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Original Article

Biochemistry Section

Comparison of Oxidative Stress Markers and Serum Cortisol between Normal Labor and Selective Cesarean Section Born Neonates

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ABSTRACT

Introduction: An imbalance between antioxidant and oxidant-generating systems in newborns can cause oxidative damage. The effect of modes of delivery on oxidative stress in neonates is not fully investigated.

Aim: This study was aimed to examine the effects of modes of delivery on oxidative stress markers and cortisol in newborns.

Materials and Methods: In this study 60 term neonates {30 born via Normal Delivery (ND) and 30 born via elective Caesarean Delivery (CS)} at birth were enrolled. Glutathione Peroxidase (GPx), Catalase (CAT) and Superoxide Dismutase (SOD) activities were determined in umbilical cord blood in all neonates. Moreover serum cortisol, uric acid and Total Antioxidant Capacity (TAC) were measured.

Results: GPx and SOD activities in cesarean born neonates were significantly higher than those of control subjects ($p<0.05$). TAC and CAT were not significantly different between the two groups. Serum cortisol was lower in caesarean born subjects as compared to normal born neonates. On the other hand uric acid concentration was higher in caesarean born neonates.

Conclusion: The obtained data indicated that babies born via caesarean section might be predisposed to pathological conditions due to altered antioxidant levels.

Keywords: Glutathione peroxidase, Newborn, Superoxide dismutase

INTRODUCTION

One of the physiological changes that occur during the birth is exposure of body to oxygen [1]. Respiration is accompanied by Reactive Oxygen Species (ROS) formation that can be harmful for the body. Oxidative stress occurs due to increased production of ROS and reduction in antioxidants [2]. Oxidative stress may disturb the metabolic pathways, damage the organ functions and consequently contribute to the pathology of some disorders. Newborns and particularly preterm infants are at high risk of ROS production and consequently oxidative stress [2,3].

The ability of ROS to react with polyunsaturated fatty acids of cell membranes, proteins, polysaccharides, and nucleic acids can damage DNA and other cell components [4]. Human body has a system to balance between antioxidant and oxidant-generating systems which contains enzymatic and non-enzymatic processes [5]. The enzymatic antioxidant defence includes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) [6].

The event of birth is a stressful experience in babies. Augmentation of oxidative stress has been shown to contribute to several neonate disorders such as chronic lung disease and neonatal respiratory distress syndrome [7]. For this reason finding the specific biomarkers of oxidative stress have been found special attention in clinical research [8].

Several studies have shown that caesarean sections are less stressful than spontaneous vaginal delivery, so blood cortisol and catecholamines levels are much lower in caesarean born neonates [9,10]. Also, Mears et al., reported significantly elevated fetal coritsol in babies born by instrumentation [11]. Tsukahara et al., measured urinary 8-hydroxy-2` deoxyguanosine as a marker of DNA damage in preterm neonates and found higher level in preterm neonates compared to healthy term neonates [7].

Alpha-tocopherol as an antioxidant molecule was found lower in premature infants compared to term neonates [7]. Gitau et al., studied cortisol level in cord venous blood of babies born via different modes of delivery; they found the lowest level in caesarean section born neonates that was different from normal vaginally delivery [10]. Another study in neonates showed that normal vaginally delivered neonates experience higher level of stress response compared to caesarian born infants [12].

Uric acid, the final product of purine metabolism in human, is another molecule possessing antioxidant activity. About two third of antioxidant activity of blood is due to uric acid.

However, increased maternal uric acid can lead to several disorders both for mother and fetus [13].

Despite published reports in this field, there is a controversy in their findings and all oxidative stress markers are not fully examined in different modes of delivery. Adekanle et al., showed no significant relationship between oxidative stress markers and mode of delivery [14]. Also Wilinska M et al., studying on 85 healthy mothers and their newborns indicated that oxidative stress intensity does not depend on the mode of delivery [15]. Nevertheless a published research on 2011 [16] indicated that oxidative stress is higher in caesarean section delivery compared to normal spontaneous vaginal delivery.

AIM

The present study aimed to examine some antioxidative enzyme activity containing SOD, GPx and catalase in newborns (both normal vaginal delivery and caesarian section). Furthermore, we determined and compared Total Antioxidant Capacity (TAC), uric acid and cortisol in these neonate groups.

MATERIALS AND METHODS

Sixty neonates with gestational age 38-42 weeks in the Neonatology Division, University Hospital of Fatemieh (Hamadan Iran), were enrolled in the study. According to the mode of delivery, they were divided into two groups: the first group comprised full-

term normal spontaneous vaginal delivery (ND) neonates that were born in a mean Gestational Age (GA) of 39 weeks (n=30) and the second group contained elective caesarean section neonates (CS) that were born with mean GA of 38 weeks (n=30). The neonates born after a normal delivery or caesarean having abnormal results in clinical examination at birth or born by mothers having diabetes or other metabolic disorders or multiple gestations were excluded. Both full-term and preterm healthy neonates irrespective of the mode of delivery, born to the healthy mothers with negative medical and obstetrical history and having a 5 min Apgar score ≥7 were included in this study. None of the subjects experienced prolonged delivery. The protocol of the study was approved by the ethical committee of Hamadan University of Medical Sciences (Hamadan, Iran). Informed consent was taken from all participated mothers.

Blood sample preparation

Blood samples were obtained from the umbilical vein post cord clamping immediately after delivery (up to 20 min) and were mixed with EDTA and immediately centrifuged at 3000×g for 15 min. The separated plasma was then stored at -20°C till analysis. Red blood cells were separated and homogenates were prepared for antioxidant enzymes assay.

Biochemical analysis

Superoxide dismutase activity in prepared haemolystae was determined using a commercial available kit (Ransod kit, Randox Laboratories Ltd, Crumlin, UK). The assay reaction in this kit was according to the superoxide production by xanthine and xanthine oxidase that react with a reagent and produces red formazon which absorbs light at 740 nm. SOD inhibits this reaction. CAT activity was measured spectrophotometrically at 240 nm by a chemical method (H_2O_2 decomposition). GPx activity was determined in the samples using reduced glutathione (GSH) as substrate (Randox kit). Plasma cortisol and antioxidant capacity were detected spectrophotometrically using IBL and Cayman kits respectively. Uric acid was measured enzymatically by Pars Azmun kit (Iran).

STATISTICAL ANALYSIS

Data are presented as mean±SD and analysed by t-test. Correlations between variables were assessed by linear regression. Statistical significance was defined as p <0.05.

RESULTS

In ND group, mean (±SD) age of mothers was 21.2 (±0.68) years with gestation period of 39 weeks, and in CS group it was 22.8 (±0.86) years with 38 weeks gestational period. [Table/Fig-1] shows that mother age and period of gestation did not differ significantly between the studied groups.

GPx activity was significantly higher (p=0.035) in CS group (average =162.18 U/ml, n= 30) compared to ND group (average =128.18U/ml, n=30) newborns [Table/Fig-2]. CAT activity did not show any significant difference between the two studied groups (p=0.91). The mean activity of this enzyme in ND group was 0.065 U/ml and in CS group was 0.067 U/ml.

The comparison of SOD activity between the two groups showed significantly higher level (p=0.045) in the caesarean born neonates (140.46 U/ml) compared to normal delivery born (125.89 U/ml).

Factor	Vaginal delivery (n=30)	Caesarean* (n=30)
Gestational age (Mean, week)	39±1.20	38±1.70
Mother age (year)	21.2±0.68	22.8 ±0.87
Birth weight (kg)	3.0± 0.180	3.0± 0.210
Apgar score	> 7	> 7
Neonate disorders	NO	NO

[Table/Fig-1]: Characteristics of the studied mothers and neonates.

We found uric acid level in neonates born via vaginal delivery (average=6.94 mg/dl) was significantly lower (p= 0.043) compared to that of the caesarean section neonates (average=8.05 mg/dl). TAC assay showed no significant difference between the two studied groups (ND group average =0.135 mM and CS group average =0.151 mM). As shown in [Table/Fig-2] cortisol level was higher in the babies born by normal delivery (mean=493.66 nmol/l), compared to those born by elective cesarean (mean=372.62 nmol/l), and the difference was statistically significant between the groups (p=0.001).

Factor	Vaginal Delivery (n=30)	Caesarean (n=30)	p-value*
	Mean (SD)	Mean (SD)	
GPx (U/ml)	128.18(44.08)	162.18(72.43)	0.035
CAT (U/ml)	0.065(0.06)	0.067(0.1)	0.91
SOD (U/ml)	125.89(18.02)	140.46(20.44)	0.045
UA (mg/dl)	6.94(1.53)	8.05(2.45)	0.043
TAC (mM)	0.135(0.06)	0.151(0.1)	0.53
Cortisol (nmol/l)	493.66(135.51)	372.62(98.42)	0.001

[Table/Fig-2]: Plasma levels of GPx, CAT, SOD, Uric acid, TAC and Cortisol in vaginal delivery and cesarean born neonates.
*Comparing between two studied groups using independent t-test.

DISCUSSION

The obtained results showed caesarean section born neonates are prone to more oxidative damage compared to normal delivery neonates. Higher activity of SOD and GPx in CS neonates indicated an increase in body defence against ROS production. The ROS are important participants in damage by virus infections, progression to cancer and neurodegenerative processes [17]. They can be toxic at molecular level and are important effectors in aging and life-span determination [18]. The studies show that breast milk plays an important role in protecting the newborn from ROS; it contains many antioxidant molecules that probably are vital for antioxidant defense at early stages of life [19,20]. It is believed that these antioxidants in breast milk contribute in scavenging free radicals [21]. Another strategy to protect babies from ROS is to promote their antioxidant defense, especially the glutathione system [22]. Since oxygen concentration in postnatal environment is higher compared to intrauterine, oxidative stress may threaten the preterm babies in the first days of birth [23].

A product of oxidative damage to DNA is 8-hydroxy-2`-deoxyguanosine (8-OHdG) that can be measured using ELISA or imunohistochemistry, has been found an application in pathology [24].

Acrolein-adduct lysine has been measured as a product of oxidative damage to protein and lipids, in healthy term and preterm neonates, indicating the higher level in preterm neonates [7,25].

Antioxidant enzymes are important in defence against ROS; it has been shown that the activity of these enzymes is low in preterm neonates [26]. Phylactos et al., measured Cu/Zn SOD in cord erythrocytes of preterm and term neonates and found that preterm neonates had only 50% of SOD activity compared to term neonates [27].

At birth there is a decline in neonates' antioxidant defenses, which is higher in lower gestational age. Robles et al., indicated that alpha-tocopherol level in preterm neonates is lower at birth comparing to that of term neonates. Also, a reduction in Co Q10 in red blood cell membrane is reported that can be related to maintaining the reduced form of vitamin E [28].

Our study indicated an association between modes of delivery and oxidative stress level. The other studies raised the question of possible correlation between the antioxidant defense of the neonate and the ways of delivery. Some of these studies have shown that prematurity and caesarean section might lead to

deficiency in antioxidant defense and production of higher level of oxidative stress [29,30].

Our results clearly show that the antioxidant defense mechanisms of the neonate are dependent on the way of delivery; distinctively the GPx activity was significantly higher in neonates born by caesarean section than in those born by spontaneous vaginal delivery. Also, we found higher SOD activity, an antioxidant enzyme and uric acid level in elective cesarean newborns than in infants with normal delivery at birth. It is similar to other studies that found uric acid as a non-enzymatic antioxidant rises in stressful conditions specially cesarean section. Indeed, we did not find significant differences in CAT activity and TAC level between the two groups.

In this study, we found higher cortisol level in normal delivery group compared to CS group. It is caused by performing potentially painful spontaneous vaginal delivery. It is well established that the normal birth process is associated with a large increase in the fetus of stress hormones such as cortisol and cathecholamines.

Taken these finding together, it can be stated that still there is controversy in this field. As it was mentioned above some researches believe that oxidative stress is dependent on mode of delivery [14,15], however, similar to our results, Siddiqui H et al., showed that "Mothers who underwent elective caesarean section and their neonates were exposed to higher oxidative stress as compared to those who had vaginal delivery" [31].

CONCLUSION

Based on our assays it can be concluded that babies born via caesarean section might be predisposed to pathological conditions due to altered antioxidant levels. Also it can be concluded that measurement of antioxidant factors can be useful in choosing a preventive way for free radical damage and selection of appropriate prophylaxis approach.

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