

## Vitamin E status of the newborn in relation to gestational age, birth weight and maternal vitamin E status

BY RAKSHA S. SHAH AND R. RAJALAKSHMI

*Department of Biochemistry, Faculty of Science, M.S. University, Baroda-390002, India*

AND R. V. BHATT, M. N. HAZRA, B. C. PATEL AND N. B. SWAMY

*Obstetrics and Gynecology Department, S.S.G. Hospital, Baroda, India*

AND T. V. PATEL

*Kalpana Clinic, Baroda, India*

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1. Studies were made on the vitamin E status of the newborn as judged by cord serum vitamin E and erythrocyte haemolysis *in vitro* in relation to gestational age, birth weight and maternal vitamin E status in subjects belonging to low (LIG)- and high (HIG)-income groups in urban Baroda.

2. In the case of full-term infants, the mean values for maternal serum vitamin E (mg/l) for LIG (*n* 73) and HIG (*n* 43) were 9.9 (SE 0.4) and 11.6 (SE 0.5). The corresponding values for cord serum vitamin E were 3.6 (SE 0.2) and 4.6 (SE 0.2) mg/l.

3. Serum vitamin E levels (mg/l) were lower in premature infants (2.3 (SE 0.2); *n* 20) and low-birth-weight full-term infants (2.9 (SE 0.2); *n* 25) than in full-term normal infants (4.2 (SE 0.1); *n* 91). This was associated with differences in maternal serum vitamin E levels (7.4 (SE 0.5), 8.2 (SE 0.5) and 11.1 (SE 0.3) respectively). The differences were more marked for LIG.

4. A negative correlation was found between serum vitamin E and erythrocyte haemolysis *in vitro* in the case of maternal blood but not in cord blood.

5. These results suggest that maternal vitamin E deficiency is one of the features associated with prematurity and intra-uterine growth retardation.

Infants and children with either severe protein-energy malnutrition or milder forms of malnutrition have been shown to develop vitamin E deficiency, although the incidence is less in the latter category (Asfour & Firzl, 1965; McLaren *et al.* 1965; Sandstead *et al.* 1965; Majaj, 1966; Thanangkul *et al.* 1966). Tocopherol depletion in these patients can be attributed to both poor vitamin intake and impaired intestinal absorption which, in turn, may be due to a combination of pancreatic insufficiency, decreased bile production and diarrhoea (Hansen (1967) quoted by Gallo-Torres (1980)). In children suffering from protein-energy malnutrition of varying degrees, serum vitamin E levels were significantly lower in those who died than in survivors (McLaren *et al.* 1969).

It is well known that plasma tocopherol levels rise during pregnancy (Ferguson *et al.* 1955; Vobecky *et al.* 1973, 1974; Horwitt *et al.* 1975; National Institute of Nutrition, 1978). In previous studies in this laboratory, serum vitamin E levels (mg/l) at term in low-income women were found to be 11.7 (SE 0.5) compared with 8.2 (SE 0.7) in non-pregnant, non-lactating women from the same social groups in urban Baroda. Even in Kerala, where tocopherol intake is low, the corresponding values for serum vitamin E were found to be 12.8 (SE 0.9) and 6.8 (SE 0.6) mg/l (Dave, 1980). However, studies by Ng & Chong (1975) suggested a fall in vitamin E levels during pregnancy. Low serum vitamin E levels have been reported in parturient women by Hassan *et al.* (1982). Vobecky *et al.* (1973, 1974) reported a fall in serum  $\alpha$ -tocopherol levels during the second trimester for women who aborted or gave birth to malformed infants. Women whose pregnancies resulted in stillbirths also tended to have lower levels of serum vitamin E (Vobecky *et al.* 1974). Plasma vitamin E

level in the neonate is found to be related to that of the mother (Leonard *et al.* 1972; Hassan *et al.* 1982). As placental transfer is limited (Martinez *et al.* 1981) any adverse effects of poor maternal status on fetal and neonatal status would not be surprising. In other studies in this laboratory, a clear-cut association has been found with regard to maternal vitamin A status, and gestational age and growth of the neonate (Shah & Rajalakshmi, 1984). These observations raise the question whether maternal deficiency of vitamin E is a contributory factor in the prevalence of prematurity and intra-uterine growth retardation.

The present study of vitamin E levels in maternal and cord serum was made in order to investigate the degree of association between the two levels and their relation to prematurity and intra-uterine growth retardation.

#### MATERIALS AND METHODS

The subjects were 136 mother–infant pairs from both low- and high-income groups (LIG and HIG respectively) in urban Baroda. Details of differences in their dietary intakes are given elsewhere (Shah & Rajalakshmi, 1984).

For the assessment of gestational age, the mother was questioned soon after delivery about the last menstrual date with the help of a local calendar, and, if necessary, using local events. Gestational age was taken as menstrual age minus 2 weeks to allow for ovulation.

Infants with a gestational age of less than 36 weeks were considered premature, and weights of less than 2.5 kg at birth in full-term infants, as low birth weights.

Mixed venous–arterial cord blood was collected in vials with and without EDTA at delivery. Maternal venous blood was collected after delivery in the same manner. Vitamin E in cord and maternal serum was determined by the method of Quaife *et al.* (1949) based on the Emmerie–Engel reaction, whereas *in vitro* erythrocyte haemolysis in the same was determined by the method of Horwitt *et al.* (1956) using a dilute solution of hydrogen peroxide. The reliability of the estimations was checked by making independent estimations of five to six portions of the same sample and the values were found to be in close agreement.

For assessment of mean differences between two groups, Student's *t* test was used. Pearson correlation coefficient was employed to assess the association between variables, its significance calculated by transformation to a *t* statistic (Ferguson, 1976).

#### RESULTS

The parity of the mothers ranged from 1 to 8 for LIG and from 1 to 4 for HIG. However, neither maternal nor cord serum vitamin E was found to vary with parity or with the sex of the infant. Also, no sex differences were found with regard to birth weight. The values were combined for these variables.

The birth weights of the newborn as well as vitamin E levels in cord and maternal serum at different gestational ages are shown in Table 1. As expected, the values for all these variables increased with the progress of gestation. However, at all gestational ages, differences were found between LIG and HIG with regard to birth weight as well as cord and maternal serum vitamin E. The product-moment correlation was calculated between gestational age and cord serum vitamin E, the values were 0.88 ( $n$  86;  $P < 0.001$ ) for LIG and 0.28 ( $n$  50;  $P < 0.05$ ) for HIG. Values in Table 1 also indicate that mothers of premature infants had lower levels of serum vitamin E than those of full-term infants. This difference seemed somewhat greater for LIG than for HIG. The birth weight differences between the two groups were greater in premature infants than in full-term infants. The relation between maternal serum vitamin E status, on the one hand, and cord serum vitamin E and birth weight on the other is shown in Table 2. The critical level seems to be

Table 1. Birth weight, cord and maternal serum vitamin E in relation to gestational age in low (LIG)- and high (HIG)-income groups from urban Baroda (Mean values with their standard errors)

Period of gestation (weeks)	No. of subjects				LIG		HIG	
	LIG	HIG	LIG Range	HIG Range	Mean	SE	Mean	SE
Birth wt (kg)								
< 32	2	—	1.15, 1.25	—	1.20	—	—	—
32-36	11	7	1.50-2.60	2.04-2.70	1.82**	0.11	2.33	0.08
36-40	68	39	1.25-3.90	2.38-3.80	2.67***†††	0.06	3.07†††	0.06
> 40	5	4	2.28-2.70	2.72-3.40	2.47***†	0.08	3.18	0.15
< 36	13	7	1.15-2.60	2.04-2.70	1.73***	0.11	2.33	0.88
> 36	73	43	1.25-3.90	2.38-3.80	2.66***†††	0.06	3.08†††	0.05
Cord serum vitamin E (mg/l)								
< 32	2	—	1.3-1.5	—	1.4	—	—	—
32-36	11	7	1.4-3.5	1.8-3.5	2.3	0.2	2.8	0.2
36-40	68	39	1.2-7.5	1.8-8.0	3.5***††	0.2	4.5†††	0.2
> 40	5	4	2.0-6.2	3.5-8.1	4.5	0.8	6.3	1.0
< 36	13	7	1.3-3.5	1.8-3.5	2.1	0.2	2.8	0.2
> 36	73	43	1.2-7.5	1.8-8.1	3.6***†††	0.2	4.6†††	0.2
Maternal serum vitamin E (mg/l)								
< 32	2	—	3.8, 4.4	—	4.1	—	—	—
32-36	11	7	4.5-10.1	5.7-12.7	7.1	0.6	8.8	0.7
36-40	68	39	4.4-17.5	6.5-20.4	9.7***†	0.4	11.4†	0.6
> 40	5	4	6.0-14.5	9.7-14.8	12.4	1.6	12.8	1.1
< 36	13	7	3.8-10.1	5.7-12.7	6.7	0.5	8.8	0.7
> 36	73	43	4.4-17.5	6.5-20.4	9.9***†††	0.4	11.6†††	0.5

Values were significantly different from HIG values: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Values were significantly different from preceding values: †  $P < 0.05$ , ††  $P < 0.02$ , †††  $P < 0.001$ .

10 mg/l. For LIG, maternal serum vitamin E values of < 10 and > 10 mg/l were associated with mean birth weights (kg) of 2.47 (SE 0.07) ( $n$  38) and 2.87 (SE 0.07) ( $n$  35) respectively. The corresponding values for cord serum vitamin E (mg/l) were 2.6 (SE 0.1) and 4.8 (SE 0.2). The product-moment correlation between maternal serum vitamin E and birth weight was  $r$  0.47 ( $n$  73;  $P < 0.001$ ) for LIG and 0.31 ( $n$  43;  $P < 0.05$ ) for HIG. The

Table 2. Birth weight and cord serum vitamin E in relation to maternal vitamin E status in full-term and premature infants in low (LIG)- and high (HIG)-income groups from urban Baroda  
(Mean values with their standard errors)

Maternal serum vitamin E (mg/l)	No. of subjects		Full-term infants						Premature infants								
	LIG	HIG	Mean	SE	LIG	Mean	SE	HIG	Mean	SE	LIG	Mean	SE	HIG	Mean	SE	
< 10	38	17	2.47***	0.07	2.97	0.08	11	5	1.76*	0.15	2.31	0.12	5	1.76*	0.15	2.31	0.12
> 10	35	26	2.87***†††	0.07	3.15	0.06	2	2	1.50, 1.65	—	2.27, 2.50	—	2	1.50, 1.65	—	2.27, 2.50	—
< 10	38	17	2.6***	0.1	3.4	0.2	11	5	2.0	0.2	2.6	0.3	5	2.0	0.2	2.6	0.3
< 10	35	26	4.8†††	0.2	5.6†††	0.3	2	2	2.1, 3.4	—	2.9, 3.5	—	2	2.1, 3.4	—	2.9, 3.5	—

Birth wt (kg)

Cord serum vitamin E (mg/l)

Values were significantly different from HIG values: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .  
Value was significantly different from preceding values: †††  $P < 0.001$ .

Table 3. *In vitro* erythrocyte haemolysis in relation to serum vitamin E for cord and maternal blood in low (LIG)- and high (HIG)-income groups from urban Baroda  
(Mean with their standard errors; mean and SE serum vitamin E values in parentheses)

Serum vitamin E (mg/l)	In vitro erythrocyte haemolysis (%)											
	Cord						Maternal					
	LIG		HIG		LIG		HIG		LIG		HIG	
	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n
< 5	13.0*	0.8	49	10.1	1.0	23	8.5	0.8	2	—	—	—
	(2.9)	(0.1)		(3.5)	(0.2)		(4.4)	(0.05)				
5-10	9.6††	0.8	11	12.5	1.6	17	7.9**	0.7	29	4.7	0.8	16
	(6.2)	(0.2)		(6.4)	(0.2)		(6.9)	(0.2)		(8.3)	(0.3)	
> 10							5.2†††	0.4	29	5.2	0.6	24
							(12.5)	(0.3)		(13.8)	(0.6)	

Values were significantly different from HIG values: \*  $P < 0.05$ , \*\*  $P < 0.01$ .  
Values were significantly different from previous values: ††  $P < 0.01$ , †††  $P < 0.001$ .

corresponding value for premature infants for the combined groups was  $r$  0.45 ( $n$  20;  $P$  < 0.05). Similarly, there was a significant relation between cord and maternal serum vitamin E, the product-moment correlations being 0.96 ( $n$  73;  $P$  < 0.001) for LIG and 0.65 ( $n$  43;  $P$  < 0.001) for HIG. All correlations were higher for LIG.

Using the converse approach, infants with birth weights less than 2.5 kg were found to have mean cord serum vitamin E levels (mg/l) of 2.9 (SE 0.2) compared with 3.9 (SE 0.2) ( $n$  49) for heavier infants. The corresponding values for maternal serum levels were 8.2 (SE 0.5) and 10.6 (SE 0.4) mg/l. (A similar analysis was not done for HIG as birth weight was less than 2.5 kg in only one case (2.4 kg). Even for comparable birth weights (i.e. above 2.5 kg) and maternal serum vitamin E levels (i.e. < 10 mg/l), the values for cord serum vitamin E tended to be higher for HIG than those for LIG. The corresponding values were 2.5 (SE 0.2) ( $n$  18) for LIG and 3.4 (SE 0.2) ( $n$  16) for HIG.

In vitro haemolysis of erythrocytes is known to increase with low levels of serum vitamin E (Gordon & De Metry, 1952; Horwitt *et al.* 1956). The values for haemolysis in cord and maternal blood were therefore analysed in relation to the respective values for serum vitamin E (Table 3). As expected, in the case of maternal blood, higher values for in vitro erythrocyte haemolysis were found with lower levels of serum vitamin E. Even for comparable serum vitamin E levels values for erythrocyte haemolysis were found to be higher in cord blood than in maternal blood. The product-moment correlation in maternal blood between serum vitamin E and erythrocyte haemolysis in vitro was  $-0.39$  ( $n$  59;  $P$  < 0.02) for LIG compared with  $-0.60$  ( $n$  42;  $P$  < 0.001) for HIG. The corresponding values in cord blood were  $-0.23$  (not significant) for LIG and  $-0.29$  (not significant) for HIG.

#### DISCUSSION

Values for maternal serum vitamin E were in the range reported by others (Ferguson *et al.* 1955; Vobecky *et al.* 1973, 1974; Dave, 1980). This was also true for cord serum values (Straumfjord & Quaife, 1946; Moyer, 1950; Wright *et al.* 1951; Nitowsky *et al.* 1956; McWhirter, 1975; Vobecky *et al.* 1976, 1982; Martinez *et al.* 1981). The observation that in both groups mothers of premature infants had lower levels of serum vitamin E than those of full-term infants suggests that maternal vitamin E deficiency may be one of the factors associated with prematurity. That premature infants have lower levels of serum vitamin E at birth has been reported by several other investigators (Wright *et al.* 1951; Hassan *et al.* 1966; Oski & Barness, 1967; Hashim & Asfour, 1968). These differences have been found to persist at 10 d (Petrich *et al.* 1976) and at 3 weeks (Gross & Melhorn, 1972) after birth. The relative deficiency of vitamin E in premature infants as judged by serum levels can be attributed to factors such as limited tissue storage (Gross & Melhorn, 1972) and, also, perhaps immature transport mechanisms. The increase in cord serum vitamin E with gestational age is consistent with the increase in fetal liver vitamin E stores (Mino *et al.* 1976). A similar pattern has also been found with regard to vitamin A (Shah *et al.* 1987). Also, whole body vitamin E was found to be 20 mg in a full-term infant weighing 3500 g compared with 3 mg in a premature infant weighing 1000 g (Dju *et al.* 1952), suggesting that the fetal accretion of vitamin E takes place mainly in late pregnancy.

The income group differences with regard to the correlations between cord serum vitamin E and gestational age may suggest the earlier achievement of a satisfactory vitamin E status for HIG because of differences in maternal status.

A positive correlation between cord and maternal serum vitamin E, as found in the present studies, has also been reported by other investigators (Nitowsky *et al.* 1956; Leonard *et al.* 1972; Hassan *et al.* 1982; Vobecky *et al.* 1982).

Values for cord erythrocyte haemolysis in vitro were similar to those reported by Hassan

*et al.* (1966) and Hashim & Asfour (1968). The inverse relation found between maternal serum vitamin E and maternal erythrocyte haemolysis *in vitro* is consistent with previously reported findings (Gordon & De Metry, 1952; Horwitt *et al.* 1956; Melhorn *et al.* 1971). The absence of a correlation between cord serum vitamin E and cord erythrocyte haemolysis *in vitro* in either full-term infants or premature infants has also been reported by Melhorn & Gross (1971) and Hassan *et al.* (1982); Melhorn & Gross (1971) established a correlation 3–4 weeks postnatally. However, for corresponding serum vitamin E values, values for *in vitro* erythrocyte haemolysis were greater in infants than in mothers, suggesting that factors other than serum vitamin E are involved in the fragility-integrity of erythrocytes. It is also interesting to note that although Gordon *et al.* (1955) found no differences with regard to serum vitamin E in infants born of affluent mothers delivering in a private hospital and those born of low-income mothers delivering in a state hospital, cord blood in the former had lower values for *in vitro* erythrocyte haemolysis than the latter.

The social class differences found with regard to cord serum vitamin E levels for corresponding birth weights and levels of maternal serum vitamin E, were similar to those observations made in other studies with regard to serum vitamin A (Shah & Rajalakshmi, 1984). These observations suggest the possible involvement of other factors such as the overall plane of nutrition, body stores of tocopherol as well as transport mechanisms.

In conclusion, the present results show that the vitamin E status of the newborn is influenced by factors such as gestational age, growth and maternal vitamin E status. They also suggest that poor vitamin E status in the mother may be associated with prematurity and intra-uterine growth retardation, especially in poorly nourished women. The findings indicate that a low serum vitamin E level in pregnancy could perhaps be considered as one of the biochemical indicators of the risk of prematurity and fetal growth retardation.

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