Serum Pro-Oxidant/Antioxidant Balance in Term Versus Preterm Neonates

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Abstract

Introduction: The oxidant/antioxidant status balance is a process that begins before birth and premature infants are particularly susceptible to oxidative stress. According to the mechanisms of oxidative stress and lack of study in this field, in this prospective study we aimed to compare the levels of serum pro-oxidant/antioxidant balance (PAB) in preterm versus term babies.

Methods: This was a prospective cross sectional study that was performed in Ghaem hospital, a university tertiary hospital, Mashhad, Iran. The study population was included of all term and preterm neonates who were admitted to the hospital within birth time.

Results: In our study, 324 neonates were included. One hundred ninety eight neonates were preterm (61.1%) and others were term (38.9%). The mean birth weight of participants was 3267.19± 446.35gr in term and 1658.78± 644.97 gr in preterm neonates. There was significant difference between PAB level in term and preterm neonates. Serum PAB level was significantly lower in preterm neonates rather than term neonates (21.86± 21.01 versus 50.33± 31.69; P=0.001). There was also significant negative correlation between PAB levels and gestational age.

Conclusion: According to previous investigations, we showed for the first time in our study that PAB is lower in preterm newborns rather than term ones. This is in line with the hypothesis that oxidative stress is higher in preterm neonates.

Introduction

Free radicals (FRs) are molecular species with an unpaired electron in the outer shell, which renders them highly reactive and unstable. FRs containing oxygen may be termed a reactive oxygen species (ROS). The accumulation of reactive FRs, beyond the capacity of the endogenous antioxidant defense system to scavenge them, results in damage to DNA, proteins, and lipids that compromises cell function, leading to cell death via apoptosis or necrosis [1]. Fetal life occurs in a relatively hypoxic environment. Hypoxia is necessary for the development and growth of the fetus. Under normal circumstances, the fetal-to-neonatal transition causes physiological oxidative stress (OS), which enhances the antioxidant defense and pulmonary surfactant maturation [2].

The oxidant/antioxidant status balance is a process that begins before birth [3], and premature infants are particularly susceptible to oxidative stress [4, 5]. Most of the complications of prematurity, such as bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and punctate white matter lesions (PWML), appear related to oxidative stress [6, 7], mostly occurring due to a mismatch among the free radical production and the anti-oxidative capacity of the premature neonate [5].

Moreover, antioxidant defense mechanisms are incompletely developed or deficient in preterm newborns [8]. Preterm infants show reduced antioxidant defense mechanisms, including decreased levels of

vitamin E, β-carotene, melatonin, ceruloplasmin, transferrin, and erythrocyte superoxide dismutase (SOD) [5]. In a study on 100 preterm and 100 full-term neonates, plasma levels of vitamin A, vitamin E, and catalase were found significantly lower while plasma level of MDA, a marker of lipid peroxidation, was significantly higher in the preterm than in the full-term newborns, especially in those ones who developed NEC or BPD [9]. Premature babies have both immature lungs and antioxidant defense systems and often require oxygen supplementation to overcome respiratory distress. The combination of hyperoxia, which enhances the generation of reactive oxygen species (ROS), and low antioxidants cause oxidative stress, inflammation, and even apoptosis, thus increasing mortality and morbidity [10].

According to the mechanisms of oxidative stress and lack of study in this field, in this prospective study we aimed to compare the levels of serum pro-oxidant/antioxidant balance (PAB) in preterm versus term babies.

Methods

Study Population

This was a prospective cross sectional study that was performed in Ghaem hospital, a university tertiary hospital, Mashhad, Iran between 2015 and 2019. The study population was included of all term and preterm neonates who were born in Ghaem hospital within birth time. This investigation was approved by the Ethical Committee of Mashhad University of Medical Sciences. We obtained parents' informed written consent before entry in the study. The term low birth weight. 'Low birth weight' (LBW) has been defined as first weight recorded within hours of birth of less than 2500 grams between 33 and 37 weeks gestational age. Very low birth weight (VLBW) is accepted as less than 1500 grams in lower than 32 weeks gestational age.

Eligibility

We recruited all babies born in our center during the study interval without any abnormal examination, known anomalies in perinatal assessment, hypotonia, bradycardia, low Apgar score, requiring cardiopulmonary resuscitation or need to use accessory oxygenation. We excluded neonates with severe birth defects, respiratory distress, positive history of eclampsia/preeclampsia in mothers, birth asphyxia, suspicious to sepsis, hemolytic hyperbilirubinemia, Rh or ABO incompatibility and pathologic jaundice neonates.

Data collection

We completed a checklist for each neonates including gestational age, gender, birth weight and Apgar score. Moreover, mothers' data included age, parity, pregnancy-related problems, mode of delivery and blood type were completed. Babies after sampling and data collection underwent routine care if necessary during their hospital admission. Blood samples were taken from umbilical cord (1 cc for each neonates) and were used for PAB measurement after centrifuge and separation of serum. For this measurement, the separated blood samples were transported to Bu-Ali Research Institute.

PAB measurement

The solutions were prepared as indicated. The standard solutions were made by mixing varying proportions (0-100%) of 250-μM hydrogen peroxide with 3mM uric acid in 10mMNaOH. In order to prepare the TMB cation, 60 mg of TMP powder was dissolved and mixed well at a ratio of 10–20 ml of the solution. Then, the prepared solution was placed in the dark for 2 hours. Next, 25 units of peroxidase enzyme were added to 20 ml of the solution, and placed at 20^{o}C. For preparation of the TMB solution, 200 ml of TMB was added into 10 ml of acetate buffer (0.05 M buffer, pH 5.8); the working solution was prepared by mixing 1 ml of TMB cation with 10 ml of TMB solution. The prepared solution was placed in a dark, dry place for 2 minutes. Next, 10 μL of each sample was mixed with 200 μL of the working solution and placed in a 96-well plate in the dark at 37^{o}C for 12 min. At the end of the process, 100 μL of 2N Hall was added to each well and measured in an ELISA plate reader at 450 nm and 620 nm wavelengths. PAB values were calculated on the standard curve as previously published [11].

Statistical analysis

Data were entered in SPSS-20 software (IBM, Chicago, IL). The continuous values were indicated as mean ± SD. Independent t-test was used for continuous variables. Parametric and non-parametric correlations were assessed using Pearson's correlation coefficients and Spearman's correlation coefficients, respectively. P value lesser than 0.05 was considered statistically significant.

Results

In our study, 324 neonates were included. One hundred ninety eight neonates were preterm (61.1%) and others were term (38.9%). Table 1 shows the demographic data of neonates.

Table 1
Demographic data of neonates

Variable	Term (n = 126) (Number, Percent)	Preterm(198) (Number, Percent)		P value
		Early (n = 101)	Late (n = 97)	
Gender	Male (76, 60.3)	Male (44, 43.5)	Male (51, 52.5)	0.141
	Female (50, 41.67)	Female (57, 56.5)	Female (46 ,47.4)	
Type of delivery	NVD (48, 41.4)	NVD (28, 24.1)	40 (34.5)	0.120
	CS (77, 37.4)	CS (72, 35)	CS (57, 27.7)	

The mean birth weight of participants was 3267.19 ± 446.35gr in term and 1658.78 ± 644.97 gr in preterm neonates. Other perinatal factors are listed in Table 2.

Table 2
Differences between perinatal factors in term and preterm neonates

Variable	Gestational age	n	Mean	Std. Deviation	P
Birth weight	preterm	197	1658.78	644.97	0.001*
	term	123	3267.19	446.35	
Apgar score of first minute	preterm	197	7.50	1.67	0.001*
	term	122	9.42	0.67	
Apgar score pf fifth minutes	preterm	196	8.91	1.31	0.001*
	term	39	9.82	0.38	
Maternal age	preterm	197	28.69	6.10	0.368*
	term	39	27.92	4.53	
Parity	preterm	198	2.12	1.27	0.002*
	term	122	2.59	1.41	
PAB level	preterm	187	21.86	21.01	0.001**
	term	126	50.33	31.69	

PAB = Pro-oxidant/Antioxidant Balance, * *Independent T-Test*, ** *Mann Whitney Test.*

As it can be seen in Table 2, there was significant difference between PAB level in term and preterm neonates. Serum PAB level was significantly lower in preterm neonates rather than term neonates (21.86 ± 21.01 versus 50.33 ± 31.69; P = 0.001). It is also shown in Fig. 1 the difference between PABp levels according to gestational age categories.

There was also significant negative correlation between PAB level and gestational age by number (r=-0.547, P < 0.001). Figure 1 shows the correlation between PAB level and gestational age.

Linear Regression analysis showed that birth weight (P = 0.005) and gestational age (P = 0.002) were predictors of PAB level in our study population.

Discussion

To best of our knowledge, this study was of limited investigation that compared the level of PAB level in term versus preterm neonates. Our findings showed that PAB level was significantly lower in preterm neonates. There are different studies in neonatal population about PAB level. In a study, it was shown that there was no significant relationship between umbilical cord blood pro-oxidant antioxidant's balance and type of delivery [12]. In another study, it was shown that a pathological increase in bilirubin levels irrespective of its neurotoxic properties can change the PAB in favor of antioxidants [13]. In asphyxia, it was demonstrated that PAB in combination with hypoxic-ischemic encephalopathy (HIE) grade might

have a better predictive value for the prognosis of asphyxiated babies and predicting future neurologic problems in asphyxiated term infants [14].

There are more data on other oxidative indexes in preterm and term neonates. It was shown in previous studies that newborn preterm neonates had high level of oxidative stress biomarkers. These markers were evaluated such as plasma MDA-hemoglobin [15], plasma F2-isoprostane [16], plasma malondialdehyde (MDA) [9, 17, 18], erythrocyte membrane hydroperoxide levels [19] and plasma lipid peroxidation [20] in preterm newborns clearly show higher levels of lipid peroxidation markers. It is also demonstrated that in comparison with full-term neonates, there are higher levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) [21], protein carbonyl [17, 22] and desferrioxamine chelatable iron [16] in preterm newborns. Compared to full-term birth, preterm newborns also enhances plasma non-protein bound iron concentration [16], [22], which can lead to oxidative damages through the Fenton reaction. Some studies reported that oxidative stress levels are negatively correlated with gestational age [16, 17, 21, 23, 24]. In our study, this correlation was also confirmed. Our findings showed that preterm neonates had lower level of PAB in comparison with term neonates. This strengthens the hypothesis that prematurity itself is at least partly capable for higher oxidative stress in preterm newborns.

This can because of lower level of different antioxidant biomarkers. It is explored that at birth, superoxide dismutase (SOD) activity in both blood [18, 25] and erythrocytes [19, 26], catalase (CAT) activity in blood [9] as well as cytosolic glutathione peroxidase (GPx) in erythrocytes [19] are lower in preterm than in full-term newborns. Preterm newborns also exhibit lower levels of non-enzymatic antioxidants such as erythrocyte vitamins [27]. Many physiological mechanisms can induce the higher oxidative stress levels observed in preterm newborns. It is known that oxidative stress level in mothers vary during the pregnancy [28] and this can because of an increased oxidative stress in the placenta [28, 29]. This oxidative stress in the placenta is probably necessary for its development by regulating the proliferation, the differentiation and the invasion of trophoblasts, promoting placental angiogenesis and regulating autophagy and apoptosis required for placentation [30]. However, a high level of systemic oxidative stress in mother can be related to preterm labor and prematurity [31]. Therefore, higher oxidative stress level in pregnant women might lead to dysfunction of placenta or other damages that lead to preterm labor and be responsible for the high oxidative stress level observed in preterm newborns by direct blood exchange in the placenta [27]. In other hand, preterm newborns have an immature antioxidant system, as the last weeks of pregnancy corresponds to the maturation and upregulation of the fetuses antioxidant system [9] and the transfer of some antioxidants from the mother to the fetus [18]. This may explain why babies born early in their third trimester exhibit lower concentration of antioxidants. Moreover, preterm newborns might need several medical interventions because of their immature state and this can also induce ROS generation.

Although in previous studies, there was many biomarkers of oxidative stress evaluated in preterm versus term newborns, there is still need to find applicable test to find this hypothesis. In this study, we used an approach with potential clinical applications in oxidant/antioxidant assays, which was pro-oxidant antioxidant balance (PAB) technique. The assay is a new strategy to determine the pro-oxidant load and

antioxidant capacity in a single assay. The assay provides general view on the oxidant/antioxidant status of the patients in one single experiment [32].

Conclusion

According to previous investigations, we showed for the first time in our study that PAB is lower in preterm newborns rather than term ones. This is in line with the hypothesis that oxidative stress is higher in preterm neonates. Targeting oxidative stress in preterm neonates as an applicable clinical index can complete the pathway of effects of oxidative stress in preterm newborns. More studies by targeting PAB is needed in the future to prevent poor prognosis of preterm newborns in chronic disorders in childhood.

Declarations

Ethics approval and consent to participate

This study was approved by the ethical committee of research and technology of Mashhad University of Medical Sciences and the names of the participants were kept confidential.

Consent for publication

The researchers obtained consent for publication from the participants' parents.

Availability of data and material

The data that support the findings of this study are available from the pediatric department of Medical faculty, Mashhad University of Medical Sciences, yet restrictions apply regarding the availability of these data, which were used under license for the current study, and are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of the corresponding author.

Competing interests

The authors declare that they had no competing interests.

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Authors' Contributions

Dr. H.B and Dr. M, Gh were responsible for the study concept, development of methodology, coordinating of the research activities, and data analysis. Dr. A.S was involved in methodological development, data collection, data input, and analysis and writing of the manuscript. All authors read and approved the final manuscript.

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Figures

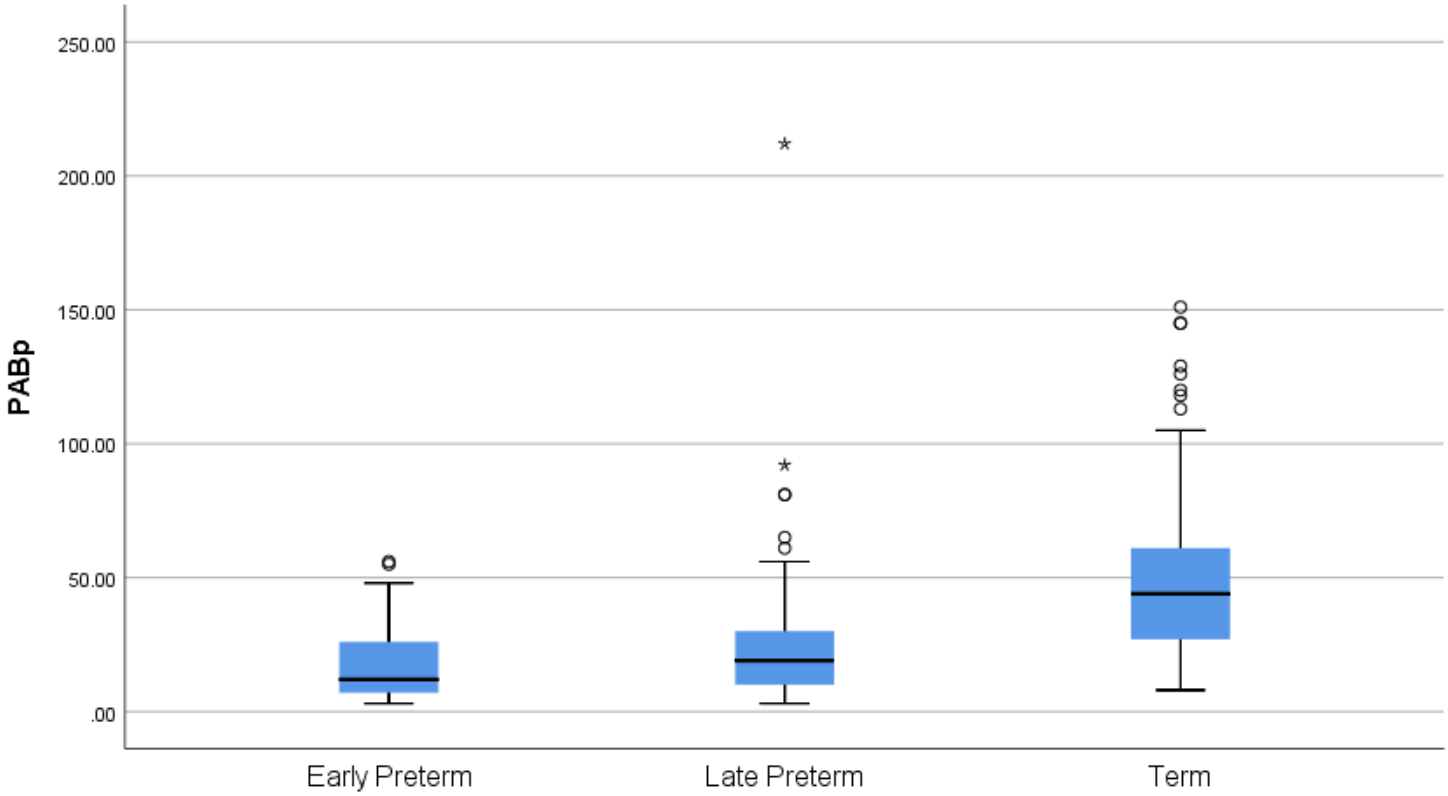

Figure 1

The difference between PABp levels according to gestational age category

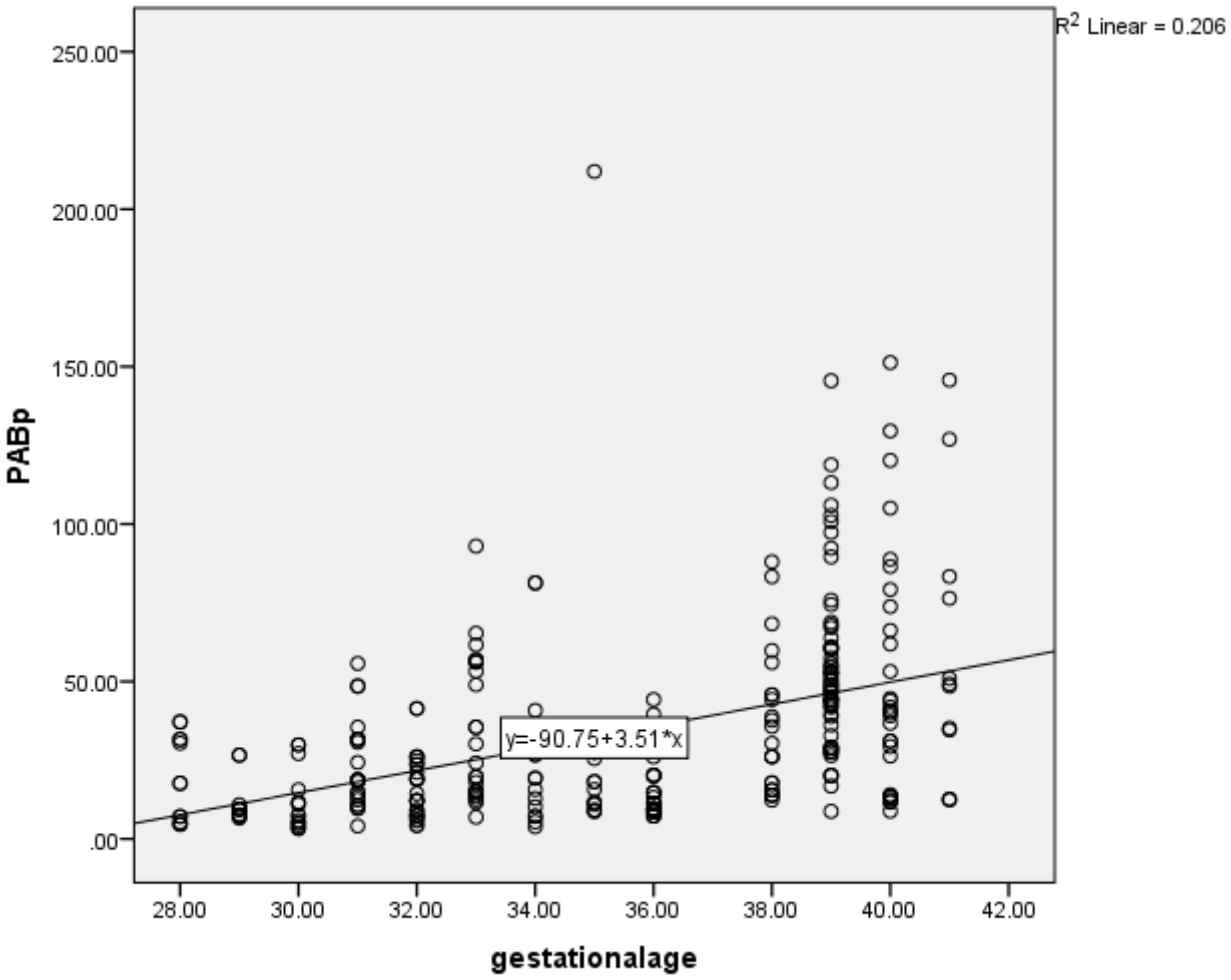

Figure 2

Correlation between PAB (Pro-oxidant/Antioxidant Balance) level and gestational age