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ORIGINAL ARTICLE

Vitamin A and E status in very low birth weight infants

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Objective: To determine vitamin A and vitamin E status in very low birth weight (VLBW) infants at the time of birth (TB), at the time of full feeding (TFF) and at term postmenstrual age (TT).

Study Design: An observational study was conducted in VLBW infants. Plasma retinol and α-tocopherol levels were measured at TB, TFF and TT. Multivitamin supplementation was given to all infants to meet the daily requirement.

Result: A total of 35 infants were enrolled. The median (interquartile range) of gestational age and birth weight was 30 (28 to 32) weeks and 1157 g (982 to 1406 g). The median of vitamin A and vitamin E intakes from TFF to TT was 832 and 5.5 IU kg^{-1} day^{-1}, respectively. Vitamin A deficiency occurred in 67.7% at birth, 51.6% at TFF and 82.1% at TT. Vitamin E deficiency occurred in 77.4% at birth, 16.1% at TFF and 35.7% at TT. Small-for-gestational age was the only risk factor for vitamin A deficiency. Lower amount of breast milk consumption was associated with higher incidence of vitamin E deficiency. No differences in vitamin A- or vitamin E-related morbidities between infants with and without vitamin deficiencies were found.

Conclusion: High prevalence of vitamin A and vitamin E deficiency was found in VLBW infants starting from birth to term postmenstrual age. Therefore, a higher dose of vitamin supplementation is required.
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Introduction

Vitamin A and vitamin E are important micronutrients. Vitamin A has a role in protein synthesis, cell growth and differentiation.[1] Vitamin A supplementation has been reported to reduce infant mortality and morbidities.[2–4] In premature infants, vitamin A has an important function in lung and visual development.[1] A systematic review of vitamin A supplementation in very low birth weight (VLBW) infants showed a significant reduction in death or oxygen requirement at 1 month of age and 36 weeks of postmenstrual age (PMA) and a trend toward reduction in retinopathy of prematurity (ROP).[2]

Vitamin E is one of the antioxidative agents. It prevents the cell membranes from lipid peroxidation and is involved in eicosanoid synthesis.[5] Therefore, it protects neonates from oxidative-related diseases such as hemolytic disease and bronchopulmonary dysplasia (BPD). A systematic review of vitamin E supplementation in preterm infants showed that vitamin E reduced the risk of ROP and intracranial hemorrhage.[6]

VLBW infants are at risk for both vitamin A and vitamin E deficiency because of low storage and intake of these vitamins, as well as poor absorption and higher requirement compared with term infants.[5] Significantly lower levels of vitamin A and vitamin E at birth and at discharge in VLBW infants were found when compared with term infants.[7]

Currently, there are various recommendations of vitamin supplementation for VLBW infants.[8] Despite vitamin supplementation, a high incidence of vitamin A and vitamin E deficiency in VLBW infants has been reported.[7,9–10] Such data are reported from developed countries that may differ from developing countries as Thailand in terms of nutritional and socioeconomical status and genetic factors. We, therefore, conducted a study with the aim to assess the vitamin A and vitamin E status of VLBW infants in our institution and to define the associated risk factors.

Methods

Study design and subjects

A prospective cohort study was conducted. All VLBW infants who were born at Ramathibodi Hospital between 1 April 2008 and 31 March 2009 were eligible. Exclusion criteria included major congenital anomalies or death within the first week of life. Informed consent was obtained from the parents. The study was approved by the Ethical Committee on Researches Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Vitamin intake

Total amount of vitamin intake was estimated from parenteral nutrition, enteral feedings (breast milk or formula) and

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supplementation. Parenteral nutrition was started within 24 to 48 h of life. Enteral feeding was initiated as soon as clinical stability was attained with an increment of 15 to 25 ml kg^{-1} day^{-1} per attending physician's discretion. Parenteral multivitamin supplementation was given at the volume of 2 ml day^{-1} in the form of OMVI (Otsuka Pharmaceutical, Naruto, Japan), consisting of vitamin A 1650 IU and vitamin E 5 IU. Enteral supplementation was given in the form of multivitamin dry syrup (The Government Pharmaceutical Organization (GPO), Bangkok, Thailand), consisting of vitamin A 1990 IU per 5 ml and vitamin E 7.5 IU per 5 ml, to increase vitamin A intake to 600 to 1000 IU/kg^{-1} day^{-1}, in accordance with the Consensus Recommendation for infants with VLBW 1993.[5,8] Oral vitamin supplementation was initiated after full feeding (total milk intake of 140 to 160 ml kg^{-1} day^{-1}). The amount of vitamins in each infant's feeding was derived from the manufacturer data for infant formula and the data from the Bureau of Nutrition, Ministry of Public Health, Thailand, for breast milk.[11]

Blood sampling and analyses

Blood samples (0.5 ml) were obtained in EDTA-containing tubes at birth (TB), at the time of full feeding (TFF) and at term PMA (TT). The blood samples were centrifuged; then, plasma was collected and stored at $-20\,^{\circ}C$ until analyses. All procedures were done under light protection. Plasma concentration of retinol was used as an indicator of vitamin A status, whereas α-tocopherol was used as a marker for vitamin E status. The analyses were performed using a high performance liquid chromatography method (Waters LC Module 1 Plus, New York, NY, USA) as described elsewhere.[12–14]

Definitions

According to the WHO (World Health Organization) criteria,[5] vitamin A deficiency is defined as retinol level $<20\,\mu g\,dl^{-1}$, and the degree of deficiency is severe if retinol level is $<10\,\mu g\,dl^{-1}$. Vitamin E deficiency is defined as α-tocopherol $<500\,\mu g\,dl^{-1}$.

Statistical analysis

Data were expressed as median and interquartile range (IQR). Univariate analysis was performed to determine factors associated with vitamin deficiency. The χ^2 or Fisher's exact test was used for categorical variables and Mann–Whitney *U*-test was used for continuous variables. The *P*-value of <0.05 was considered statistically significant.

Results

Patient characteristics

In all, 44 VLBW infants were eligible. Nine were excluded (4 deaths in the first week of life, 1 Hydrops fetalis and 4 parental refusals). Therefore, 35 infants were enrolled in the study with 31, 31 and 28 blood samples at TB, TFF and TT, respectively. Of these, 23 were males and 12 were females. The median (IQR) of birth weight and gestational age was 1157 g (982 to 1406 g) and 30 (28 to 32) weeks, respectively. Apgar scores were 5 at 1 min and 9 at 5 min. All attained full feeding within 10 days.

Nutritional data

Most infants (97%) received parenteral nutrition with multivitamin supplementation. Median (IQR) duration of parenteral nutrition was 6.5 (4.8 to 8) days. Infants were fed either breast milk or premature formula or a combination of both. Median (IQR) of daily vitamin A intake was 1066 IU day^{-1} (893.7 to 1337; or 820 IU kg^{-1} day^{-1} (698 to 1122)) from TB to TFF and 1585 IU day^{-1} (1169 to 1893; or 832 IU kg^{-1} day^{-1} (516.3 to 1087)) from TFF to TT. Median (IQR) of daily vitamin E intake was 5.9 IU day^{-1} (3.4 to 7.5; or 4.6 IU/kg/$^{-1}$$day^{-1}$ (3.3 to 6.0)) from TB to TFF and 10.6 IU day^{-1} (6.8 to 13.7; or 5.5 IU kg^{-1} day^{-1} (3.4 to 7.5)) from TFF to TT.

Vitamin status

Vitamin A. At birth, 67.7% of enrolled infants had vitamin A levels $<20\,\mu g\,dl^{-1}$ (vitamin A deficiency) with a median (IQR) plasma retinol of 15.5 $\mu g\,dl^{-1}$ (10.5 to 24.9). Incidence of vitamin A deficiency declined to 51.6% at TFF and increased to 82.1% at TT (Table 1). Small-for-gestational age (SGA) was the only risk factor for vitamin A deficiency at birth and remained significant at TFF (Table 2). Vitamin A levels were very low at birth in SGA infants and slightly increased over time to term PMA (Figure 1). Interestingly, appropriate-for-gestation age preterm infants had higher vitamin A levels that progressively decreased over time to term PMA. Infants in the vitamin A-deficient group received significantly less vitamin A than those in the vitamin A-normal group; median vitamin A intake was 942.6 IU day^{-1} (835 to 1090) vs 1258.8 IU day^{-1} (1061 to 1422) ($P=0.02$). There were no correlations between vitamin A intake and breast milk consumption and duration of human milk fortifier.

Vitamin E. At birth, 77.4% of enrolled infants had plasma α-tocopherol $<500\,\mu g\,dl^{-1}$ (vitamin E deficiency), with a median (IQR) of 283.7 $\mu g\,dl^{-1}$ (226.3 to 469). Incidence of vitamin E deficiency markedly declined to 16.1 and 35.7% at TFF and at TT, respectively (Table 1). There were no differences in birth weight, gestational age, antenatal steroid or proportion of SGA infants between normal and vitamin E-deficient group. At TFF and at TT, infants with normal vitamin E levels had a significantly higher percentage of breast milk consumption, although they had less vitamin E intake at both time frames compared with those in the deficient group (Table 3).

Clinical outcomes

There was 1 infant who died of severe BPD; 13 had BPD, 2 had periventricular leucomalacia and 3 had ROP. Median (IQR)

Table 1 Vitamin status at different time intervals

Vitamin status	*At time of birth*, N = *31*	*At time of full feeding*, N = *31*	*At term PMA*, N = *28*
Vitamin A			
Plasma retinol ($\mu g\,dl^{-1}$)[a]	15.5 (10.5–24.9)	19.8 (12.5–28.3)	13.75 (9.4–18.8)
Deficiency, *n* (%) ($<20\,\mu g\,dl^{-1}$)	21 (67.7)	16 (51.6)	23 (82.1)
Severe deficiency, *n* (%) ($<10\,\mu g\,dl^{-1}$)	6 (19)	5 (16)	8 (28.6)
Vitamin E			
Plasma α-tocopherol ($\mu g\,dl^{-1}$)[a]	283.7 (226.3–469)	871 (570.4–1421)	879 (289–1281)
Deficiency, *n* (%) ($<500\,\mu g\,dl^{-1}$)	24 (77.4)	5 (16.1)	10 (35.7)

Abbreviation: PMA, postmenstrual age.
[a]Data are presented as median (interquartile range (IQR)).

Table 2 Factors associated with vitamin A deficiency

Factor	*Deficient group*	*Normal group*	P-*value*
At time of birth	*N* = 21	*N* = 10	
Gestational age (weeks)[a]	30.5 (29–33)	29.5 (29–31)	0.48
Birth weight (g)[a]	1120 (975–1440)	1180 (1055–1362.5)	0.70
SGA, *n* (%)	13 (62)	1 (10)	0.01[b]
Antenatal steroid, *n* (%)	4 (19)	1 (10)	0.601
At time to full feeding	*N* = 16	*N* = 15	
SGA, *n* (%)	10 (59)	4 (28.5)	0.04[b]
Duration of TPN (days)[a]	5 (6–10)	7 (4.7–8)	0.37
Breast milk consumption[a] (% of total milk intake)	38 (2.2–58)	21 (1.6–61)	0.89
Vitamin A intake from birth[a] ($IU\,day^{-1}$)	942.6 (835–1090)	1258.8 (1061–1422)	0.02[c]
Vitamin A intake from birth[a] ($IU\,kg^{-1}\,day^{-1}$)	693 (598–995)	882 (744–1077)	0.036[c]
At time to term PMA	*N* = 23	*N* = 5	
SGA, *n* (%)	9 (40)	3 (60)	0.63
Breast milk consumption[a] (% of total milk intake)	29 (5–70.3)	15.8 (5–41)	0.38
Vitamin A intake from full feeding to term PMA[a] ($IU\,day^{-1}$)	1589.5 (1423–1.899)	1186.4 (938–1983)	0.40
Vitamin A intake from full feeding to term PMA[a] ($IU\,kg^{-1}\,day^{-1}$)	897 (627–1167)	571 (421–1019)	0.275

Abbreviations: GA, gestational age; PMA, postmenstrual age; SGA, small-for-gestational age; TPN, total parenteral nutrition.
[a]Data are presented as median (IQR).
[b]*P*-value <0.05, Fisher's exact test.
[c]*P*-value <0.05, Mann–Whitney *U*-test.

duration of oxygen therapy was 1 (0 to 46) days and median (IQR) duration of mechanical ventilation was 0 (0 to 2.5) days.

Regarding the clinical outcomes related to vitamin A status, there were no differences in clinical outcomes in terms of BPD, ROP, death rate or length of hospital stay between infants with normal vitamin A levels and those with vitamin A deficiency. However, infants in the normal vitamin A group had a higher percentage of weight gain ((Body weight at TT−birth weight)/birth weight × 100) compared with those in the vitamin A deficient group, 156 (137 to 211) vs 79 (71 to 192) %, $P = 0.015$.

Regarding the clinical outcomes related to vitamin E status, there were no differences in clinical outcomes in terms of BPD, ROP, hematocrit at TT, number of blood transfusions, length of hospital stay or duration of oxygen supplementation between infants with normal vitamin E levels and those with vitamin E deficiency.

Discussion

Body fat is increased during late gestation; therefore, VLBW infants are born with lower storage of fat-soluble vitamins.[5] Thus, these infants are at high risk of fat-soluble vitamin deficiency. Moreover, fat absorption in these infants is impaired,[5] and they usually have a higher requirement than term infants. Previous studies have

consistently shown high incidence of fat-soluble vitamin deficiency, especially vitamin A in VLBW infants.[15] In breast-fed preterm and term infants, Henriksen *et al*.[7] reported that plasma retinol concentrations in preterm infants at discharge were significantly lower than term counterparts at 4 weeks of age. However, there was no significant difference in plasma α-tocopherol between the two groups.

In our study, despite a high dose of vitamin supplementation according to the Consensus Recommendation for infants with VLBW,[5] we found unexpectedly high incidence of vitamin A and vitamin E deficiency starting from birth through discharge. The incidence of vitamin A and vitamin E deficiency decreased from TB to TFF but increased from TFF to TT. This finding may be explained by the fact that parenteral vitamin supplementation is given during the first period and changed to enteral supplementation in the second period. This is because absorption of fat-soluble vitamins depends on fat absorption that needs the presence of food, bile salt and pancreatic lipase activity.[5] These components may not function properly in premature infants. Moreover, in our institution, vitamin A supplementation is usually given in the form of retinyl ester that has to be hydrolyzed by a specific esterase and activated by bile salt before being absorbed.

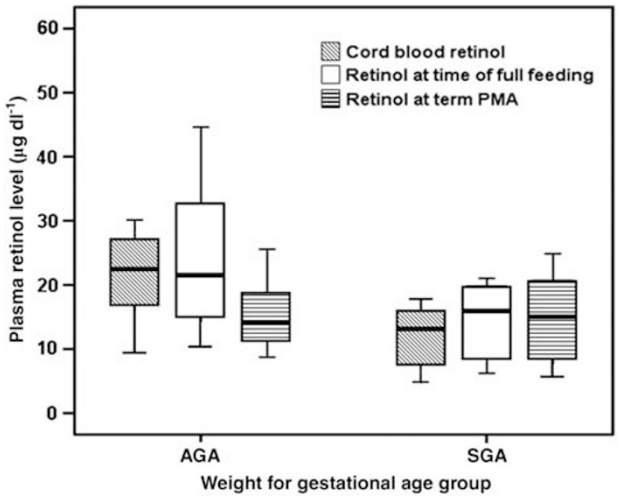

Figure 1 Comparison of plasma retinol levels between appropriate-for-gestational age and small-for-gestational age infants.

We found that SGA infants had significantly lower plasma retinol levels. This may be because of several reasons. First, SGA infants may be born from vitamin A-deficient mothers, as birth weight is significantly correlated with maternal vitamin A status.[16] Second, SGA infants have poor nutritional supply *in utero*, and therefore the placental transfer of vitamin A is decreased. Last, SGA infants have less body fat that affects vitamin storage. Plasma retinol levels in appropriate-for-gestation age infants were found to decrease over time. This is possibly caused by an insufficient *ex utero* vitamin supplementation to maintain vitamin A levels. In contrast, SGA infants who had deficient nutritional supply *in utero* had slightly increased plasma vitamin A levels after birth.

Tyson *et al*.[17] found that vitamin A 5000 IU intramuscularly 3 times per week for 28 days significantly improved vitamin A status and decreased the risk of chronic lung disease of extremely low birth weight infants. But this recommendation could not be applied in many developing countries because of no intramuscular

Table 3 Factors associated with vitamin E deficiency

Factor	*Deficient group*	*Normal group*	P-*value*
At time of birth	$N=24$	$N=7$	
Birth weight (g)[a]	1132 (1002–1417.5)	1210 (830–1400)	0.72
SGA, *n* (%)	13 (54)	2 (28)	0.39
Antenatal steroid, *n* (%)	17 (70)	5 (71)	1
At time to full feeding	$N=5$	$N=26$	
SGA, *n* (%)	2 (40)	4 (46)	1
RDS with surfactant replacement, *n* (%)	0 (0)	4 (15)	0.8
Vitamin E intake from birth[a] (IU day^{-1})	7.7 (6.3–8.7)	5.6 (3.4–8)	0.11
Breast milk consumption (% of total milk intake)[a]	0.7 (0–9.5)	48 (12–61)	0.006[b]
At time to term PMA	$N=10$	$N=18$	
SGA, *n* (%)	3 (33)	9 (50)	0.68
Breast milk consumption (% of total milk intake)[a]	5 (2.3–28.9)	30 (13.3–76.9)	0.048[b]
Vitamin E intake from full feeding through term PMA[a]	12.2 (9.1–16.3)	8.7 (6.2–13.67)	0.26

Abbreviations: PMA, postmenstrual age; RDS, respiratory distress syndrome; SGA, small-for-gestational age.
[a]Data are presented as median (IQR).
[b]*P*-value <0.05, Mann–Whitney *U*-test.

injection form of vitamin A available. In addition, the sequential measurement of serum retinol and α-tocopherol could not be routinely performed in limited-resource laboratories. Our findings may provide information on the amount of enteral vitamin A supplementation for VLBW infants.

The percentage of breast milk consumption was the only factor that affected vitamin E status at TFF and TT. Like vitamin A, vitamin E absorption depends on fat absorption. Fat absorption from breast milk is better than that from formula because of its composition, especially bile salt-stimulated lipoprotein lipase.[18] Moreover, breast milk also has antioxidative effect. A study by Shoji *et al*.[19] found that breast-fed infants had lower excretion of 8-hydroxy-2′-deoxguanosine than formula-fed infants, suggesting lower oxidative DNA damage in breast-fed infants.

Kaempf *et al*.[20] studied α- and γ-tocopherol levels in 14 healthy breast-fed premature infants whose birth weight was between 1090 and 2170 g from birth through day 42 after birth. They found that plasma α-tocopherol increased to a normal range within 6 weeks after birth without vitamin supplementation. In contrast to their study, we found 35.7% of enrolled infants having vitamin E deficiency at TT. This may be explained by a difference in patient characteristics. Their study was conducted in healthy preterm infants with neither severe complications related to prematurity nor diseases that were possibly linked to oxygen free radicals (such as birth asphyxia, severe respiratory distress, BPD and ROP). Furthermore, the infants in their study were bigger than those in ours and could have better vitamin E absorption.

Our study was not designed to compare clinical outcomes between infants with normal vitamin levels and those having vitamin A or vitamin E deficiency. We were unable to demonstrate any differences in relevant outcomes such as BPD, ROP and length of hospital stay. However, we found better weight gain of infants with normal vitamin A status at TFF. Possibly, this may be the effect of vitamin A on cell growth and differentiation.

There are some limitations in our study. We used multivitamin dry syrup from GPO, Thailand, which have no data of oral bioavailability. The gold standard for diagnosis of vitamin A deficiency is vitamin A relative-dose-response test,[21] which is technically difficult in tiny premature babies. Therefore, we used plasma retinol that is widely acceptable to represent vitamin A status in a clinical setting. However, it may underestimate the incidence of deficiency. Regarding vitamin E status, there are several ways to determine vitamin E sufficiency such as vitamin E/ total lipid, cellular vitamin E concentrations and plasma α-tocopherol. In our study, we used plasma α-tocopherol because of the ease of using this test, and it has a clear cutoff for deficiency according to the WHO criteria.

In conclusion, there were unexpectedly high incidences of vitamin A and vitamin E deficiency starting from birth through term PMA. SGA was the risk factor for vitamin A deficiency, whereas lower amount of breast milk consumption was associated with higher incidence of vitamin E deficiency. Therefore, high-risk preterm infants should be closely monitored for their vitamin status. A higher dose of vitamin supplementation may also be considered, especially for those who are at risk for deficiency.

Conflict of interest

The authors declare no conflict of interest.

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