Plasma Ascorbate and Ceruloplasmin Levels in Thai Premature Infants

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Background: *Free radicals have been implicated in the pathogenesis of some complications among premature infants. Even though ascorbate is an important anti-oxidant in human plasma, it can also act as a pro-oxidant at high concentrations in the presence of metal ions, which causes oxidative damage in premature infants.*
Objective: *To determine plasma ascorbate and ceruloplasmin levels in premature infants (and their mothers) and full-term infants and to compare between groups.*
Material and Method: *Premature (n = 27) and full-term infants (n = 24) and the mothers of the premature infants (n = 13) admitted to Srinagarind Hospital, Khon Kaen University, Thailand, were enrolled in the study. Plasma ascorbate and ceruloplasmin levels were determined and compared among various clinical presentations.*
Results: *Plasma ascorbate has negative correlation with gestational age of infants. Its level on day 1 of the premature infants was significantly higher than full-term group (52.62 vs 39.00 μmol/L) and then decline after birth. Premature infants receiving oxygen therapy had lower plasma ascorbate than premature infants without oxygen therapy ($p = 0.017$). Plasma ascorbate in premature infants who died was higher than in those that survived ($p = 0.029$). Premature infants with poor outcomes had a higher ratio of plasma ascorbate to ceruloplasmin than those with good outcomes ($p < 0.05$).*
Conclusion: *This study shows that high plasma ascorbate and low ceruloplasmin levels are associated with poor outcomes of premature infants; that is, ascorbate can act as either an anti-oxidant or a pro-oxidant.*

Keywords: *Vitamin C, Ascorbate, Ceruloplasmin, Premature Infants, Pro-oxidant, Anti-oxidant, Thai*

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Vitamin C or ascorbate is a powerful anti-oxidant in human plasma. Premature infants are born with high levels of plasma ascorbate, which decline soon after birth[1-3]. Some researchers reported that ascorbate can act as either an anti-oxidant or a pro-oxidant depending on its concentration and the presence of metal ions[4,5]. However, there are many binding proteins in human plasma which prevent metal ions from participating in free radical reactions such as transferrin and ceruloplasmin.

Ceruloplasmin, a copper-binding protein, prevents copper from participating in reactions with radicals and plays a major role in iron metabolism as ferroxidase enzyme, which catalyzes oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions for binding to transferrin. It was found that transferrin[6-9] and ceruloplasmin[10] levels are low in infants, resulting in higher circulating Fe^{2+} [6-9]. In addition, the pro-oxidant effect of ascorbate may inhibit ferroxidase activity of ceruloplasmin by reducing cupric (Cu^{2+}) to cuprous (Cu^{+}) at the active site of the enzyme[11] so that Fe^{2+} can not be

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oxidized to Fe^{3+} Therefore, the Fe^{2+} ion can react with hydrogen peroxide (by the Fenton reaction) to form a hydroxyl free radical ($^{\bullet}OH$).

The $^{\bullet}OH$ is extremely reactive and generates numerous free radicals. The increase in free radicals in infants may lead to complications such as bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of pre-maturity, respiratory distress syndrome and intraventricular hemorrhage[12-16].

Since maternal and infant nutritional status and genetic background may differ from Western countries, the authors' main objective was to measure the level of ascorbate and ceruloplasmin in plasma of premature infants and to compare it with their mothers and full-term infants. The association between plasma ascorbate and clinical outcomes were also examined.

Material and Method

Design

The present study was designed using a descriptive study with a control group. The test group comprised premature infants and full-term infants as control group.

Subjects

The study group comprised premature infants (gestation age 29-36 weeks), their mothers and full-term infants ($\geq$ 37 weeks gestation) admitted to Srinagarind Hospital, Khon Kaen University, Thailand, between June 1997 and December 1998. The exclusion criteria were: Rhesus hemolytic disease and congenital malformations incompatible with life.

The premature infants were grouped according to the presence or absence of oxygen-radical-related diseases including respiratory distress syndrome, intraventricular hemorrhage, retinopathy of pre-maturity and necrotizing enterocolitis.

Respiratory distress syndrome was diagnosed in premature infants presented with respiratory distress within 4 hours of age with characteristic chest radiograph finding (decreased lung volume, air bronchograms and a reticulogranular pattern). Intraventricular hemorrhage was diagnosed by ultrasonography performed when clinical indications appeared or routine screening in infants less than 1500 g or gestational age less than 32 weeks. Retinopathy of prematurity was diagnosed by retinal examination with indirect ophthalmoscopy performed by an ophthalmologist when infants was 4-6 weeks old or 31-33 weeks postmenstrual age. Necrotizing enterocolitis was diagnosed in premature infants with systemic or abdominal signs and had abnormal gas pattern from abdominal radiograph.

Ethics

The Ethics Committee, Faculty of Medicine, Khon Kaen University, approved the study protocols and mothers gave informed consent.

Sample collection

Three hundred microlitres of blood samples of premature and full-term infants were collected by venipuncture blood sampling on day 1 (within 6 hr after birth), 3 and 7 after birth. It was added into heparinized tubes and centrifuged at 10,000 g at 4°C for 10 min.

A 50 μL sample of plasma was aliquoted and mixed directly with an equal volume of pre-cooled 10% metaphosphoric acid (MPA) for ascorbate analysis. All samples were stored at -70°C until analyzed.

Ascorbate determination

Ascorbate was determined by an ion-paired, reverse phase, high-performance liquid chromatograph (HPLC) system coupled with an electrochemical detector (model LC-4C, Bioanalytical Systems). The applied potential was + 0.6 V. The method was modified from Heiliger[17] using ascorbate as the external standard.

The mobile phase comprised 80 mL of methanol, 720 mL of deionized water, 6.4 mL of glacial acetic acid, 540 mg of disodium ethylenediamine tetraacetate (Na_2EDTA) and 460 mg of sodium chloride. The pH was adjusted to 3.5 using 2 mol/L of sodium hydroxide. Hexadecyl trimethyl ammoniumbromide (2.9 g) was added to the mobile phase as the ion pair agent, then delivered to the ODS-2 column (diameter 5 μm, 250 x 4.6 mm, Phenomenex) at 1 mL/min by an isocratic pump (model CM 3500; Thermo Separation Products). The sample was injected into the HPLC system with a 5 μL injector (model 7125, Rheodyne). Ascorbate was eluted at a retention time of 4.64 min. Data were analyzed using the BDS program.

Ceruloplasmin determination

The ferroxidase activity of ceruloplasmin was determined as per Johnson et al[18]. The mixture contained 170 μL of 1.2 mol/L acetate buffer pH 6.0, 270 μL deionized water, 250 μL of 2% ovotransferrin stock solution. Ten microliters of the plasma sample were mixed in a glass tube then incubated for 3 min at 30°C. The reaction was initiated by adding 300 μL of stock solution of 0.4 mmol/L of ferrous ammonium

sulfate and immediately mixed. The solution was monitored by a spectrophotometer at 460 nm every 1 min for 5 min and the rate of change determined by the change of absorbance per time (ΔOD/min).

Statistical analysis

The history and neonatal course, including the date of birth, gender, gestational age, birthweight, Apgar scores, chemistry laboratory data, type of feeding, blood transfusion, antibiotic administration and oxygen therapy were collected prospectively from the medical records.

The data was analyzed with SPSS Version 7.5 (SPSS Inc., Chicago, USA). Data were presented as means $\pm$ SD. A p-value < 0.05 was considered statistically significant. Clinical characteristics of premature and full-term infants were compared using t test and χ^2test or Fisher's Exact test.

The change over time of the biochemical measurements within and between groups were assessed using ANOVA. The Least-Significant Difference (LSD) was used for a multiple pairwise comparison.

The relationship between the biochemical measurement and birthweight and gestational age were measured using both the correlation coefficient and linear regression. The change in biochemical measurements of the premature infants with and without clinical setting was evaluated using the Mann-Whitney U non-parametric test.

Results

Clinical settings of the infants

Premature (n = 27) and full-term infants (n = 24) admitted to Srinagarind Hospital, Khon Kaen University, Thailand, were enrolled in the present study. The mothers of the premature infants (n = 13) were willing to give their blood samples for the present study. The clinical manifestations of the infants were tabulated (Table 1). The authors observed no difference in premature vs full-term infants vis- -vis phototherapy, blood transfusion, birth asphyxia (Apgar score < 7 at 1 and/or 5 min) and death. But oxygen therapy, respiratory distress syndrome, as expected, was increased in premature infants (p < 0.001).

Plasma ascorbate level in premature vs full-term infants and mothers of premature infants

The post-natal changes in plasma ascorbate in premature, full-term infants and mothers of premature infants are shown in Table 2. Plasma ascorbate in infants was determined on day 1, 3 and 7 of life; it declined within a few days after birth. Ascorbate levels on day 1 in premature and full-term infants is shown in Fig. 1. In the premature group, plasma ascorbate on day 1 was significantly higher than on day 3 or 7 (p = 0.046). In the full-term group, plasma ascorbate on day 1 was also higher than day 3 (p = 0.016) but lower than in the premature infants (p = 0.033). In the mothers of the premature infant group, plasma samples were collected on day one postpartum. Maternal ascorbate levels were lower than premature infant groups but not statistically significant (p = 0.083). The plasma ascorbate levels of all three groups were in the same range of the other studies[19-22].

Correlation between plasma ascorbate and gestational age and birthweight of premature vs full-term infants

The negative correlation between plasma ascorbate on day 1 and gestational age was r = -0.348 (p = 0.017) (Fig. 3); however, there was no correlation between plasma ascorbate and birthweight.

Table 1. Clinical settings and clinical outcomes of the infants

	Premature (n = 27)	Full-term (n = 24)	p-value
Gender (Male/Female)[c]	17/10	11/13	0.524
Gestational age (wk)[a, b]	32.76 $\pm$ 2.38	38.45 $\pm$ 1.23	0.000
Birthweight (g) [a, b]	1770 $\pm$ 420.86	2569 $\pm$ 608.53	0.001
Phototherapy, n (%)[c]	15 (53.57%)	9 (40.90%)	0.322
Blood transfusion, n (%)[d]	1 (3.57%)	1 (4.54%)	0.932
Birth asphyxia, n (%)[d]	3 (10.71%)	3 (13.63%)	0.739
Oxygen therapy, n (%)[d]	16 (57.14%)	0	<0.001
Respiratory distress syndrome, n (%)[d]	7 (25.92%)	0	<0.001
Death, n (%)[d]	3 (11.11%)	1 (4.54%)	0.722

[a] = computed as mean $\pm$ SD, [b] = using ANOVA or χ^2-test as appropriate, [c] = using χ^2-test, [d] = using Fisher's Exact test

Comparison of mean plasma ascorbate levels on the first day after birth in premature infants with clinical settings

A comparison of mean plasma ascorbate on the first day after birth in premature infants and the clinical manifestations are presented (Table 3). No difference in plasma ascorbate was found among the premature infants and the clinical settings, viz.: the male to female ratio, phototherapy, blood transfusion, birth asphyxia and respiratory distress syndrome.

Plasma ascorbate in the premature infants receiving oxygen therapy ($FiO_2 \geq 0.4$) was significantly lower than in premature infants without the therapy ($p = 0.017$). The premature infants who died had significantly higher plasma ascorbate than the premature infants who survived ($p = 0.029$).

Table 2. Plasma ascorbate and ceruloplasmin (μmol/L) in premature and full-term infants and the mothers of premature infants

	Ascorbate (μmol/L)	Ceruloplasmin (μmol/L)
Premature group		
day1	52.62±18.06 (n = 27)	0.47±0.17 (n = 17)
day3	26.00±12.10* (n = 8)	0.77±0.04* (n = 2)
day7	12.03±4.08* (n = 2)	-
Full-term group[a]		
day1	39.00±22.46 (n = 24)	0.91±0.33 (n = 14)
day3	22.28±15.70* (n = 6)	1.36±0.12* (n = 3)
Mother of premature infants group	29.87±6.78 (n = 13)	1.51±0.49 (n = 10)
Group difference (p value)	<0.05	<0.05

* = Significantly different from day 1 ($p < 0.05$)

[a] = full-term infants on day 7 were discharged from the Neonatal Unit before sample collection There is no reference values for ascorbate and ceruloplasmin in Thai infants. The ascorbate or ceruloplasmin values from other studies are shown in references[20-22,32].

Table 3. Comparison of mean plasma ascorbate and ceruloplasmin levels (μmol/L) one day after birth of premature infants with clinical settings

	Ascorbate (μmol/L)	Ceruloplasmin (μmol/L)
Gender		
- Male	52.88 ± 17.60 (n = 17)	0.60 ± 0.33 (n = 10)
- Female	52.19 ± 19.72 (n = 10)	0.56 ± 0.19 (n = 7)
Phototherapy		
- with	50.81 ± 19.71 (n = 15)	0.65 ± 0.30 (n = 10)
- without	55.07 ± 16.12 (n = 12)	0.45 ± 0.23 (n = 7)
Blood transfusion		
- with	45.91 (n = 1)	0.31 (n = 1)
- without	52.88 ± 18.38 (n = 26)	0.61 ± 0.29 (n = 26)
Birth asphyxia		
- with	66.48 ± 21.30 (n = 3)	0.31 ± 0.04 (n = 3)
- without	50.81 ± 17.31 (n = 24)	0.61 ± 0.29* (n = 14)
Oxygen therapy		
- with	45.77 ± 17.98 (n = 16)	0.63 ± 0.32 (n = 10)
- without	63.58 ± 12.24* (n = 11)	0.50 ± 0.18 (n = 7)
Respiratory distress syndrome		
- with	53.18 ± 18.64 (n = 7)	0.30 ± 0.01 (n = 3)
- without	52.41 ± 18.65 (n = 20)	0.64 ± 0.28* (n = 14)
Death		
- with	73.60 ± 11.35 (n = 3)	0.26 ± 0.03 (n = 3)
- without	50.89 ± 16.30* (n = 24)	0.51 ± 0.15* (n = 14)

* = Significantly different from "with"

Plasma ceruloplasmin levels in premature and full-term infants and the mothers of premature infants

The post-natal changes of plasma ceruloplasmin levels in the premature and full-term infants and the mothers of premature infants are shown (Table 2). The plasma ceruloplasmin of the infants was determined on day 1 and 3 of life. Ceruloplasmin levels on day 1 in premature and full-term infants is shown in Fig. 2. The plasma ceruloplasmin on day 1 was significantly lower than the level on day 3 in both premature ($p = 0.001$) and full-term infants ($p = 0.021$). The plasma ceruloplasmin level in full-term group was higher than in the premature group on day 1 and 3 of birth.

Plasma samples were collected in the mother group during day 1 postpartum. In maternal group, the plasma ceruloplasmin was higher than premature infant groups. The plasma ceruloplasmin in the present study was slightly lower than the previous study (i.e. 1.5 to 3.0 μmol/L)[23]. Plasma ceruloplasmin among the premature and full-term infants was not correlated with the level of plasma ascorbate.

Correlation between plasma ceruloplasmin with gestational age and birthweight of premature vs full-term infants

A positive correlation was found between plasma ceruloplasmin on day 1 and gestational age ($r = 0.736$) ($p < 0.001$) (Fig. 4), but none between plasma ceruloplasmin and birthweight.

Comparison of plasma ceruloplasmin at birth in premature infants with various clinical manifestations

Comparisons of the mean plasma ceruloplasmin levels on day 1 in premature infants and the associated clinical manifestations are presented in Table 3. The plasma ceruloplasmin in premature infants with birth asphyxia was significantly lower than infants without birth asphyxia ($p = 0.026$). The premature infants who died had significantly lower plasma ceruloplasmin than those who survived ($p = 0.011$). The premature infants with respiratory distress syndrome had significantly lower plasma ceruloplasmin than premature infants without this condition ($p = 0.037$).

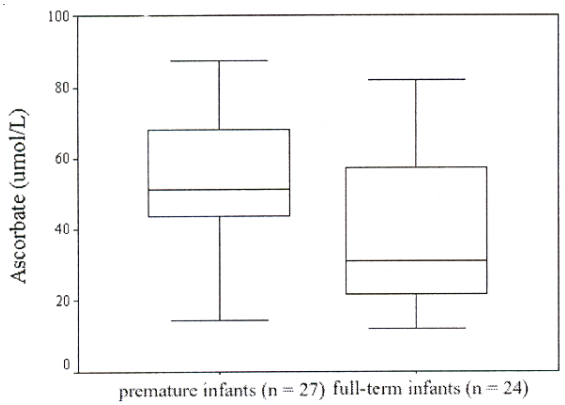

Fig. 1 Ascorbate level on day 1 in premature and full-term infants

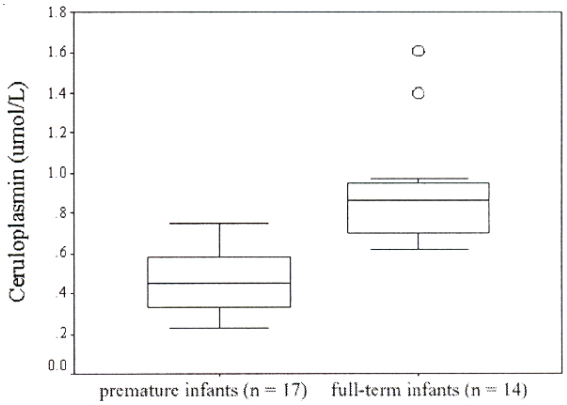

Fig. 2 Ceruloplasmin level on day 1 in premature and full-term infants

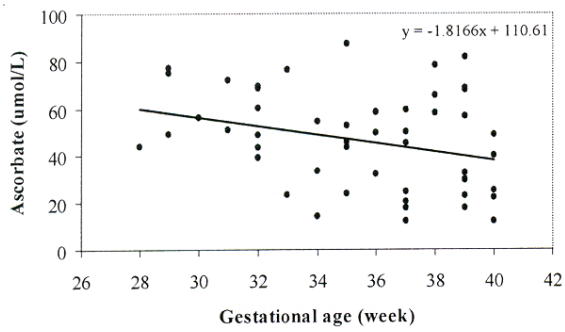

Fig. 3 Association between ascorbate level on day 1 and gestational age of the premature and full-term infants

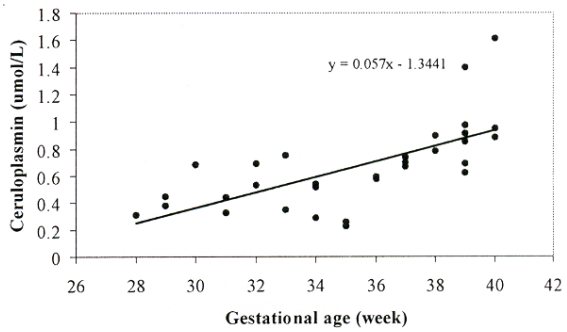

Fig. 4 Association between ceruloplasmin level on day 1 and gestational age of the premature and full-term infants

No differences were found in the clinical settings of gender, phototherapy, blood transfusion and oxygen therapy.

Plasma ascorbate to ceruloplasmin ratio in premature infants who died, survived, with and without respiratory distress syndrome vs mother groups and the full-term infants

Ascorbate can decrease the ferroxidase activity of ceruloplasmin. When the molar ratio of ascorbate to ceruloplasmin was greater than 200, loss of ferroxidase activity occurred[11]. Thus, Fe^{2+} cannot be oxidized to Fe^{3+} resulting in generating of free radicals by Fenton reaction. The plasma 'ascorbate to ceruloplasmin' ratios in the premature infants are shown in Table 4. There were 3 premature infants died during this study. The first and the second infants were 34 and 35 weeks gestation with 1330 g and 2000 g body weight died from septicemia with septic shock at 16 days and 8 days of age respectively. The third infants, who was 35 weeks gestation, 2200 g body weight, died at 18 days of age from accidental suffocation. The 'plasma ascorbate to ceruloplasmin' ratio (on day 1) in premature infants who died was significantly higher than in those who survived ($p < 0.001$). The ratio in premature infants who died and survived was 283.01 and 99.78, respectively. The ratio in premature infants with respiratory distress syndrome was higher than in infants without respiratory distress syndrome, but not statistically significant.

Discussion

At birth, premature infants are more susceptible to oxidative injury than full-term infants because their anti-oxidant defense system is immature. In the present study, the most common complications among premature infants was respiratory distress syndrome (n = 7) due to a deficiency of surfactant-coated alveoli to prevent collapsing and leakage of plasma across the alveolar wall. All of these infants needed oxygen therapy. High concentrations of oxygen may contribute to many complications such as bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage and retinopathy of prematurity[24].

There are several reports on plasma ascorbate levels among premature vs full-term infants[2,3,25-28]. The authors found that plasma ascorbate among premature infants on day 1 was 52.62 ± 18.06 μmol/L, which is much lower than previous studies.

Moreover, the authors found plasma ascorbate among premature infants was significantly higher than in full-term infants, perhaps because the ascorbate is more actively transported across the placenta at an earlier gestational age[29,30]. Thus, concentrations of ascorbate in the plasma of some infants, especially premature infants at birth, were higher than normal adult range (i.e. 30 to150 μmol/L).

Post-natally, plasma ascorbate in both premature and full-term infants rapidly declined during the first 3 days of life, perhaps as post-natal renal excretory function improved[31]. In addition, high levels of oxidative stress may contribute to the rapid turnover and loss of ascorbate. However, previous studies indicated that after the maximal fall (on day 7), the ascorbate levels gradually rose to normal adult values by day 42[25].

Plasma ascorbate among premature infants in the present study was higher than in their mothers. This observation is supported by numerous studies which have shown that plasma ascorbate in the cord blood of infants is higher than in the maternal blood[32,33], indicating ascorbate is actively transported across the placenta such that the infant maintains a certain ascorbate level even if the mother has a low plasma ascorbate level[2]. The mean plasma ascorbate level in mothers of premature infants was 29.87 ± 6.78 μmol/L, which is much lower than previous reports of mothers of full-term infants (range, 40 to 66 μmol/L)[9].

Table 4. Plasma ascorbate to ceruloplasmin ratio (on day 1) in premature infants who died, survived, with and without respiratory distress syndrome compared to full-term infants and mothers of premature infants

Groups	Ascorbatemean (μmol/L)	Ceruloplasminmean (μmol/L)	Ascorbate: ceruloplasmin ratio
Premature infants			
Death	73.60 (n =3)	0.26 (n = 3)	283.01
Survived	50.89 (n = 24)	0.51 (n = 14)	99.78
with respiratory distress syndrome	53.18 (n = 7)	0.30 (n = 3)	177.27
without respiratory distress syndrome	52.41 (n = 20)	0.64 (n = 14)	81.89
Full-term infants	39.00 (n = 24)	0.91 (n = 14)	42.86
Mothers of premature infants	29.87 (n = 13)	1.51 (n = 10)	19.78

The present study may indicate a poor nutritional status among mothers of the premature infants admitted to Srinagarind Hospital or low ascorbate level in these mothers may be due to low ascorbate intake. Only 13 mothers of premature infants has ascorbate level measured, whereas 27 premature infants were measured ascorbate level. The premature infants of these 13 mothers showed no statistically significant ascorbate level with the other 14 premeture infants ($p = 0.206$).

The negative correlation between plasma ascorbate and gestational age was confirmed by Berger et al[9]; they found plasma ascorbate at birth in premature infants higher than in full-term infants, and highest in the most immature infants. Zalani et al found ascorbate concentrations in fetal tissues related to the development of organ and gestational age[34]. Ascorbate has an important anti-oxidant function in the womb because of low enzymatic anti-oxidants synthesized from immature liver. Moreover, ascorbate acts as a cofactor of prolyl and lysyl hydroxylase in the biosynthesis of collagen, a process which occurs at a high rate during this period of rapid fetal growth[35].

The authors found that plasma ascorbate in premature infants receiving oxygen therapy was significantly lower than in those without oxygen therapy (Table 3). Possibly, ascorbate acts as an anti-oxidant in premature infants exposed to hyperoxic environmental and mechanical ventilation after birth. The ascorbate would be oxidized to dehydroascorbate. This condition was also found in rheumatoid arthritis[36], adult respiratory distress syndrome and critically ill patients[37].

The plasma ascorbate levels among premature infants with RDS are similar to premature infants without respiratory distress syndrome as per many other investigators. Zoeren-Grobben et al found that plasma ascorbate in infants with and without respiratory distress syndrome was not different[38]. Berger et al described that in the event of a plasma iron-overload in premature infants, ascorbate can delay iron-induced lipid peroxidation in a dose-dependent manner[39].

Drury et al found that increased lipid peroxidation was associated with low extracellular anti-oxidant and low extracellular anti-oxidant was not associated with adverse effects (i.e. intraventricular hemorrhage, retinopathy of pre-maturity or death) in infants[40].

In the present study, plasma ceruloplasmin (Table 2) in premature infants was lower than the full-term infants and mother groups, respectively. A correlation between plasma ceruloplasmin and gestational age was found, perhaps because hepatic function with respect to ceruloplasmin synthesis is more developed in adults than in infants[20]. Plasma ceruloplasmin in the fetus increased after 26 weeks gestation but was low compared with the adults[21].

Plasma ceruloplasmin among the premature infants with respiratory distress syndrome was lower than infants without ($p < 0.05$). Similarly, Airede found that sick premature infants had significantly lower mean ceruloplasmin levels at birth compared to well premature infants (5 μmol/L and 125 μmol/L)[22]. Thus, an insufficiency of ceruloplasmin would reduce the protective effect against free radicals in the lungs and other tissues. However, Moison et al found that plasma ceruloplasmin in premature infants with or without respiratory distress syndrome was not significantly different[8]. The authors confirmed that plasma ceruloplasmin level in premature infants with asphyxia was lower than infants without asphyxia. The high non-protein bound iron (Fe^{2+}) presented in premature-infants due to low transferrin and ceruloplasmin can be related to the post-asphyxial injury as was found in Dorrepeaal et al[41].

The ratio of plasma ascorbate to ceruloplasmin in premature infants with poor outcomes was significantly higher than in infants who survived (Table 4). The combination of high ascorbate and low ceruloplasmin in the premature infants who died may have promoted free radical generation, which in turn contributed to an adverse outcome. Ascorbate may inhibit the ferroxidase activity of ceruloplasmin, thus, the anti-oxidant activity of the plasma ceruloplasmin would be impaired. Ascorbate supplementation to premature infants requires caution since it may cause poor outcomes.

Conclusion

This is the first study to investigate plasma ascorbate and ceruloplasmin in both premature and full-term Thai infants admitted to Srinagarind Hospital. High levels of plasma ascorbate were found in premature infants who died, so under this condition ascorbate may act as a pro-oxidant. In premature infants with poor outcomes, the ratio of 'plasma ascorbate to ceruloplasmin' was higher than premature infants with better outcomes. The combination of high levels of ascorbate and low levels of ceruloplasmin could alter the ascorbate's role to that of a pro-oxidant. The results imply that plasma ascorbate in

premature infants may function as both an anti-oxidant and pro-oxidant, therefore, ascorbate supplementation in premature infants should be done with caution.

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References

1. Heinonen K, Mononen I, Mononen T, Parviainen M, Penttila I, Launiala K. Plasma vitamin C levels are low in premature infants fed human milk. Am J Clin Nutr 1986; 43: 923-4.
2. Arad I, Glick B, Dgani Y, Salpeter L, Eyal F. The vitamin C status of formula-fed preterm infants. Am J Perinatol 1986; 3: 104-6.
3. Lindeman JH, van Zoeren-Grobben D, Schrijver J, Speek AJ, Poorthuis BJ, Berger HM. The total free radical trapping ability of cord blood plasma in preterm and term babies. Pediatr Res 1989; 26: 20-4.
4. Gutteridge JM. Fate of oxygen free radicals in extracellular fluids. Biochem Soc Trans 1982; 10: 72-3.
5. Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. Arch Biochem Biophys 1990; 280: 1-8.
6. Lindeman JH, Houdkamp E, Lentjes EG, Poorthuis BJ, Berger HM. Limited protection against iron-induced lipid peroxidation by cord blood plasma. Free Radic Res Commun 1992; 16: 285-94.
7. Evans PJ, Evans R, Kovar IZ, Holton AF, Halliwell B. Bleomycin-detectable iron in the plasma of premature and full-term neonates. FEBS Lett 1992; 303: 210-2.
8. Moison RM, Palinckx JJ, Roest M, Houdkamp E, Berger HM. Induction of lipid peroxidation of pulmonary surfactant by plasma of preterm babies. Lancet 1993; 341: 79-82.
9. Berger HM, Mumby S, Gutteridge JM. Ferrous ions detected in iron-overloaded cord blood plasma from preterm and term babies: implications for oxidative stress. Free Radic Res 1995; 22: 555-9.
10. Rosenfeld W, Concepcion L, Evans H, Jhaveri R, Sahdev S, Zabaleta I. Serial trypsin inhibitory capacity and ceruloplasmin levels in prematures at risk for bronchopulmonary dysplasia. Am Rev Respir Dis 1986; 134: 1229-32.
11. Gutteridge JM. Plasma ascorbate levels and inhibition of the antioxidant activity of caeruloplasmin. Clin Sci (Lond) 1991; 81: 413-7.
12. Warner BB, Wispe JR. Free radical-mediated diseases in pediatrics. Semin Perinatol 1992; 16: 47-57.
13. Kelly FJ. Free radical disorders of preterm infants. Br Med Bull 1993; 49: 668-78.
14. Sullivan JL. Iron, plasma antioxidants, and the 'oxygen radical disease of prematurity'. Am J Dis Child 1988; 142: 1341-4.
15. Johnson L, Quinn G, Abbasi S, Gerdes J, Bowen F, Bhutani V. Severe retinopathy of prematurity in infants with birth weights less than 1250 grams: incidence and outcome of treatment with pharmacologic serum levels of vitamin E in addition to cryotherapy from 1985 to 1991. J Pediatr 1995; 127: 632-9.
16. Hittner H, Godio L, Rudolph A, Adams J, Garcia-Prats J, Friedman Z, et al. Retrolental fibroplasia: efficacy of vitamin E in a double-blind clinical study of preterm infants. N Engl J Med 1981; 305: 1365-71.
17. Heiliger F. Current separation. (Heilinger F, ed). Pub BSC Inc. USA. 1980.
18. Johnson DA, Osaki S, Frieden E. A micromethod for the determination of ferroxidase (ceruloplasmin) in human serums. Clin Chem 1967; 13: 142-50.
19. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr 1995; 62(Suppl 6): S1315-21.
20. Hilderbrand DC, Fahim Z, James E, Fahim M. Ceruloplasmin and alkaline phosphatase levels in cord serum of term, preterm, and physiologically jaundiced neonates. Am J Obstet Gynecol 1974; 118: 950-4.
21. Gitlin D, Biasucci A. Development of gamma G, gamma A, gamma M, beta IC-beta IA, C 1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, alpha 1-antitrypsin, orosomucoid, beta-lipoprotein, alpha 2-macroglobulin, and prealbumin in the human conceptus. J Clin Invest 1969; 48: 1433-46.
22. Airede AI. Serial copper and ceruloplasmin levels in African newborns with emphasis on the sick and stable preterm infant, and their antioxidant capacities. Early Hum Dev 1998; 52: 199-210.
23. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER, Eds. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: Saunders Company; 1999: 477-540.
24. Saugatad O. Mechanisms of tissue injury by oxygen radicals; implication for neonatal disease. Acta Paediatr 1996; 85: 1-4.
25. van Zoeren-Grobben D, Lindeman JH, Houdkamp E, Brand R, Schrijver J, Berger HM. Postnatal changes in plasma chain-breaking antioxidants in healthy preterm infants fed formula and/or human milk. Am J Clin Nutr 1994; 60: 900-6.
26. Gopinathan V, Miller NJ, Milner AD, Rice-Evans CA. Bilirubin and ascorbate antioxidant activity in neonatal plasma. FEBS Lett 1994; 349: 197-200.
27. Silvers KM, Gibson AT, Russell JM, Powers HJ. Antioxidant activity, packed cell transfusions, and outcome in premature infants. Arch Dis Child Fetal Neonatal Ed 1998; 78: F214-9.

28. Baydas G, Karatas F, Gursu MF, Bozkurt HA, Ilhan N, Yasar A, et al. Antioxidant vitamin levels in term and preterm infants and their relation to maternal vitamin status. Arch Med Res 2002; 33: 276-80.
29. Berger T, Rifai N, Avery M, Frei B. Vitamin C in premature and full-term human neonates. Redox Report 1996; 2: 257-62.
30. Choi JL, Rose RC. Transport and metabolism of ascorbic acid in human placenta. Am J Physiol 1989; 257(1 Pt 1): C110-3.
31. Kallner A, Hartmann D, Hornig D. Steady-state turnover and body pool of ascorbic acid in man. Am J Clin Nutr 1979; 32: 530-9.
32. Heinz-Erian P, Achmuller M, Berger H, Brabec W, Nirk S, Rufer R. Vitamin C concentrations in maternal plasma, amniotic fluid, umbilical cord blood, the plasma of newborn infants, colostrum and transitory and mature breast milk. Padiatr Padol 1987; 22: 163-78.
33. Dvir M, Kohelet D, Arbel E, Goldberg M. Umbilical ascorbic acid levels in fetal distress. J Nutr Sci Vitaminol (Tokyo) 1992; 38: 511-5.
34. Zalani S, Rajalakshmi R, Parekh LJ. Ascorbic acid concentration of human fetal tissues in relation to fetal size and gestational age. Br J Nutr 1989; 61: 601-6.
35. Levine M. New concepts in the biology and biochemistry of ascorbic acid. N Engl J Med 1986; 314: 892-902.
36. Lunec J, Blake DR. The determination of dehydro-ascorbic acid and ascorbic acid in the serum and synovial fluid of patients with rheumatoid arthritis (RA). Free Radic Res Commun 1985; 1: 31-9.
37. Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH, Sanderson MJ, et al. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am J Clin Nutr 1996; 63: 760-5.
38. van Zoeren-Grobben D, Lindeman JH, Houdkamp E, Moison RM, Wijnen JT, Berger HM. Markers of oxidative stress and antioxidant activity in plasma and erythrocytes in neonatal respiratory distress syndrome. Acta Paediatr 1997; 86: 1356-62.
39. Berger TM, Polidori MC, Dabbagh A, Evans PJ, Halliwell B, Morrow JD, et al. Antioxidant activity of vitamin C in iron-overloaded human plasma. J Biol Chem 1997; 272: 15656-60.
40. Drury JA, Nycyk JA, Baines M, Cooke RW. Does total antioxidant status relate to outcome in very preterm infants? Clin Sci (Lond) 1998; 94: 197-201.
41. Dorrepaal CA, Berger HM, Benders MJ, van Zoeren-Grobben D, Van de Bor M, Van Bel F. Nonprotein-bound iron in postasphyxial reperfusion injury of the newborn. Pediatrics 1996; 98: 883-9.

ระดับพลาสมาวิตามินซีและเซรูโลพลาสมินในทารกไทยที่เกิดก่อนกำหนด

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***ความเป็นมา**: วิตามินซีเป็นแอนติออกซิแดนท์ที่สำคัญตัวหนึ่งในร่างกาย ขณะเดียวกันวิตามินซีสามารถทำหน้าที่เป็นโปรออกซิแดนท์ได้ด้วยในกรณีที่มีความเข้มข้นสูงร่วมกับมีไอออนของโลหะหนักซึ่งอนุมูลอิสระที่เกิดขึ้นอาจทำให้เกิดภาวะแทรกซ้อนในทารกเกิดก่อนกำหนดได้*

***วัตถุประสงค์**: เพื่อวัดระดับพลาสมาวิตามินซีและเซรูโลพลาสมินในทารกเกิดก่อนกำหนดเปรียบเทียบกับทารกครบ กำหนด*

***วัสดุและวิธีการ**: วัดระดับพลาสมาวิตามินซีและเซรูโลพลาสมินในทารกเกิดก่อนกำหนด 27 ราย และมารดา 13 ราย เปรียบเทียบกับทารกครบกำหนด 24 ราย ที่โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น*

***ผลการศึกษา**: ระดับวิตามินซีในพลาสมาของทารกเกิดก่อนกำหนดมีความสัมพันธ์ผกผันกับอายุครรภ์ ระดับวิตามินซีในวันแรกหลังคลอดสูงกว่าในทารกครบกำหนด (52.62 และ 39.00 ไมโครโมลต่อลิตร ตามลำดับ) และลดลงหลังจากนั้น วิตามินซีในทารกเกิดก่อนกำหนดที่จำเป็นต้องได้รับออกซิเจนมีระดับต่ำกว่าทารกที่ไม่ได้รับออกซิเจน ($p = 0.017$) ทารกเกิดก่อนกำหนดที่เสียชีวิตมีระดับวิตามินซีสูงกว่า ($p = 0.029$) และมีสัดส่วนของวิตามินซีต่อเซรูโลพลาสมินสูงกว่าทารกที่รอดชีวิต ($p < 0.05$)*

***สรุป**: สำหรับทารกเกิดก่อนกำหนดระดับวิตามินซีที่สูงและเซรูโลพลาสมินที่ต่ำมีความสัมพันธ์กับการพยากรณ์โรคที่ไม่ดี เนื่องจากวิตามินซีสามารถทำหน้าที่เป็นได้ทั้งแอนติออกซิแดนท์และโปรออกซิแดนท์*