

Serum paraoxonase and arylesterase values as antioxidants in healthy premature infants at fasting and posprandial times

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Abstract. – OBJECTIVE: Newborn infants, particularly preterm infants, are at greater risk of oxidative stress because of an imbalance between high oxidant loads and immature antioxidant defenses. In several studies, the activities of serum paraoxonase (PON) and arylesterase (ARE) have been found to decline in patients under increased oxidative stress. We investigated the relationships between PON-1 and ARE with fasting and postprandial in premature newborns in this study.

PATIENTS AND METHODS: Serum paraoxonase-1 and arylesterase levels were investigated in premature infants less than 37 weeks, after birth while they were fasting and postprandial.

RESULTS: The paraoxonase-1 and arylesterase values of infants in fasting were significantly lower than the values in postprandial (for paraoxonase-1, $p = 0.034$, 0.002 , and 0.002 , respectively; for arylesterase, $p < 0.001$, 0.002 , and $p < 0.001$, respectively).

CONCLUSIONS: In premature infants, paraoxonase-1 and arylesterase values are increased in postprandial and reduced in fasting, showing that these neonates are subjected to oxidative stress. Thus, starting feeding as soon as possible in premature newborns is vital to protect them from oxidative damage.

Key Words:

Premature newborn, Paraoxonase-1, Arylesterase.

Introduction

Newborn infants, particularly preterm infants, are at greater risk of oxidative stress because of an imbalance between high oxidant loads and immature antioxidant defenses¹. Poor fetal growth affects the development of antioxidant defenses in preterm neonates, predisposing them to higher oxidative stress, which, in turn, may account in part for the increased morbidity and mortality in these infants². It has been reported that oxidative damage to the tissues of premature newborns may be due to immature antioxidant defense mechanisms and a deficiency of antioxidant substances provided to the fetus by the mother during the final period of pregnancy because most antioxidant molecules do not cross the placenta until the third trimester of gestation³.

The premature infant is especially susceptible to reactive oxygen species (ROS)-induced damage because of inadequate antioxidant stores at birth, as well as impaired upregulation in response to oxidant stresses. Thus, the premature infant is at increased risk for the development of ROS-induced diseases of the newborn, such as bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, and periventricular leukomalacia⁴⁻⁶.

Free radicals and related species are derived mainly from ROS⁷. Enzymatic protection against ROS is provided by many systems, such as superoxide dismutase, catalase, and glutathione peroxidase. Other than these important enzymatic antioxidants, paraoxonase-1 (PON-1) also appears to have antioxidant properties⁸. Paraoxonase is known to retard the oxidation of low-density-lipoprotein cholesterol (LDL-C), preventing the generation of lipid peroxides⁹. PON-1 is known to have antioxidant/anti-inflammatory properties; it also decreases during the inflammatory response and so is classified as a negative acute-phase protein⁸. It has been shown that PON-1, along with other antioxidants, is decreased in marasmic children⁹. In several studies, the activities of serum paraoxonase and arylesterase (ARE) have been found to decline in patients under increased oxidative stress¹⁰⁻¹⁴.

To our knowledge, there is no previous report of the relationship between fasting and postprandial and PON-1 and ARE values in premature newborns. Thus, we investigated in this study the relationships between PON-1 and ARE with fasting and postprandial in premature newborns.

Patients and Methods

Patients

The study included 30 neonates hospitalized and treated with a diagnosis of prematurity at Erzurum Nenehatun Obstetrics and Gynecology Hospital Neonatal Intensive Care Unit (NICU) between June 2010 and December 2010. The study was initiated after obtaining Local Ethics Committee approval and written informed consent from the families. Measurements of body weight, height, and head circumference of infants were performed at birth. Neonates with asphyxia, respiratory distress syndrome (RDS), transient tachypnea of newborn (TTN), pneumonia, intrauterine growth retardation (IUGR) and small-for-gestational-age (SGA), congenital somatic anomalies, severe congenital heart disease, those whose mothers were active or passive smokers, had a medical history of rupture of membranes for more than 24 h, vaginitis, and/or chorioamnionitis were excluded. Neonates where hemodynamically significant patent ductus arteriosus was detected during hospitalization, neonates where sepsis and/or necrotizing enterocolitis, feeding intolerance, and/or gastrointestinal bleeding and intracranial hemorrhage developed,

neonates with severe apnea and hypoglycemia, where blood transfusion was performed, or who died during the study were also excluded.

The neonates that could be fed with breast milk were started on breast-feeding and those without breast milk were started on feeding with infant formula. The neonates who could not be fed enterally were fed first with total parenteral nutrition (TPN) and then minimal enteral nutrition; enteral nutrition was subsequently increased gradually. TPN in neonates in which feeding reached 100 mL/kg/day was stopped and full enteral feeding was started.

Blood Samples

The first blood samples were taken after birth, immediately after admission to the NICU after breast-feeding or use of TPN, in postprandial. The second blood samples were taken on first samples than 3 h after feeding, in fasting. The blood samples for paraoxonase and arylesterase values of the neonates were taken from peripheral veins. Blood samples for hormone analysis were drawn into no-additive Vacutainer tubes and centrifuged (3500 g, 10 min, 4°C); the resulting serum samples were stored at -80°C until assayed. All samples were run in duplicate, and the two measurements were averaged for statistical analyses.

Measurement of PON-1 and ARE Activities

PON-1 and ARE activities were measured by methods described previously, with some modifications¹¹. All measurements were performed in 96-well UV plates and the kinetic absorbances were measured using a spectrophotometric microplate reader and its software (PowerWave XS, KC Junior; Bio-Tek Inc. Winooski, VT, USA). For the measurements of PON-1 and ARE activities, diethyl-p-nitrophenyl phosphate (Sigma Co., Newport, UK) and phenyl acetate (Sigma Co., UK) were used as substrates, respectively. Molar absorption coefficients were used in the calculation of PON-1 and ARE activities were 17100 M⁻¹ cm⁻¹ and 1310 M⁻¹ cm⁻¹, respectively. One unit of PON-1 activity was defined as 1 nmol 4-nitrophenol/mL serum/min and that of ARE activity as 1 mol phenol/mL serum/min.

Statistical Analysis

The NCSS 2007 and PASS 2008 software (Utah, USA) were used for statistical analyses. The Mann-Whitney U-test was used to compare quantitative data with non-normal distributions.

Yates' continuity correction test (Yates' corrected χ^2) was used to compare qualitative data. The Wilcoxon signed-rank test was used to assess changes between parameters. Spearman's correlation analysis was used to evaluate the correlations between parameters. Significance was evaluated at the $p < 0.01$ and $p < 0.05$ levels.

Results

In total, 37 neonates were included. Three neonates were excluded due to the diagnosis of sepsis and four neonates due to severe congenital heart disease. The mean gestational age of the 30 infants analyzed was 30.1 ± 2.1 (28-36) weeks. There were 17 (56.7%) female and 13 (43.3%) male infants. Sixteen infants (53%) were born by the vaginal route, and 14 (47%) by caesarean section. The mean birth weight was 1654 ± 342 (1040-2350) g; the body weight on the fifth day was 1884 ± 428 (1240-2900) g. The mean height at birth was 41.4 ± 2.6 (38-48) cm, the mean height on the fifth day was 42.2 ± 2.7 (38.4-48.7) cm. The mean PON-1 and ARE values were 25.8 ± 21.6 (2.2-78.8) U/mL and 15 ± 19.6 (2.2-75) U/mL respectively fasting at birth. The mean PON-1 and ARE values were 36.6 ± 21.7 (2.4-105.6) U/mL and 29.4 ± 36.0 (3-155.6) U/mL respectively postprandial at birth. The mean PON-1 and ARE values of neonates in fasting were lower than postprandial at birth the difference was significant ($p = 0.034$, $p = 0.001$, respectively).

Discussion

We demonstrated that the values of PON-1 and ARE, which have anti-oxidant properties, were low while the neonates were fasting but increased when the babies were satiated. This shows that fasting resulted in oxidative stress. Newborn infants are at greater risk of oxidative stress because of an imbalance between high oxidant loads and immature antioxidant defenses¹.

Negi et al² found increased lipid peroxidation, protein oxidation, with decreased values of vitamin A, E, C, and total antioxidant capacity in the cord blood of preterm low-birth-weight (LBW) neonates. They stated that observations of negative correlations between malondialdehyde (MDA) and protein carbonyls with antioxidants vitamins A, E, and C, and total antioxidant status

pointed to the existence of oxidative stress in the preterm LBW newborns.

Baydas et al¹⁵ reported that levels of lipid-soluble antioxidant vitamins E and A in cord blood were lower than maternal values. Conversely, the cord blood water-soluble vitamin C level was higher than maternal levels. Significantly higher levels of vitamins E, A, and C were found in term neonates compared with those born preterm.

Negi et al¹⁶ found significant elevation in the levels of 8-OHdG along with MDA in preterm LBW newborns. Serum 8-OHdG was found to be significantly and negatively correlated with the birth weight and gestational age of the newborn. They suggested that these results provided evidence of increased oxidative stress in the form of DNA damage and lipid peroxidation in premature LBW newborns, which might be responsible for some of the complications associated with prematurity. In a review¹⁷, it was suggested that improving early postnatal protein nutrition and the formulation of parenteral nutrition fluids could reduce oxidative stress and enhance the antioxidant defenses of extremely premature newborns.

In another study¹⁸, hydroperoxide concentrations were found to be high, especially in premature infants, in erythrocyte membranes at birth and in the initial days of life. The erythrocyte membrane has been found to contain low levels and/or low activities of antioxidant defense mechanisms, which are more evident in premature newborns, where α -tocopherol levels are significantly lower than in full-term infants.

Some authors do not subscribe to the view that there is increased morbidity of premature infants with low anti-oxidant capacity. Rogers et al¹⁹ found that cord serum antioxidant capacity correlated with gestational age but did not predict oxygen radical diseases of prematurity, such as bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, or retinopathy of prematurity.

In a study conducted in very preterm infants, Drury et al²⁰ separated them into two groups. They found a negative correlation between total antioxidant status and the lipid peroxidation marker MDA in plasma during the first 11 days of life in the very premature newborns in the first group. They found that in the second group, total antioxidant status was significantly related to plasma urate and bilirubin levels, but not to adverse neonatal outcomes, such as chronic lung disease, intraventricular hemorrhage, retinopathy of prematurity, or death.

Table 1. Paraoxonase-1 and arylesterase values of neonates.

Variables	Fasting at birth	Postprandial at birth	p values
Paraoxonase (U/mL) mean ± SD (range)	25.8 ± 21.6 (2.2-78.8)	36.6 ± 21.7 (2.4-105.6)	p = 0.034
Arylesterase (U/mL) mean ± SD (range)	15 ± 19.6 (2.2-75)	29.4 ± 36.0 (3-155.6)	p = 0.001

Aydin et al⁹ investigated the oxidant/antioxidant status, paraoxonase activity, and leptin levels in children with marasmic malnutrition. They showed that malnourished children had significantly lower leptin and PON-1 activity than controls. A significant negative correlation was found between PON-1 and total peroxide and a positive correlation between antioxidant activity and body mass index. Children with marasmic malnutrition had increased pro-oxidant and decreased antioxidant statuses.

These studies showed that the antioxidant capacity of premature newborns is insufficient, consistent with the results of our study. In our work, PON-1 and ARE values in infants who were born prematurely, and did not have other problems beyond prematurity, were found to be lower in fasting and higher in postprandial. To our knowledge, this is the first reported study to examine the relationships between PON-1 and ARE and fasting and postprandial in preterm infants.

Conclusions

Reductions in PON-1 and ARE levels, which are involved in antioxidant systems, in premature infants and their normalization with postprandial indicate that these babies, whose antioxidant systems are insufficient, are subjected to oxidative stress in fasting. Thus, starting the feeding as soon as possible in premature newborns is vital for protection against oxidative damage. Finally, we want to underline that oxidant agents such as lipid hydroperoxide, TAS, TOS, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, HDL, LDL, oxidative stress index, lipid hydroxylperoxide, free sulfhydryl groups, MDA could not be analyzed due to refusals by families to allow the frequent collection of blood.

Conflict of interest

The Authors declare that are no conflict of interests.

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