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SERUM MALONDIALDEHYDE CONCENTRATION AS A MEASURE OF OXYGEN FREE RADICAL DAMAGE IN PRETERM INFANTS*

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SUMMARY: Yiğit Ş, Yurdakök M, Kılınç K, Oran O, Erdem G, Tekinalp G. (Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum malondialdehyde concentration as a measure of oxygen free radical damage in preterm infants. Turk J Pediatr 1998; 40: 177-183.

Lipid peroxidation was measured in 35 preterm infants by determining serum malondialdehyde (MDA) levels during the fist week of life. Serum concentrations of MDA were measured at one hour, 24 hours, 48 hours and one week of age. There were no correlations between gestational age, birth weight, and serum malondialdehyde levels at one, 24, or 48 hours, or on day seven. Serum MDA levels were higher in infants requiring mechanical ventilation compared to those breathing spontaneously, but the difference was not statistically significant. There were no correlations between MDA levels and corresponding fraction of inspired oxygen (FIO_2) levels and arterial oxygen tension. No significant difference in serum MDA concentration was seen in relation to intravenous feeding with lipid emulsions. Serum MDA concentrations of infants with sepsis were not different at hours one, 24 and 48, but were significantly higher on day seven, when compared with infants without sepsis. MDA levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to those born by cesarean section. *Key words: malondialdehyde, oxygen free radical damage, preterm infants.*

Free radical reactions are part of many biological processes. Molecular oxygen, present in all aerobic organisms, readily accepts an electron, resulting in the formation of oxygen free radicals[1]. Radicals are known to injure membranes, mainly by lipid peroxidation, which leads to structural alterations in protein and DNA. Oxygen free radicals are implicated as pathologic mediators in many disease processes. It has been suggested that bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intra ventricular hemorrhage (IVH), and patent ductus arteriosus (PDA) can be grouped together as oxygen radical diseases in newborn infants[1-4].

Oxygen radicals, of which a number of different species are known such as the superoxide and hydroxyl radicals), can be produced from different sources: normal energy metabolism, by hypoxanthine-xanthine oxidase, metabolism of

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catecholamine and arachidonic acids, and so fort[1]. The body has defense mechanisms for protection against the toxicity of oxygen free radicals: enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (e.g. several antioxidant vitamins)[5-7]. Furthermore, a series of compounds and metabolites, such as mannitol, uric acid, bilirubin and others, act as oxygen radical scavengers[6, 8, 9].

As shown by studies[10-16], the concentration of most antioxyenzymes increases during pregnancy. Phylactos et al.[17] have demonstrated that preterm babies have lower erythrocyte superoxide dismutase activitiy than term babies. Therefore, a premature infant may be at increased risk of radical damage.

It has been shown that malondialdehyde (MDA) is a highly reactive metabolite of free radical-induced lipid peroxidation[18]. Studies have shown that MDA levels are high in infants who develop BPD[19, 20]. The results of these studies[19, 20], concerning factors that influence MDA levels (i.e., birth weight, oxygen treatment, intravenous nutrition), are conflicting. This study to evaluate factors involved in free radical damage in preterm infants was, therefore, conducted by determination of MDA levels.

Material and Methods

The study group consisted of 35 preterm neonates (27-35 weeks gestation) who were born in Hacettepe University Hospital. All infants under 2500 g and of less than 36 weeks gestation were eligible for inclusion. Full details of the pregnancy, birth history, Apgar scores and neonatal course, including details of nutrition, bilirubin levels at the time of sampling, oxygen supplementation, and ventilatory support, were collected prospectively. Infants with severe congenital malformation or birth asphyxia were excluded from the study. Sepsis was diagnosed in infants by positive blood culture, in addition to clinical findings. Parenteral nutrition with glucose and amino acids was started on day three and lipid emulsions were started on day five for patients in whom sufficient enteral feeding had not been established.

Serum concentrations of MDA were measured at one hour, 24 hours, 48 hours and one week of age. Blood sampless were centrifuged at 3000 rpm for three minutes within 15 minutes of collection. Serum samples were stored at -20 °C and analyzed within two months.

Serum malondialdehyde levels were measured by the thiobarbituric acid (TBA) test, as described by Wade and van Rij[21]. Although the reactivity of TBA is not limited to malondialdehyde, this method is one of the most common used for detecting oxidative modification of lipids, and hence, oxidative stress. For detecting thiobarbituric acid reacting substances (TBARS), 0.2 ml of trichloroacetic acid (TCA, 25 gr TCA in 10 ml distilled water) was added into 1 ml serum, with vigorous shaking. After centrifugation at 1000 g for 10 minutes,

the supernatant was neutralized with 4 M NaOH. One ml of neutralized supernatant was added to 1 ml TBA (0.67%) and the mixture was then heated at 100 °C for 30 minutes. The absorption of cooled samples was measured at 532 nm. Tetramethoxy propane was used as the standard. MDA levels (measured as TBARS) were calculated as μmol/L[21].

Results are given as mean ± standard deviation (SD). Differences among groups were tested by Student's t test and chi-square analysis. Correlation analysis was used for determination of relationship between variables. Differences between two samples were tested by paired t-test.

Results

Some characteristics of the study group are shown in Table I. Artificial ventilation had been performed at least 24 hours by intermittent mandatory ventilation on 25 of 35 infants. There were no correlations between gestational age, birth weight, and MDA levels at one, 24 or 48 hours or on day seven ($r = 0.03$, 0.17, 0.11 and 0.07).

Table I: Study Group (n = 35)

Male/Female	17/18
Gestational age (wk)	31.9 ± 2.1 (27-35)
Birth weight (g)	1653 ± 508 (702-2460)
Delivery route CS/spontaneous vaginal	26/9
Maternal age (year)	28.3 ± 5.1 (20-38)
No. of infants ventilated more than 24 hours	25 (71.6%)
No. of infants who received parenteral nutrition	7 (20%)

Mean ± standard deviation (range).

Three groups were formed according to birth weight: low-birth-weight infants (1500-2500 g), very low-birth-weight infants (1000-1500 g) and extremely-low-birth-weight infants (< 1000 g). MDA levels were higher in extremely-low-birth-weight infants, but no statistically significant difference was shown in the other groups ($p > 0.05$). (Table II).

Malondialdehyde levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to those born by cesarean section ($p < 0.05$) and $p < 0.05$) respectively. This difference was slight between samples at 48 hours and there was no difference between samples on day seven ($p > 0.05$) (Table II).

Apgar scores did not correlate with MDA levels ($p > 0.05$). MDA levels were not different in infants who were resuscitated at birth.

Table II: MDA Levels According to Some Factors

	MDA Levels (mmol/L)*			
	1 Hour	24 Hours	48 Hours	7 Days
Male (n: 17)	3.9 ± 1.0	5.1 ± 1.3	5.9 ± 2.1	5.9 ± 1.9
Female (n: 18)	3.9 ± 1.1	4.7 ± 1.2	6.2 ± 2.7	4.9 ± 1.7
Gestational age				
< 32 weeks (n: 12)	3.9 ± 1.3	5.1 ± 1.1	6.3 ± 2.8	6.2 ± 1.8
> 32 weeks (n: 23)	3.9 ± 1.0	4.8 ± 1.3	5.9 ± 2.3	5.0 ± 1.8
Birth weight				
< 1000 g (n: 3)	4.3 ± 0.7	5.1 ± 1.3	7.7 ± 5.5	5.7 ± 2.5
1000-1500 g (n: 12)	3.9 ± 1.0	5.0 ± 1.1	6.1 ± 1.8	5.2 ± 2.0
1501-2500 g (n: 20)	3.8 ± 1.2	4.8 ± 1.3	5.8 ± 2.2	5.4 ± 1.8
Delivery route				
Spontaneous vaginal (n: 9)	4.6 ± 1.0[a]	5.7 ± 1.3[b]	7.2 ± 2.7	5.9 ± 2.1
Cesarean section (n: 26)	3.7 ± 1.0[a]	4.6 ± 1.1[b]	5.6 ± 2.2	5.2 ± 1.7
Resuscitation at birth				
Yes (n: 15)	3.7 ± 0.8	4.8 ± 1.3	5.9 ± 2.5	5.1 ± 1.7
No (n: 20)	4.0 ± 1.2	4.9 ± 1.2	6.1 ± 2.4	5.6 ± 2.0
Ventilation > 24 hours				
Yes (n: 25)	4.0 ± 1.1	5.0 ± 1.3	6.3 ± 3.5	5.7 ± 1.7
No (n: 10)	3.6 ± 0.8	4.8 ± 1.2	5.9 ± 1.7	5.2 ± 2.3
FiO_2 requirement at the time of sampling				
< 40%	3.7 ± 1.1 (n: 14)	4.9 ± 1.2 (n: 23)	5.9 ± 1.6 (n: 25)	5.4 ± 1.9 (n: 33)
> 40%	4.0 ± 1.1 (n: 21)	4.9 ± 1.2 (n: 12)	6.4 ± 3.9 (n: 10)	4.5 ± 1.5 (n: 2)
Sepsis				
Present (n: 5)	3.9 ± 1.4	5.6 ± 1.1	7.5 ± 3.7	8.1 ± 0.7[c]
Absent (n: 30)	3.9 ± 1.0	4.8 ± 1.2	5.8 ± 2.1	5.0 ± 1.7[c]
Intravenous lipid				
given (n: 7)	–	–	–	6.4 ± 1.6
not given (n: 28)	–	–	–	4.9 ± 2.0

* Mean ± standard deviation, a: $p < 0.05$, b: $p < 0.05$, c: $p < 0.01$.
Abbreviations: MDA malondialdehyde, FiO_2 fraction of inspired oxygen.

Serum MDA levels were higher in infants requiring mechanical ventilation compared to those breathing spontaneously, but the difference was not statistically significant. There was no correlation between MDA levels and corresponding FiO_2 levels or arterial oxygen tension ($p > 0.05$) (Table II).

Parenteral feeding with lipid emulsions created no significant change in serum MDA concentration ($p > 0.05$).

Sepsis was diagnosed on the third day of life in two infants, on the fourth day of life in one infant, and on the tenth day of life in two infants. Serum MDA concentrations of infants with sepsis were not different at hours one, 24 and 48, but were significantly higher on day seven when compared with infants without sepsis ($p < 0.01$) (Table II). Because only one patient had been diagnosed as having BPD, no statistical analysis could be performed.

Discussion

The antioxidant status of the premature infant has been shown to be poorly developed[13-16]. In the present study, although MDA levels were highest in extremely-low-birth-weight infants, MDA concentrations could not be related directly to immaturity.

The concentration of MDA in cord blood samples of the premature infants who developed chronic lung disease, was found to be higher than that in the others without chronic lung disease[19]. Gladstone and Levine[22] have shown that protein oxidation is enhanced in bronchoalveolar lavage samples in babies exposed to high oxygen concentrations compared with samples from babies with low oxygen supplementation.

No difference could be found in this study, between MDA levels in infants requiring assisted ventilation and those breathing spontaneously. Furthermore, there was no correlation between serum MDA levels and breathing oxygen concentrations. This might be due to the fact that serum MDA levels might not reflect oxidation of lipids in the lung. Similar to the results of our study, no correlation was shown between urinary MDA levels and mean airway pressure, arterial oxygen tension, or ventilatory index in the study of Schlenzig et al.[20].

It has been demonstrated that a lipid emulsion used in parenteral nutrition undergoes peroxidation that causes free-radical-mediated damage to human cells in vitro[23]. In contrast, it has also been shown that the amount of MDA administered to an infant, when infused with 0.5-1.5 g/kg/day of a lipid emulsion, is considered insignificant[20]. In accordance with this latter finding, MDA levels were not higher in infants infused with intravenous lipid than the levels in the others in our study who were not infused.

Serum MDA concentrations of infants with sepsis were significantly higher on day seven when compared with concentrations in infants without sepsis. The activated phagocyte might contribute to tissue injury by an excess production of oxygeen radicals as part of the defense against microorganisms[24, 25]. This might explain why MDA levels were found higher in infants with sepsis than in the others.

Malondialdehyde levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to levels in those born by cesarean section. We could not find any factor which could affect serum MDA levels in infants who were born after spontaneous delivery. A more detailed study has been planned to investigate this issue.

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