Antioxidant profiles and markers of oxidative stress in preterm neonates

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Background: Preterm birth is associated with an increased oxidant burden which places these infants at a higher risk of injury.

Aims: This prospective study aimed to assess levels of antioxidants and a marker of oxidative stress in preterm neonates.

Objectives: (i) To compare levels of anti-oxidants [vitamin A, vitamin E, catalase, total anti-oxidant status (TAS)] as well as malondialdehyde level (MDA) (a marker of lipid peroxidation) between preterm and full-term neonates; (ii) to determine changes in the values of measured vitamins at birth and at discharge among preterm neonates; and (iii) to compare levels of anti-oxidants with MDA levels in relation to complications of prematurity and outcome.

Methods: The study was undertaken in 100 preterm neonates and 100 full-term neonates as a control group. MDA was estimated by a thiobarbituric acid-reactive technique; TAS was determined using a Randox assay kit; catalase activity was measured spectrophotometrically and vitamin A and E levels were estimated by high performance liquid chromatography.

Results: The plasma levels of vitamin A, vitamin E, TAS and catalase were significantly lower in the preterm than in the full-term group (P<0.01), and the plasma level of MDA was significantly higher in preterm than full-term neonates (P<0.01). Vitamin A and E levels in preterm neonates were significantly higher at discharge than at birth (P<0.01). Vitamin A, vitamin E and catalase levels at birth were significantly lower in patients who developed necrotizing enterocolitis or bronchopulmonary dysplasia than in those who did not.

Conclusion: Preterm neonates are exposed to increased oxidant stress at birth and are susceptible to anti-oxidant deficiencies. A higher dose of enteral vitamin A supplementation in preterm neonates might reduce morbidity and improve outcome. Further studies are warranted to evaluate the appropriate dose of oral vitamin E supplementation for preterm neonates.

Keywords: Preterm neonates, Oxidative stress, Antioxidants, Vitamin A, Vitamin E, Total antioxidant status, Malondialdehyde, NICU

Introduction

In premature infants, oxygen radical injury is thought to be one of the common mechanisms for several neonatal diseases.¹ At birth, neonates have an increase in markers of oxidative stress and a decrease in anti-oxidant defences.² Cellular oxidative damage is caused primarily by free radicals and reactive oxygen species (ROS).³ There are complex anti-oxidant defense systems, including both enzymatic and non-enzymatic components, against the effects of oxygen free radicals on biological macromolecules such as proteins, nucleic acids, carbohydrates and lipids.⁴ Vitamins such as E, A and C are among the non-enzymatic anti-oxidants.¹ Vitamin E is an effective lipid membrane anti-oxidant. It is also essential in maintaining the integrity and functional ability of the plasma membrane in a role distinct from the anti-oxidant properties.⁵ Vitamin A plays an important role in cellular function, development and maintenance of normal visual acuity.⁶,⁷ In addition, vitamin A is known to be an important natural anti-oxidant.⁸ Evidence for oxidative injury comes from measurements of biochemical markers of lipid peroxidation and protein oxidation.³ The aim of the study was to determine the level of malondialdehyde (MDA), a marker of lipid peroxidation, and the anti-oxidants’ levels of catalase, vitamin A, vitamin E and total anti-oxidant status (TAS) in the cord blood of preterm, low-birthweight (LBW) infants in order to compare them with levels in full-term infants and relate them to complications of prematurity and outcome.
Methods

Subjects

This prospective observational study was undertaken in the neonatal intensive care unit (NICU) of Kasr El Ainy Hospital, Cairo University during the 9-month period from September 2013 to May 2014. Blood samples were collected at birth from 100 preterm infants who were admitted to the NICU and treated according to standard protocols, and from 100 healthy full-term controls. A second blood sample was drawn from preterm patients at the time of discharge to measure serum vitamin A and E levels. Exclusion criteria included gestational diabetes, major congenital anomalies, small-for-gestational-age neonates and death within the first week of life.

Gender, gestational age, birthweight, type of delivery, Apgar score at 5 minutes, duration of admission, oxygen support, mechanical ventilation, complications of prematurity including bronchopulmonary dysplasia (BPD), intraventricular haemorrhage (IVH), necrotizing enterocolitis (NEC), median intake of vitamin A and E per day and outcome were recorded for each newborn infant.

Necrotizing enterocolitis was diagnosed on the basis of clinical and radiological modified Bell staging criteria.9 Bronchopulmonary dysplasia was defined according to the National Institutes of Health (NIH) consensus definition for BPD.10 IVH was diagnosed by real-time portable cranial ultrasound.11

Parenteral nutrition was commenced for all the preterm neonates within 24-48 hours of life. Parenteral, lipid soluble multivitamin supplementation was not available. Enteral feeds were begun as soon as possible and administered in non-nutritive volumes (1 ml/2-4-hourly) until feeding could be advanced as tolerated to reach 150-180 ml/kg/day using continuous or bolus feeds. Preterm formula (Similac®; Neosure® Powder) containing vitamin A 346 IU per 100 ml and vitamin E 2.7 IU per 100 ml of constituted formula at standard dilution was used for the preterm neonates. Oral vitamin supplementation was commenced when the infant was tolerating full oral feeding using 0.5 ml of multivitamin oral drops containing 1500 IU/ml of vitamin A and 5 mg/ml of vitamin E. The average daily intake of vitamins was calculated for each infant from the amount of daily enteral intake in addition to the amount of oral vitamin supplementation.

Informed consent was obtained from the parents of all the study subjects. The study design was approved by the Scientific Research Committee of the Pediatric Department, Faculty of Medicine, Cairo University. Data confidentiality was preserved according to the revised Helsinki Declaration of Bioethics.12

Laboratory methods

Blood samples (2 ml) were taken using EDTA-containing tubes at birth for both the pre- and full-term groups to measure MDA, catalase, TAS and vitamins A and E in the cord blood, and, in pre-term neonates, a second sample was taken at the time of discharge to measure vitamin A and E. The blood samples were centrifuged and the plasma collected and stored at -20°C until analysis. The red blood cell (RBC) phase was washed twice with two volumes of isotonic saline solution at pH 7.0. RBCs were haemolysed by adding distilled water in the ratio 1:9.13 All the procedures were undertaken under light protection.

Analysis of vitamins A and E by high performance liquid chromatography (HPLC): sample extraction

100 µl of serum was mixed with ethanol. The micro-nutrients were extracted from the aqueous phase into hexane and dried under vacuum. The extract was re-dissolved in ethanol and acetonitrile and was filtered to remove any insoluble materials.

HPLC condition for vitamin A

20 µl of the filtrate was injected onto a C18 reversed phase column (25 cm × 10.00 mm, 5 µm particle size) and isocratically eluted with a mobile phase consisting of ethanol/acetonitrile 50:50 with 0.1% triethylamine and was delivered at a flow rate of 1 ml/min. UV detection was performed at 325 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas vs the corresponding concentrations. The concentration in samples was obtained from the curve.

HPLC condition for vitamin E

20 µl of the filtrate was injected onto a C18 reversed phase column (15 cm × 10.00 mm, 5 µm particle size) and the thermostat was adjusted to 30°C with a mobile phase consisting of 100% methanol delivered at a flow rate of 1 ml/min. A fluorescence detector was used and performed at wavelengths of 295 nm and 330 nm. Serial dilutions of standards were injected, and their peak areas were determined. A linear standard curve was constructed by plotting peak areas vs the corresponding concentrations. The concentration in samples was obtained from the curve.14

Analysis of total anti-oxidant status, malondialdehyde and catalase activity

Total anti-oxidant status was determined using a Randox assay kit. The assay was based on the principle that ABTS [2, 2’-Azino-di-(3-ethylbenzthiazoline sulphonate)] is incubated with a peroxidase and H2O2 to produce the radical cation ABTS+. This has a relatively stable blue–green colour which is measured at 600 nm. Antioxidants in cord blood cause suppression of this
colour production to a degree which is proportional to the concentration.\textsuperscript{15} Plasma MDA levels were assayed by the thiobarbituric acid-reactive substances technique.\textsuperscript{16} In order to determine the catalase activity, erythrocyte haemolysates (100-fold dilution) were used. The blank sample was 3 ml of phosphate buffer (pH 7.0) in a quartz cuvette. The incubation mixture contained 2 ml of the phosphate buffer (warmed to 37°C), 5 μl of haemolysate and 1 ml of H₂O₂ solution. Catalase activity was measured spectrophotometrically at 240 nm.\textsuperscript{13}

**Statistical methods**

Data management and analysis were performed using the Statistical Package for Social Sciences (SPSS) version 17. Data were statistically described in terms of frequencies (number of cases) and percentages. The study groups were compared by means of the \( \chi^2 \) test, but Fishers’ Exact test was used when the expected frequency was less than five. Non-normally distributed numerical variables were compared between the groups using the Mann–Whitney test. Spearman’s correlation coefficients were used to measure the association between non-normally distributed variables. All \( P \)-values were two-sided. \( P \)-values < 0.05 were considered to be significant.

**Results**

Characteristics of the pre-term group and the term (control) group are summarised in Table 1. The majority of the pre-term neonates were of very low birthweight (VLBW) (birthweight < 1500 g) (88%). The median gestational age of the pre-term group was 31 weeks (27–34), while the median gestational age of the full-term neonates was 38 weeks (37–40) \( (P<0.01) \). The median birthweight of the pre-term group was 1300 g (800–2000), and the median birthweight of the full-term group was 3200 g (2750–3750) \( (P<0.01) \). The median duration of hospital stay of the study population was 20.50 days (7–49).

Plasma vitamin levels and levels of TAS, MDA and catalase in the pre-term and full-term neonates are shown in Table 1. Plasma level of vitamin A, vitamin E, TAS and catalase were significantly lower in the pre-term than in the full-term group \( (P<0.01) \), while the plasma level of MDA was significantly higher in the pre-term neonates \( (P<0.01) \).

The median intake of vitamin A by the pre-term group during admission was 644.2 IU/kg/day (513–769), and median intake of vitamin E during admission was 2.93 IU/kg/day (2–6).

Measurement of vitamin A and E levels was repeated on discharge for the 72 pre-term neonates who survived. There was a statistically significant difference between vitamin A levels at birth and at discharge \( [\text{mean (SD)} 0.264 \mu\text{mol/L (0.198)} \text{ vs } 0.624 \mu\text{mol/L (0.217)}] \), respectively, \( P<0.01 \). There was also a statistically significant difference in the level of vitamin E at birth and at discharge \( [\text{mean (SD)} 6.03 \mu\text{mol/L (4.36)} \text{ vs } 29.32 \mu\text{mol/L (14.74)}] \), respectively, \( P<0.01 \) (Table 2).

The levels of vitamin A and E at birth were significantly higher in pre-term neonates whose mothers had received antenatal corticosteroids than in those whose mothers had not \( [\text{mean (range)} 0.401 \mu\text{mol/L (0.069–0.907)} \text{ vs } 0.160 \mu\text{mol/L (0.069–0.523)}] \) and \( 8.35 \mu\text{mol/L (0.32–13.69)} \text{ vs } 3.27 \mu\text{mol/L (0.30–13.69)} \), respectively, \( P<0.01 \) and \( <0.01 \), respectively. There was no statistically significant difference in TAS or catalase between the groups \( [\text{median (range)} 1.89 \text{nmol/L (1–3)} \text{ vs } 1.78 \text{nmol/L (1–3)} \text{ and 179 units/mg Hb (168–198)} \text{ vs } 173 \text{ units/mg Hb (161–198)}] \), respectively, \( P=0.72 \) and 0.08, respectively.

The relationship between the levels of vitamins and anti-oxidants at birth and complications of prematurity are shown in Table 3, and the relationship between vitamin levels at discharge in the pre-term neonates who survived and complications of prematurity are shown in Table 4.

Serum vitamin A level was significantly lower at birth in patients who developed BPD and those who developed NEC than in those who did not, and these differences persisted at discharge. Vitamin A was also significantly lower in patients who developed IVH than in those who did not, but the level did

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**Table 1 Clinical and laboratory data in pre-term and full-term neonates**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm neonates ( n=100 )</th>
<th>Full-term neonates ( n=100 )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wks</td>
<td>31 (27–34)</td>
<td>38 (37–40)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Birthweight, g</td>
<td>1300 (800–2000)</td>
<td>3200 (2750–3750)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male, ( n ) (%)</td>
<td>50 (50)</td>
<td>56 (56)</td>
<td>0.39</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVD</td>
<td>62 (62)</td>
<td>60 (60)</td>
<td>0.77</td>
</tr>
<tr>
<td>CS</td>
<td>36 (36)</td>
<td>40 (40)</td>
<td></td>
</tr>
<tr>
<td>Serum vitamin A at birth, ( \mu\text{mol/L} )</td>
<td>0.176 (0.083–0.924)</td>
<td>0.907 (0.436–3.367)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum vitamin E at birth, ( \mu\text{mol/L} )</td>
<td>5.22 (3.01–13.69)</td>
<td>14.87 (9.98–29.48)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TAS, ( \mu\text{mol/L} )</td>
<td>1.81 (1.16–2.81)</td>
<td>3.67 (3.20–3.93)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Catalase, units/mg Hb</td>
<td>177 (161–198)</td>
<td>214 (183–224)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MDA, ( \mu\text{mol/L} )</td>
<td>1.86 (1.23–3.78)</td>
<td>1.11 (0.74–1.25)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

NVD, normal vaginal delivery; TAS, total anti-oxidant status; MDA, malondialdehyde. Values are expressed as median (range).
Henriksen found that vitamin A 5000 IU given n 0.06, 25 23 et al. The mechanism by which corticosteroids 1,20 et al. also found significance was not statistically significant 

\[ \text{Vitamin level at birth} (\text{nmol/L, mean (SD)}) \]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin level at birth n=72</th>
<th>Vitamin level at discharge n=72</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, μmol/L, mean (SD)</td>
<td>0.264 (0.198)</td>
<td>0.624 (0.217)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vitamin E, μmol/L, mean (SD)</td>
<td>6.03 (4.36)</td>
<td>29.32 (14.74)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

not differ significantly between the two groups at discharge (Tables 3 & 4).

Serum vitamin E level at birth was significantly lower in patients who developed BPD than in those who did not. Although vitamin E at discharge was still lower in patients who developed BPD, the difference between the two groups was not statistically significant (Tables 3 & 4). Serum vitamin E at birth was unexpectedly higher in patients who developed NEC than in those who did not, but the difference in vitamin E level between the two groups was not significant at discharge (Tables 3 & 4).

Catalase level at birth was significantly lower in patients who developed NEC, those who developed BPD and those who developed IVH than in those who did not (Table 3). TAS level at birth was significantly lower in patients who developed BPD and IVH than in those who did not (Table 3).

There was no statistically significant difference in the levels of vitamin A, vitamin E, TAS and catalase at birth between patients who survived and those who did not (Table 3).

Although the MDA level at birth was lower in patients who survived than in those who died, the difference was not statistically significant [median (range) 1.78 nmol/L (1.30–2.81) vs 1.94 nmol/L (1.22–3.77)] (Table 3). MDA levels at birth were significantly higher in patients who developed BPD (\(P=0.03\)).

There was a statistically significant negative correlation between MDA levels and vitamin E and TAS levels at birth (\(r = -0.35, \ P<0.01\) and \(r = -0.24, \ P=0.01\), respectively). On the other hand, there was a statistically non-significant weak negative correlation between MDA level at birth and both vitamin A and catalase levels at birth (\(r = -0.11, \ P=0.25\) and \(r = -0.10, \ P=0.37\), respectively).

There was a statistically significant positive correlation between birthweight and TAS and vitamin A level at birth (\(r=0.26, \ P=0.01, \ r=0.39, \ P<0.01\), respectively). A non-significant weak positive correlation was found between birthweight and vitamin E and catalase levels at birth (\(r=0.06, \ P=0.55, \ r=0.17, \ P=0.08\), respectively), while a significant weak negative correlation was found between birthweight and MDA level at birth (\(r = -0.20, \ P=0.04\)).

**Discussion**

The oxidant/anti-oxidant balance in the fetus and newborn is delicate. In the case of pre-term birth, this balance can tip towards oxidant injury. Premature delivery often occurs before the normal upregulation of anti-oxidant systems and other ROS scavengers such as glutathione and ceruloplasmin. This is in addition to relatively deficient utero-placental transfer of nutrients important to anti-oxidant defenses which places the newborn at particular risk of ROS-induced injury.

Plasma vitamin A levels were significantly lower in the pre-term than in the full-term group. Similarly, a lower concentration of vitamin A was previously found in pre-term infants compared with term infants, reflecting low hepatic stores.

The plasma level of vitamin E was also significantly lower in the pre-term than in the full-term group. This is in agreement with previous studies which demonstrated that cord blood vitamin E concentrations in term infants were higher than in pre-term infants. Henriksen et al. also found significantly lower levels of vitamins A and E at birth in VLBW infants compared with term infants.

Levels of vitamins A and E at birth were significantly higher in pre-term neonates whose mothers had received antenatal corticosteroids. A similar effect of antenatal corticosteroids was found in cord vitamin A levels. Antenatal and postnatal corticosteroids significantly increase plasma concentrations of retinol in pre-term infants. The mechanism by which corticosteroids elevate vitamin A levels is not known but is likely to be owing to an increase in plasma retinol binding protein levels secondary to enhanced hepatic protein synthesis. This results in an increased release of vitamin A from the liver into the plasma.

Vitamin A is required in the fetal lung for both cellular differentiation and surfactant synthesis. With regard to respiratory outcome, levels at birth of vitamins A and E, TAS and catalase were significantly lower in patients who developed BPD than in those who did not. In a study by Inder et al., there was a trend toward lower vitamin A levels at all sample times in infants who died or developed chronic lung disease. However, in the current study, there was no statistically significant difference in the levels of vitamin A or E at birth between patients who died and those who survived.

Tyson et al. found that vitamin A 5000 IU given intramuscularly three times per week for 28 days to extremely low-birthweight infants significantly improved vitamin A status and decreased the risk.
of chronic lung disease. This recommendation is not appropriate for many developing countries because of the unavailability of intramuscular vitamin A.

Despite administration of oral vitamin A supplementation after enteral feeding had been established, the level of vitamin A at discharge was still significantly lower in pre-term neonates who had developed BPD and NEC than in those who had not. Vitamin E is a potent anti-oxidant in cell membranes. Some investigators have claimed that vitamin E supplementation may decrease the incidence of retinopathy of prematurity, while other reports have demonstrated that vitamin E may be helpful in the course of IVH and BPD.

In accordance with these findings, serum vitamin E levels at birth were significantly lower in patients who subsequently developed BPD than in those who did not, and although they were still lower at discharge, the difference between the two groups at discharge was not statistically significant. Serum vitamin E levels were significantly higher at birth in patients who developed NEC than in those who did not. Since only ten patients developed NEC, a type I error could not be excluded. Moreover, this difference did not persist at discharge.

MDA is a global marker of lipid peroxidation, and although isoprostanes are the most specific markers of lipid peroxidation, they are also the most difficult to measure. Similar to other studies, MDA levels measured at birth were significantly higher in pre-term than in full-term neonates, which might indicate that peroxidation and protein oxidation processes are intensified in pre-term newborns. In agreement with the above study, significantly higher levels of different blood oxidative stress markers – MDA and protein carbonyl – were detected in pre-term newborns. In concordance with these findings, there was a significant positive correlation between MDA and vitamin A levels at birth. It has been observed previously that cord serum vitamin A concentrations increased with birthweight. In the current study, there was a statistically significant positive correlation between birthweight and MDA levels at birth.

Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vitamin A μmol/L</th>
<th>Vitamin E μmol/L</th>
<th>TAS nmol/L</th>
<th>Catalase units/mg Hb</th>
<th>MDA nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC</td>
<td>Yes (n=10)</td>
<td>0.087 (0.069-0.244)</td>
<td>10.21 (8.12-11.84)</td>
<td>1.82 (1.16-1.83)</td>
<td>172 (168-172)</td>
</tr>
<tr>
<td></td>
<td>No (n=90)</td>
<td>0.184 (0.069-0.907)</td>
<td>3.48 (0.30-13.69)</td>
<td>1.80 (1.31-2.61)</td>
<td>179 (161-198)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.53</td>
</tr>
<tr>
<td>BPD</td>
<td>Yes (n=20)</td>
<td>0.115 (0.069-0.139)</td>
<td>3.01 (0.30-10.21)</td>
<td>1.36 (1.26-1.57)</td>
<td>164 (161-172)</td>
</tr>
<tr>
<td></td>
<td>No (n=80)</td>
<td>0.186 (0.069-0.907)</td>
<td>6.52 (0.32-13.69)</td>
<td>1.81 (1.34-2.81)</td>
<td>181 (165-198)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>IVH</td>
<td>Yes (n=24)</td>
<td>0.115 (0.069-0.244)</td>
<td>3.48 (0.32-11.84)</td>
<td>1.62 (1.16-2.61)</td>
<td>170 (162-196)</td>
</tr>
<tr>
<td></td>
<td>No (n=76)</td>
<td>0.184 (0.069-0.907)</td>
<td>6.26 (0.30-13.69)</td>
<td>1.81 (1.34-2.61)</td>
<td>179 (162-198)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Outcome</td>
<td>Discharge (n=72)</td>
<td>0.174 (0.069-0.837)</td>
<td>6.26 (0.30-13.69)</td>
<td>1.81 (1.16-2.81)</td>
<td>177 (161-198)</td>
</tr>
<tr>
<td></td>
<td>Mortality (n=28)</td>
<td>0.181 (0.069-0.907)</td>
<td>3.04 (0.32-12.30)</td>
<td>1.77 (1.34-2.21)</td>
<td>179 (165-197)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.46</td>
<td>0.23</td>
<td>0.92</td>
<td>0.59</td>
<td>0.06</td>
</tr>
</tbody>
</table>

NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; IVH, intraventricular haemorrhage; TAS, total anti-oxidant status; MDA, malondialdehyde. Values are expressed as median (range).
There was significant negative correlation between total anti-oxidant status and the lipid peroxidation marker MDA \((r=-0.24, P=0.01)\). Similarly, in another study, there was a weak negative correlation between total anti-oxidant status in pre-term infants and the lipid peroxidation marker MDA \((r=-0.24, P=0.05, n=89)\) during the first 11 days of life.  

Most neonatal units administer oral vitamin supplements to pre-term infants once enteral feeding has been established, but doses vary and are not generally adjusted in favour of the smallest, most immature infants. The American Society for Clinical Nutrition recommends 910 IU/kg/day as the minimum parenteral vitamin A dose suitable for pre-term infants, and suggests evaluation of a higher dose; oral vitamin A supplementation of 4000 IU/kg/day has been recommended for VLBW infants from the establishment of full enteral feeding until discharge from the neonatal unit. Unfortunately, there are too few data to provide solid guidelines for oral vitamin E therapy.  

In summary, pre-term birth is associated with an increased oxidant burden which puts infants at much greater risk of free radical damage. Vitamin A levels are lower at birth in those who subsequently developed NEC and BPD, and since these differences persisted at discharge, a higher dose of enteral vitamin A supplementation in pre-term neonates should be evaluated to reduce morbidity and improve outcome. Further studies are warranted to determine the appropriate dose of oral vitamin E supplementation for pre-term neonates.  

Preterm neonates are exposed to increased oxidant stress at birth and are susceptible to anti-oxidant deficiencies, which places them at greater risk of morbidity and mortality. The study highlights the relationship between vitamin/anti-oxidant levels at birth and the risk of neonatal morbidities including bronchopulmonary dysplasia, intraventricular haemorrhage, necrotizing enterocolitis and outcome.  

**Disclaimer Statements**  

**Contributors**  

EAA conceived and designed the study, helped in data acquisition, and revised the manuscript for important intellectual content. WAA helped in manuscript drafting and in data acquisition, and revised the manuscript for important intellectual content. AAE helped in manuscript drafting, analyzed and interpreted the data and revised the manuscript for important intellectual content. ERY and JSH helped in data acquisition and preformed the laboratory tests, and revised manuscript for important intellectual content. The final manuscript was approved by all authors.

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None.

**Conflicts of interest**  

The authors declare that they have no conflict of interest.

**Ethics approval**  

The study design was approved by the Scientific Research Committee of the pediatric Department, Faculty of Medicine, Cairo University. Informed consent was obtained from the parents of all the study subjects.

**References**  


**Table 4** Levels of vitamins A and E at discharge in relation to complications of prematurity among pre-term neonates who survived  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)=72</th>
<th>Vitamin A at discharge, (\mu)mol/L median, range</th>
<th>Vitamin E at discharge, (\mu)mol/L median, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC</td>
<td>Yes ((n=8))</td>
<td>0.253 (0.139–0.349)</td>
<td>24.03 (12.07–35.99)</td>
</tr>
<tr>
<td></td>
<td>No ((n=64))</td>
<td>0.732 (0.349–0.907)</td>
<td>32.97 (7.89–57.35)</td>
</tr>
<tr>
<td></td>
<td>(P)-value</td>
<td>&lt;0.01</td>
<td>0.61</td>
</tr>
<tr>
<td>BPD</td>
<td>Yes ((n=18))</td>
<td>0.349 (0.349–0.767)</td>
<td>10.44 (7.89–40.63)</td>
</tr>
<tr>
<td></td>
<td>No ((n=54))</td>
<td>0.732 (0.139–0.907)</td>
<td>35.52 (9.98–57.35)</td>
</tr>
<tr>
<td></td>
<td>(P)-value</td>
<td>&lt;0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>IVH</td>
<td>Yes ((n=8))</td>
<td>0.560 (0.349–0.767)</td>
<td>23.22 (10.44–35.99)</td>
</tr>
<tr>
<td></td>
<td>No ((n=64))</td>
<td>0.715 (0.139–0.907)</td>
<td>32.97 (7.89–63.62)</td>
</tr>
<tr>
<td></td>
<td>(P)-value</td>
<td>0.42</td>
<td>0.42</td>
</tr>
</tbody>
</table>

NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; IVH, intraventricular haemorrhage.
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