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Original Article

Physiology Section

Oxidative Stress in Preterm Neonates: An Analysis of Oxidative Stress Biomarkers and Antioxidant Profiles

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ABSTRACT

Introduction: Oxidative stress is a complex event determined genetically and induced by an in- utero stressor. Oxidants are composed of reactive free radicals like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which are manifested by several macromolecules of lipid, protein and DNA, causing deleterious effects in several organs. Antioxidant defense mechanism and its ability to be induced by hyperoxia is relatively impaired in preterm neonates.

Aim: To study oxidative stress and antioxidants in preterm neonates.

Materials and Methods: This study is an observational analytical study, which included preterm babies (25 males and 20 females) delivered vaginally from October 2012 to October 2013. Cord blood was collected in citrate bulbs immediately after vaginal delivery and stored at 4°C until processed. Malondialdehyde (MDA), Nitrates, Vitamin C and Vitamin E, levels were measured in cord blood. Statistical z-test was applied.

Results: High levels of oxidative stress biomarkers like MDA and Nitrites along with decreased levels of antioxidants, Vitamin C and Vitamin E in preterm neonates was observed. MDA and Nitrates levels were significantly higher in males ($p<0.05$) than females. Vitamin C and Vitamin E levels were not significant ($p>0.05$) in both.

Conclusion: This study results may conclude that preterm neonates have more oxidative stress especially in males affecting their life survival.

Keywords: Biomarkers, Cord blood, Malondialdehyde, Nitrates, Preterm newborns

INTRODUCTION

Oxidative stress is the common end point for a complex of events that either are genetically determined or triggered by an in-utero stressor [1]. Oxidative stress is conceptually defined as the imbalance between generations and clearances of oxidant [2]. Oxidants are composed of reactive free radicals like Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) causing deleterious effects in several organs [3]. Antioxidant defense mechanism and its ability to be induced by hyperoxia are relatively impaired in preterm infants [4]. Antioxidants enzymes participate in a complex interaction that defines the cellular milieu necessary for maintaining cellular, placental, foetal and postnatal growth [5].

During the transition from foetal to neonatal life, at birth the foetus is transferred from an intrauterine hypoxic environment with the oxygen tension PO_2 of 20-25 mmHg to an extra-uterine normoxic environment with a PO_2 of 100 mmHg. This increase induces an elevated production of ROS as well as RNS [6]. Oxygen vital, to survival is highly damaging to foetal tissues which are poorly equipped to neutralise its toxic derivatives so it currently recognise that oxidative stress is elevated when resuscitation is performed with 100% oxygen [7]. Many neonatal diseases are closely connected with oxidative stress. An early sign of oxidative stress is the appearance of lipid peroxidation products, can ultimately lead to cell death [8]. Oxidative stress can be measured by using various parameters. Malonaldehyde is one such product and used as oxidative stress biomarker.

Malonaldehyde measurements can provide a sensitive index of lipid peroxidation and oxidative stress. Nitric Oxide (NO) is synthesised in an oxygen dependent reaction catalysed by Nitric Oxide Synthetase (NOS). Measurement of nitrous oxide radical itself is difficult due to its poor stability with a very short half life. Plasma levels of inorganic nitrites and nitrates representing the stable and final metabolites of the NO-metabolic pathway, can be measured with calorimetric assays [9].

In human, antioxidants maintain a state of health. The antioxidant like Vitamin C traps free radicals and ROS. Under all types of oxidising conditions, ascorbic acid (Vitamin C) completely protects lipids in plasma and low density lipoproteins against oxidative damage. Antioxidant vitamin C in plasma protects lungs, lipids and low density lipoproteins from oxidative damage [10].

Vitamin E acts as a free radical scavenger, reducing peroxidation of membrane polyunsaturated fatty acids. Antioxidant Vitamin E has ability to stabilise highly reactive free radical. Preterm babies are particularly sensitive to oxidative stress involving an imbalance in the production of oxygen free radicals and the antioxidant defense system. Deficiency of antioxidant molecules can occur in tissues and cause damage to proteins, carbohydrates, lipids and DNA, which made the authors to take-up the study [11]. Saugstad OD suggested the phrase “oxygen radical diseases of neonatology” as adverse effects of oxidative stress has been postulated to be implicated in several newborn conditions like bronchopulmonary dysplasia, chronic lung disease, retinopathy of prematurity and necrotising enterocolities [12]. The stable and final metabolites of the NO-metabolic pathway present in the form of inorganic nitrites and nitrates in the plasma, its level can be measured with calorimetric assays. Later Haynes RL et al., in their study proved that free radicals are also involved in peri-ventricular leucomalacia, in regulating the ductus arteriosus and pulmonary circulation [13]. Hence, the present study was conducted with the aim of studying preterm neonatal oxidative stress and antioxidant profile using cord blood.

MATERIALS AND METHODS

Analytical observational experiment in Physiology, October 2012 to October 2013. Ethical approval letter no. 17/2012, MGIMS, Sevagram. Informed consent in proforma was taken from parents. Healthy 45 preterm newborns delivered vaginally were selected, 25 males and 20 females. Gestational age- 34 to 36 weeks.

Gestational age of the newborns was calculated using NewBallard Scoring System for assessment of gestational age [14].

Inclusion criteria: The mothers who don't have any medical or obstetric complications and the newborns who cried immediately after birth, required no resuscitation. Birth weight of newborn to range between 2.2 kg to 2.5 kg.

Exclusion criteria: Newborns with Congenital malformations, Birth asphyxia.

Sample collection: Newborn's 10 mL of cord blood was collected in EDTA bulb immediately after its delivery in labor room. Blood and the plasma was separated and processed within 6 hours of sample collection. All the tests were read on the Syntronics uv-vis spectrophotometer in the department.

Four parameters were measured- Malonaldehyde, Nitrates, Vitamin C and Vitamin E from cord blood of newborns. MDA was measured by action of thiobarbituric acid with MDA. It forms a pink chromogen compound, which was calculated using standard formula, whose absorbance at 530 nm was recorded [15]. The concentration of MDA (nmol/mL) was calculated using standard curve obtained from the reaction between varying MDA concentrations. Nitrites were measured using the GriessReagent assay method. In acid solution, nitrite is converted in to nitrous acid (HNO_2) which is reacted with sulphonamides [16].

This sulphonamide- Dizonium salt is then reacted with 1 Naphthyl-Ethylene Diamine (NED) to produce chromophore, which is measured at 540 nm concentration of nitrites (μM/dL), calculated from standard curve obtained from the reaction between varying nitrite concentrations. Vitamin C was measured by its reaction with Phosphotungstate (PTA) [17].

The acid PTA used in this method as plasma protein precipitant as well ascorbic acid extractant and colour developing agent, as it gets reduced to tungstate blue by ascorbic acid. Blue colour is measured in a spectrophotometer at 700 nm.

Vitamin C concentration (mg/dL) was calculated using standard formula. The estimation of Vitamin E levels in cord blood was done by Emmerie Engel procedure in which tocopherol is oxidised to tocopherol quinone by ferric chloride and resultant ferrous ion complexed with 2,2'-dipyridyl to produce a red coloured compound whose absorbance was measured at 520 nm [18]. Vitamin E level-were determined from standard curves already calculated from different concentrations of Vitamin E.

STATISTICAL ANALYSIS

Statistical software- EPI INFO version 7 was used for statistical analysis. All values were expressed as Mean±SD values in each group. Z-test was used for calculating the significance.

RESULTS

All the included subjects had gestational age between 34 to 36 weeks. Males were 25 and females were 20. Birth weight ranged between 2.2 to 2.5 kg. The present study showed significantly increased levels of MDA (8.36±0.07 μmol/dL vs. 8.19±0.05 μmol/dL; p<0.05) and Nitrites (30.46±0.43 μmol/dL vs. 28.78±2.21 μmol/dL; p<0.05) in the preterm males as compared to preterm female babies. No significant differences were noticed in the levels of antioxidants Vitamin C and E levels in the preterm male and female newborns (p>0.05), shown in [Table/Fig-1] below.

Parameters	Mean	Female (n=20)	Male (n=25)	z-value	p-value
MDA nmol/mL	8.27	8.19±0.05	8.36±0.07	8.14	<0.001
Nitrates μmol/dL	29.62	28.78±2.21	30.46±0.43	3.64	<0.001
Vitamin C mg/dL	79.8	80.12±3.31	79.48±2.61	0.69	0.49
Vitamin E mg/dL	6.65	6.58±0.61	6.73±0.48	0.88	0.38

[Table/Fig-1]: Oxidative stress parameters and antioxidant status in preterm babies. MDA: Malondialdehyde; p-value <0.05 to be considered significant

DISCUSSION

Present study showed significantly higher levels of oxidative stress biomarkers like MDA and Nitrates in the preterm neonates. This leads to preterm neonates more prone to oxidative stress. Study findings are similar to Thomas C et al., reported more production of highly toxic hydroxyl radical leading to more oxidative stress in preterm neonates [19].

MDA levels were increased in preterm infants due to increased oxidant burden and exposes preterm neonates to increased oxidative stress [20,21], it coincides with the present study. Maulik D et al., in their study has stated that the preterm baby is more susceptible to oxidative damage than full term, due to exposure to high oxygen [22]. So, both endogenous and passively acquired exogenous antioxidant defence system do not accelerate in maturation till late trimester. Preterm delivery is the most important contributor to the neonatal mortality rate as proved by Yasmin S et al., [23]. As such preterm newborns have higher incidence of mortality and morbidity as compared to term newborns. This increased mortality and morbidity could be related to increased oxidative stress and low levels of oxygen in preterm babies.

Greater incidence of problems in pregnancy and complications of delivery, birth asphyxia and neurological signs in neonatal period [24] a higher incidence of infections occurring after prolong rupture of membranes [25]. Congenital malformations have been reported in males, more commonly hyaline membrane disease and bronchopulmonary dysplasia. These studies reported that preterm males have increased incidence of complications and poor survival as compared to preterm females. Since, these diseases have been linked to toxicity of ROS, hence an increased oxidative stress in preterm males. In general, males have increased morbidity and mortality than females in preterm groups. Since the diseases have been linked to toxicity of ROS, the increased oxidative stress in preterm neonates as is observed in this study can partly explain the differences in perinatal and postnatal morbidity and mortality in male neonates. Thus, this study is in accordance with the above mentioned studies and proves that preterm male babies have more oxidative stress as compared to females. Newborns and in particular preterm neonates have less protection and are very susceptible to free radical oxidative damage.

Negi D et al., in their study states that Vitamin C and Vitamin E, act as first line of defense against free radical attack [26]. These antioxidants Vitamin C and Vitamin E react with activated oxygen species and thereby prevent the propogation of free radical chain reactions. Their low levels result in decreased antioxidant defense system in preterm neonates. However, there can be presence of association between oxidative stress biomarkers and antioxidants in preterm neonates. The concept may be revealed by the observation that, the expression of antioxidant enzymes reaches the appropriate level only at the end of gestation and preterm infants are therefore vulnerable to oxidative stress as studied by Buonocore G et al., [27]. Thus, several important factors contribute to augmented oxidative stress in the preterm neonates. In this study, it may be concluded that an impaired antioxidant defense, may be a factor for more oxidative stress in preterm neonates.

Limitation(s)

Maternal levels of antioxidant Vitamins were not taken for consideration. Sample size (N) is limited, more size, provides significance Age group variation can lead to good results.

CONCLUSION(S)

Preterm neonates are exposed to increased oxidative stress at birth and are susceptible to reduced antioxidants comparatively more in males, may be related to hormonal basis.

In future, oxidative damage can be controlled clinically by hastening impaired antioxidant defense system of the foetus, with the provision

of antioxidant, Vitamin C and Vitamin E to the mother during pregnancy. Also, to be given to the baby after birth as a nutritional intervention. Therefore, further intensive studies are needed to frame proper management strategies for preterm neonates and their diseases.

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