UA by Uricase/POD method with a semi auto analyser using a commercially available Kit-Liquid Check Uric Acid S.L of AGAPPE Company, MDA was estimated as TBARS (Thiobarbituric Acid Reactive Substances) [8] and assessed spectrophotometrically, IMA was estimated by method proposed by Bar-Or et al., [9].

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 17. Basic socio-demographic data of the study participants were expressed in terms of frequency and percentage for categorical variables and means with standard deviation for continuous variables.The biochemical markers were analysed using Chi square test and Fischers Exact test, unpaired 't'-test and ANOVA. The p-value less than 0.05 was considered as significant and less than 0.01 as highly significant.

RESULTS

Full term normal newborns with birth weight ≥2.5 kg of both sexes, born VD and elective CS were included in the study. Average APGAR score after 5 minutes was 9-10. All mothers of neonates included in the study were normotensive, normoglycemic, free from any complications. There were a total of 92 samples analysed. The two study groups were comparable inmost of demographic variables shown in [Table/Fig-1-3].

On comparison of the oxidative parameters in both the study groups it was found that IMA among normal VD babies was

Variable	Study Groups	N	Mean±SD	p-value
Maternal Age (in years)	Normal Vaginal Delivery	46	27.3±4.1	0.219 (NS)
	Caesarean Section	46	28.4±4.6	
Maternal Height (in cms)	Normal Vaginal Delivery	46	155.0±5.7	0.219 (NS)
	Caesarean Section	46	153.8±6.6	
Maternal Weight (in Kg)	Normal Vaginal Delivery	46	57.4±7.9	0.601 (NS)
	Caesarean Section	46	58.3±8.0	
Period of Gestation	Normal Vaginal Delivery	46	39.0±1.1	0.001 (Sig)
	Caesarean Section	46	38.0±1.6	
Foetal Birth Weight (in Kg)	Normal Vaginal Delivery	46	2.9±0.4	0.471 (NS)
	Caesarean Section	46	2.9±0.4	

[Table/Fig-1]: Comparison of the demographic variables between the two study groups [n=92].
Statistical test used: Student 't' test; Level of significance p<0.05 is considered significant; p>0.05 is non significant (NS). The Mean ± Standard deviation (SD) of the demographical variables of the study groups such as: Maternal age; Maternal height; Maternal weight; Period of gestation; Foetal birth weight were compared using Student 't' test.

		Study Groups		Total	Chi square test/Fischer's exact test p-value
		Normal Vaginal Delivery	Caesarean section		
Birth order	Multi	26 (57.4%)	33 (72.4%)	59 (64.9%)	0.130 (NS)
	Primi	20 (42.6%)	13 (27.7%)	33 (35.1%)	
	Total	46 (50%)	46 (50%)	92 (100%)	

[Table/Fig-2]: Association of birth order between the study groups [n=92].
Statistical test used: Chi-square test; Level of significance p<0.05 is considered significant; p>0.05 is non significant (NS).

significantly higher at 0.63±0.36 U/mL than babies of CS at 0.52±0.07 U/mL (p<0.05) [Table/Fig-4]. Although MDA in normal VD cases was lower (5.8±0.32 U/mL) than CS cases (6.13±7.4 U/mL) the difference was not statistically significant (p >0.05).The antioxidant analysis among the two groups showed that SOD in normal VD cases (1.2±0.2 U/mL) were significantly lower (p<0.05) when compared to CS cases (1.8±0.39 U/mL) [Table/Fig-5]. Serum uric acid levels in normal VD cases (2.9±0.7 mg/dL) were also significantly lower (p <0.05) when compared to CS cases (3.5±0.3 mg/dL).

Pearson's correlation analysis showed a weak negative correlation of IMA with antioxidants, uric acid (r=-0.319) and SOD (r=-0.557) which were statistically significant (p<0.05). The negative correlation was stronger with SOD than uric acid [Table/Fig-6]. This showed that whenever there was oxidative stress there was probable insufficient antioxidant defence to combat it. Another interesting finding was that IMA levels negatively correlated with the period of gestation(r=-0.318; p<0.05) [Table/Fig-6].

ROC-IMA-The ROC curve of IMA [Table/Fig-7] shows that at a cut off value of 0.4005, the specificity is 100% and the sensitivity is 97.9%. The area under the IMA ROC curve is 0.985 [Table/Fig-8]. Hence, IMA would be considered to be "accurate and excellent" parameter at separating normal VD from CS. It depicts that there is a significant difference in the oxidative stress levels among both the groups.

		Study Groups		Total	Chi square test/Fischer's exact test p-value
		Normal Vaginal Delivery	Caesarean section		
Birth presentation	Breech	0 (0.0%)	12 (25.5%)	12 (12.8%)	0.001 (Sig)
	Cephalic	46 (100.0%)	33 (72.3%)	79 (86.2%)	
	Transverse	0 (0.0%)	1 (2.2%)	1 (1.1%)	
	Total	46 (50%)	46 (50%)	92 (100%)	

[Table/Fig-3]: Association of frequency of birth presentation between the study groups [n=92].
Statistical test used: Chi-square test; Level of significance p<0.05 is considered significant (Sig).

Variable	Study Groups	Mean±SD	p-value
Ischemia Modified Albumin (IMA) in U/mL	Normal Vaginal Delivery	0.63±0.36	<0.001 (Sig)
	Caesarean Section	0.52±0.07	
Malondialdehyde (MDA) in U/mL	Normal Vaginal Delivery	5.8±0.3	0.750 (NS)
	Caesarean Section	6.1±7.4	

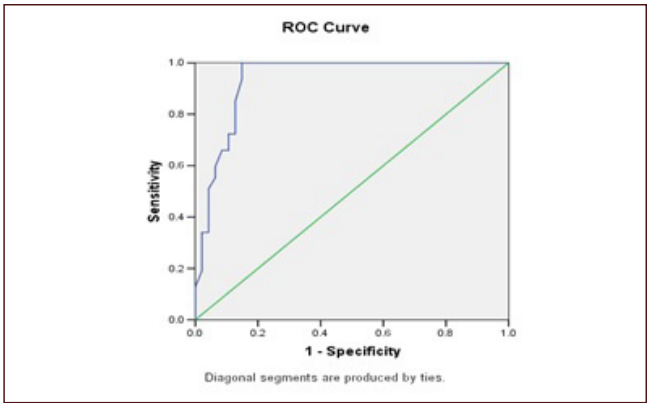
[Table/Fig-4]: Comparison of the cord blood levels of the oxidative parameter between the two study groups [n=92].
The Mean ± Standard deviation (SD) of the cord blood levels of oxidative parameters in the two study groups were compared using Student 't' test.Level of significance p<0.05 is considered significant; p>0.05 is non significant (NS).

Variable	Study Groups	N	Mean±SD	p-value
Uric Acid (mg/dL)	Normal Vaginal Delivery	46	2.9±0.7	<0.001 (Sig)
	Caesarean Section	46	3.5±0.3	
Superoxide dismutase (SOD) (U/mL)	Normal Vaginal Delivery	46	1.2±0.2	<0.001 (Sig)
	Caesarean Section	46	1.8±0.39	

[Table/Fig-5]: Comparison of the cord blood levels of the antioxidant parameters between the two study groups [n=92].
The cord blood levels (Mean ± SD) of antioxidant parameters in the two study groups were compared with Student 't' test. Level of significance p<0.05 is considered significant; p>0.05 is non significant (NS).

	Oxidant and antioxidant parameters	Correlation coefficient	p-value
IMA	POG	-0.318	0.002 (sig)
	URIC ACID	-0.319	0.002 (sig)
	MDA	0.136	0.190 (non sig)
	SOD	-0.557	<0.001 (sig)

[Table/Fig-6]: Correlation between Ischemia modified albumin (IMA) and other oxidative and antioxidant parameters of this study [n=92].
Level of significance p<0.05 is considered significant; p>0.05 is non significant (NS).



[Table/Fig-7]: ROC Curve for IMA.

Area	Std. Error[a]	Asymptotic Sig[b]	Asymptotic 95% confidence Interval	
			Lower bound	Upper bound
0.935	0.027	0.000	0.881	0.988

[Table/Fig-8]: Receiver operating characteristic curve of ischemia modified albumin.
The test result variable IMA has at least one tie between the positive actual state group and the negative actual state group. Statistics maybe biased.
[a] Under the non parametric assumption.
[b] Null hypothesis: true area=0.5

DISCUSSION

Oxidative stress in cellular metabolism occurs when generation of free radicals (oxidants) cannot be counteracted by available antioxidants or due to insufficient antioxidant production in the system even when there is minimal generation of free radicals. Most of these ROS have a detrimental effect on cell organelles causing lipid peroxidation [10,11]. During pregnancy there is increased oxidative stress and foetus has less protection against the oxidative stress as a result of which ROS can cause complications during early or late stages of pregnancy [11]. Serum MDA is a by-product of free radical induced membrane lipid injury [3].

Also when serum albumin is exposed to high levels of free radicals there is a transient modification of the N-terminal of the amino acid chain which reflects as IMA in the serum. Hence, these two biochemical markers are measured to interpret oxidative stress [9].

This process may be considerable in the baby or in the mother or in both due to the underlying pathology in cases of emergency CS. However, in elective CS cases babies are mostly free of oxidative injury. Hence, it is apparent that in VD due to labor, the foetus is likely to be exposed to higher levels of oxidative stress [12].

In this study, a total of 92 samples of cord blood were analysed, 46 in each of the study groups i.e., vaginal and CS delivery. It was observed that the two study groups were comparable in most of the socio-demographic features of the both the groups such as mean age of the mothers, parity, anthropometry of the mother and birth weight of the babies. All the maternal factors that can influence oxidative stress were excluded to avoid any bias among the study groups. The most common form of foetal presentation at delivery was seen to be cephalic. There were no breech deliveries conducted vaginally. The commonest indication of the caesarean section was observed to be previous CS. However, the period of gestation in two of groups were significantly different i.e., VD group being 39.0±1.1 weeks as compared to the 38.0±1.6 weeks in the CS group, showing the gestation period is longer by a week in the babies of VD than in CS ($p<0.05$) [3]. This study observed no significant differences in the plasma levels of MDA in cord blood of babies among the studied groups, (5.8±0.32 U/mL in VD vs 6.13±7.4 U/mL in elective CS) [Table/Fig-4] which is in accordance with the study done by Adekanle DA et al., where SVD vs elective CS, was 5.78 ± 1.56 U/mL Vs 5.01±1.21U/mL respectively [3]. However, even though not significant there are higher levels of MDA noted in VD which may indicate there is a notable increase in free radical generation in this group. On the contrary Yigit S et al., also determined that cord blood MDA levels in babies born via spontaneous VD were higher compared to those of babies born via CS [12].

On the contrary a study done by Kirimi E et al., states that there is increased oxidative damage in caesarean deliveries as compared to normal vaginal deliveries and the variation MDA levels does not have any correlation with gestational age [13].

Study done by Gupta P et al., stated intrauterine malnutrition is associated with significant oxidative stress in small for gestational age neonates born at term to malnourished mothers [14]. Kamath U et al., have shown that free radical generation in the mother reflects on the newborn [15] this observation may not mislead the interpretation of the findings of this study because clinical conditions like pre-eclampsia, gestational diabetes mellitus that have been linked to free radical generation were excluded. All mothers recruited in this study had apparently normal pregnancy and were exposed to free radical generation observed in normal pregnancy. Hence, mothers had only free radical generation noted in normal pregnancy.

On the other hand, there were significantly higher values of cord blood IMA levels of babies born via VD as compared to elective CS as seen in [Table/Fig-4]. Study done by Biswas S et al., also had similar findings [2]. Gugliucci A et al., also showed similar results where complicated deliveries including IUGR, foetal distress, preterm deliveries, PROM, Meconium stained liquor) had a IMA levels of 0.96±0.59 U/mL and uncomplicated deliveries showed 0.64±0.24 U/mL [16]. IMA levels are higher in pregnancies with abnormal foetal Doppler evaluation and can be used as a marker for perinatal asphyxia [17]. Thus, it can be considered as a reasonable indicator of oxidative damage. Another interesting finding which came to the forefront was that the serum IMA levels correlated positively with the period of gestation i.e., the longer the period of gestation, the higher values of IMA were noted. This probably shows that pregnancies proceeding to post-term have greater stress on the baby and higher incidence of meconium stained liquor is noted in those deliveries which is an indication of foetal distress [18].

Antioxidants play an important role in preventing the formation of and scavenging of free radicals. Measurement of the antioxidant capacity of cord blood sample provides better picture of the capability to counteract oxidative stress induced diseases [2]. In this study we analysed SOD and UA to know the antioxidant status. UA is the end product of purine metabolism and also a major antioxidant in the human plasma, correlates well with conditions associated with oxidative stress.

UA is noted to have a dual role in terms of potentiating oxidant effects inside the cell and as an antioxidant in the plasma, but our study lays emphasis only on its antioxidant effect [19]. Plasma SOD values and serum uric acid levels were higher in cord blood of CS born newborns than that of normal VD born newborns [Table/Fig-5], suggesting that there is increased

antioxidant status (SOD and UA) in cord blood of caesarean section babies. The studies which are in agreement with these results are of Biswas S et al., Sazzad Y et al., and Inanc F et al., [2,20,21]. But there are studies not in favour of this i.e., some say that the antioxidant reserve is more in term neonates born via vaginal route in comparison to preterm neonates [22]. There are other studies which concluded that the mode of delivery has no association with oxidative stress but the sample size was less (n=41) when compared to the present study (n=92) which probably explains the lack of significant difference [3].

This study also brought out the negative correlation between the oxidant and antioxidant parameters, where oxidative parameter, IMA, levels was high, SOD and UA levels were significantly lower in normal VD than the CS [Table/Fig-6]. This could be explained with increased consumption of antioxidants in conditions of greater oxidative stress.

So, according to the present as well as majority of studies done earlier in other parts of the world, CS proves to be better than normal VD, as in CS the oxidative stress is less and antioxidant level is better [2]. But Siddiqui H et al., have reported differently and this is probably because they have used a single marker MDA for estimation of oxidative damage and both the groups had unequal number of samples [23].

LIMITATION

Outcome of the neonates with increased oxidative parameters could not be followed up. The further variation in levels in neonates with perinatal problems especially birth asphyxia couldn't be studied to decide if this could be a significant marker. Oxidative stress which is directly related to foetal outcome needs to be explored in relation to economic background, ethnicity, genetic preponderance, diet, nutritional status. This was not studied.

CONCLUSION

There is increased oxidative stress and decreased antioxidant status in neonates born via normal VD as compared to CS. There is decreased oxidative stress during caesarean delivery as depicted by decreased Ischemia Modified Albumin and higher antioxidant reserve (SOD and UA) in the cord blood of these babies which in turn is a protective mechanism. Further studies are recommended to know the use of antioxidant supplements antenatally in high risk mothers for the benefit of babies.

REFERENCES

[1] Betteridge DJ. What is oxidative stress? Metabolism. 2000;49(2 Suppl 1):03-08.

[2] Biswas S, Bhattacharyya S, Ghosh C, Banerjee S, Mukherjee K, Basu A. Assessment of oxidative stress and antioxidant status among newborns in relation to mode of delivery. IJCRR. 2014;6(7):65-73.

[3] Adekanle DA, Oparinde DP, Atiba AS, Akintayo AA. Effect of different modes of delivery on cord blood oxidative stress markers. Int J Biomed Sci. 2013;9(4):249-54.

[4] Toescu V, Nuttall SL, Martin U, Kendall MJ, Dunne F. Oxidative stress and normal pregnancy. Clin Endocrinol (Oxf). 2002;57:609-13.

[5] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gel. Anal Biochem. 1971;44(1):276-87.

[6] Sun Y, Oberley LW, Ving U. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988; 34(3):497-500.

[7] Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-10.

[8] Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. Eur J Biochem. 2001;268(1):42-47.

[9] Hracsko Z, Safar Z, Orvos H, Novak Z, Pal A, Varga IS. Evaluation of oxidative stress markers after vaginal delivery or caesarean section. In Vivo. 2007;21:703-706.

[10] Atiba AS, Abbiyesuku FM, Niran-atiba TA, Oparinde DP, Ajose OA, Akindele RA. Free radical attack on membrane lipid and antioxidant vitamins in the course of pre-eclamptic pregnancy. Ethiop J Health Sci. 2014;24(1):35-42.

[11] Boles H. Pathophysiology of diseases in the newborn caused by reactive oxygen species. Johann Wolfgang Goethe University - University Frankfurt. 1999.

[12] Yiğit S, Yurdakök M, Kilinç K, Oran O, Erdem G, Tekinalp G. Serum malondialdehyde concentration as a measure of oxygen free radical damage in preterm infants. Turk J Pediatr. 1998;40(2):177-83.

[13] Kirimi E, Peker E, Tuncer O. Increased serum malondialdehyde level in neonates with hypoxic- ischaemic encephalopathy: prediction of disease severity. J Int Med Res. 2010;38(1):220-26.

[14] Gupta P, Narang M, Banerjee B, Basu S. Oxidative stress in term small for gestational age neonates born to undernourished mothers: a case control study. BMC Pediatrics. 2004;4:14.

[15] Kamath U, Rao G, Kamath SU, Rai L. Maternal and foetal indicators of oxidative stress during pregnancy and pregnancy induced hypertension (PIH). IJABPT. 2011; 2(1);405-10.

[16] Gugliucci A, Hermo R, Monroy C, Numaguchi M, Kimura S. Ischemia-modified albumin levels in cord blood: a case-control study in uncomplicated and complicated deliveries. Clin Chim Acta. 2005;362(1-2):155-60.

[17] Kumral A, Okyay E, Guclu S, Gencpinar P, Islekel GH, Oguz SS, et al. Cord blood IMA:Is it associated with abnormal Doppler findings in complicated pregnancies and predictive of perinatal asphyxia? J Obstet Gynaecol Res. 2013;39(3):663-71.

[18] Galal M, Symonds I, Murray H, Petraglia F, Smith R. Postterm pregnancy. Facts, Views Vis ObGyn. 2012;4(3):175-87.

[19] Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. Curr Pharm Des. 2005;11(32):4145-51.

[20] Sazzad Y, Leonard M, Doyle M. Antioxidant levels in the cord blood of term foetus. J Obstet Gynaecol. 2000;20(5):468-71.

[21] Inanc F, Kilinc M, Kiran G, Guven A, Kurutas EB, Cikim IG, et al. Relationship between oxidative stress in cord blood and route of delivery. Foetal Diagn Ther. 2005;20(5):450-53.

[22] Buhimschi IA, Buhimschi CS, Pupkin M, Weiner CP. Beneficial impact of term labor: nonenzymatic antioxidant reserve in the human foetus. Am J Obstet Gynecol. 2003;189(1):181-88.

[23] Siddiqui H, Noor N, Moin S, Parveen S. Evaluation of oxidative stress markers in maternal and cord blood: Vaginal delivery versus Elective caesarean section. IJMSC. 2014;3(2):24-27.

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