

# Plasma vitamin E values in the newborn

D. LABADARIOS, G. S. SHEPHARD, J. GREEN, M. E. HUTCHISON, P. A. H. HENNING, P. B. HESSELING

## Summary

Plasma vitamin E values of newborn infants in a Coloured population of Cape Town have been determined. The mean plasma  $\alpha$ -tocopherol value was  $3,2 \pm 0,8$  mg/l. A significant correlation between the plasma  $\alpha$ -tocopherol level and plasma total lipid content ( $r = 0,45$ ;  $P < 0,001$ ) and plasma cholesterol level ( $r = 0,65$ ;  $P < 0,001$ ) has been found. However, maternal and neonatal plasma vitamin E levels were unrelated.

*S Afr Med J* 1984; 66: 477-480.

Vitamin E, discovered in the 1920s as an antisterility factor in the rat, is the generic name for a group of chemically similar, fat-soluble alcohols of which the most abundant and also the most biologically active is  $\alpha$ -tocopherol. It is found in the cellular and subcellular membranes where it acts as an anti-oxidant.

The exact role of vitamin E in human metabolism is not well defined, and no clinical evidence of a primary human deficiency state has been reported.<sup>1,2</sup> However, secondary vitamin E deficiency presenting as a reduction in erythrocyte stability in gastro-intestinal malabsorption syndromes has been reported.<sup>1</sup> Similarly, infants are susceptible to vitamin E deficiency because of poor transport of the vitamin across the placenta<sup>3</sup> and their low blood lipid concentration. Premature infants are especially vulnerable in this regard because of gastro-intestinal immaturity and low tissue stores of the vitamin. The deficiency syndrome is characterized by restlessness; noisy breathing; watery nasal discharges; oedema of the entire face, lower limbs, genitalia and sometimes the trunk; a disturbed sleep pattern; gradual but progressive skin changes with lesions on the scalp, sides of the face, neck, back, shoulders, upper arms and, in extreme cases, the lower part of the arms, thighs and knees; papular erythema with dryness and depigmentation of the skin; seborrhoea of the scalp in severe cases; and haemolytic anaemia with reticulocytosis, pyknocytosis, the presence of red cell fragments and shortened red cell survival times.<sup>4,5</sup> Vitamin E supplementation in these infants reduces the severity of but does not eliminate this haemolytic condition.<sup>1,6</sup>

Metabolic Unit, Department of Internal Medicine, University of Stellenbosch and Tygerberg Hospital, and MRC National Research Institute for Nutritional Diseases, Parowvallei, CP

D. LABADARIOS, PH.D.

G. S. SHEPHARD, PH.D.

J. GREEN, M.B. CH.B.

M. E. HUTCHISON, B.SC.

Department of Paediatrics, Tygerberg Hospital and University of Stellenbosch, Parowvallei, CP

P. A. H. HENNING, M.B. CH.B., M.MED. (PAED.)

P. B. HESSELING, M.B. CH.B., M.MED. (PAED.), M.D.

The present study was undertaken to define a normal range for plasma vitamin E levels in the newborn in an urbanized Coloured population of Cape Town since no such data are available for any South African population group.

## Subjects and methods

### Subjects

**Mothers.** The mean age of the 52 mothers was  $25,5 \pm 0,81$  (1 SEM) years (range 17-40 years). Sixteen of the mothers were primigravidas, the highest parity of the rest being 9 in a 40-year-old. Monthly income varied from R30 to R860. All except 2 mothers regularly attended antenatal clinics and none developed any serious antenatal complications. Weight gain during pregnancy had been satisfactory and all mothers received iron (250 mg ferrous gluconate 3 times daily) and folic acid supplements (5 mg/d). In addition, 11 mothers were taking Multivite as a supplement (1 tablet daily), which does not contain vitamin E. Three of the mothers gave a history of occasional alcohol intake and 1 of moderate intake; the rest had abstained. Twenty-four of the mothers were non-smokers, 18 smoked less than 10 cigarettes a day and only 8 smoked more than 10 cigarettes daily (smoking status of 2 of the mothers was unknown).

**Babies.** Six of the 52 babies (27 males, 25 females) studied were small for dates (weight below the 10th percentile for gestational age), all the rest being born at term. All babies were assessed for gestational age within 24 hours of birth.<sup>7</sup> Fetal distress had been absent in all cases and only 7 babies had needed assisted delivery (4 with forceps, 3 by suction). The mean ( $\pm$  1 SEM) body weight of the babies was  $3\ 110 \pm 90$  g, mean height  $50,5 \pm 0,5$  cm and mean head circumference  $34,1 \pm 0,2$  cm. No major placental abnormalities were noted, and the mean ( $\pm$  1 SEM) placental weight was  $590 \pm 20$  g.

### Material and methods

Alpha-tocopherol and  $\alpha$ -tocopheryl acetate were purchased from Sigma Chemical Co. (St Louis, Mo., USA). Absolute ethanol was obtained from Merck (Darmstadt, Federal Republic of Germany), and methanol and hexane were obtained from Waters Associates (Milford, Mass., USA). Solvents were used without further purification. The SP8000B high-performance liquid chromatograph (HPLC) (Spectra Physics, Santa Clara, Calif., USA) was fitted with a 100  $\mu$ l injector loop and variable-wavelength ultraviolet light detector (Spectra Physics SP8400 UV/vis detector, wavelength 292 nm). The HPLC column was a prepacked 250 x 4,5 mm Spherisorb ODS (5  $\mu$ m particle size), supplied by Phase Separations (Clwyd, Wales, UK). The column was eluted with methanol at a flow rate of 1,0 ml/min.

Maternal venous and cord blood was drawn immediately after birth and plasma was obtained by centrifugation. The analysis of vitamin E ( $\alpha$ -tocopherol) in plasma was based on a previously reported HPLC method.<sup>8</sup> The following sample preparation was performed before injection onto the HPLC column. To plasma (250  $\mu$ l) absolute ethanol (250  $\mu$ l) and an ethanolic solution of  $\alpha$ -tocopheryl acetate (250  $\mu$ l, concentration approximately 35

mg/l) were added. The mixture was vortexed (5 s) and hexane (2 ml) was added. After vortexing (30 s), the samples were spun at 2000 g for 5 minutes at 4°C. The supernatant was removed and the aqueous phase was re-extracted with further hexane (2 ml). The supernatants were combined and evaporated to dryness under a stream of nitrogen at room temperature. The dry residue was taken up in 200 µl methanol for injection. Quantitation was achieved by peak area measurement relative to the internal standard ( $\alpha$ -tocopheryl acetate). The HPLC-detector response was calibrated using a standard solution of  $\alpha$ -tocopherol (concentration 12,0 mg/l).

Plasma total cholesterol and triglyceride levels were analysed using the Monotest Cholesterol kit (CHOD-PAP) and the Peridochrom Triglycerides kit supplied by Boehringer Mannheim.

## Results

The form of vitamin E measured by this HPLC method is  $\alpha$ -tocopherol. This is the biologically most active vitamin and also the most abundant form in plasma. Reproducibility studies on 9 individual determinations of a single plasma sample from a normal adult gave a coefficient of variation of 2,0% with a mean level of 11,6 mg/l, while for the babies five determinations of a single plasma sample gave a coefficient of variation of 7,2% with a mean level of 2,8 mg/l. Analytical recoveries were calculated by adding a known amount of  $\alpha$ -tocopherol to the plasma before extraction. Average recoveries were  $96,2 \pm 2,7\%$  for the mothers and  $92,1 \pm 3,2\%$  for the babies (mean  $\pm$  1 SEM for 6 determinations of each).

The distribution of vitamin E levels in mothers and their newborn infants is shown in Fig. 1. The mean plasma  $\alpha$ -tocopherol value of the babies was  $3,2 \pm 0,8$  mg/l and that of the mothers  $15,4 \pm 0,6$  mg/l (mean  $\pm$  1 SEM). Since the plasma

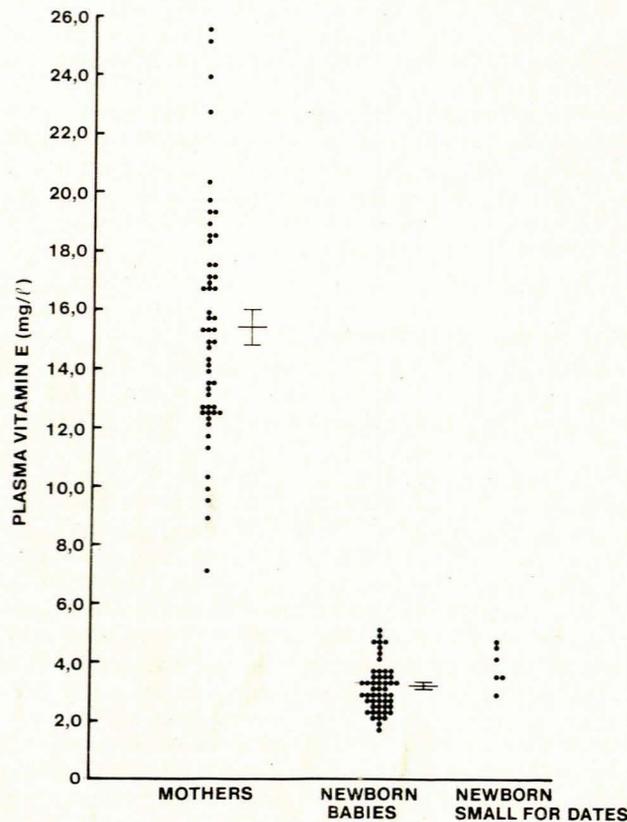


Fig. 1. Vitamin E levels in mothers and full-term and small-for-dates newborn infants.

vitamin E concentration of the 6 small-for-dates babies was within the range of the term ones (Fig. 1), no distinction was subsequently made between term and small-for-dates babies in this study. No relationship was found between maternal vitamin E levels and those of the newborn infants.

The mean plasma cholesterol level of the mothers was  $5,90 \pm 0,19$  mmol/l and that of their newborn babies  $1,55 \pm 0,05$  mmol/l (mean  $\pm$  1 SEM). The mean plasma triglyceride value of mothers was  $1,91 \pm 0,09$  mmol/l and that of the infants  $0,40 \pm 0,02$  mmol/l (mean  $\pm$  1 SEM).

The coefficient of variation for these determinations was better than 5%, as measured on 6 replicates.

The plasma vitamin E levels of the newborn infants were found to correlate with their plasma cholesterol levels (Fig. 2) and also with their total plasma lipid values (cholesterol + triglycerides) (Fig. 3), but no correlation was found with plasma triglycerides. Similar correlations were found in the mothers (Figs 4 and 5). In addition, a correlation was found between the mean maternal plasma vitamin E level and the mean plasma triglyceride level (vitamin E (mg/l) 2,94, triglycerides (mmol/l) + 9,8;  $N = 52$ ,  $r = 0,45$ ,  $P < 0,01$ ).

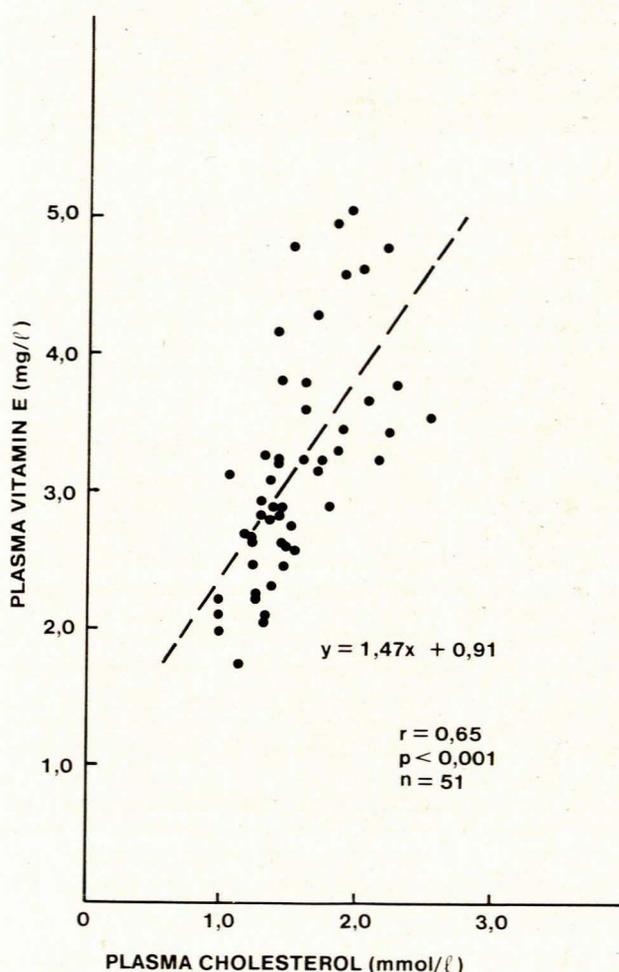


Fig. 2. Correlation between plasma vitamin E levels and plasma cholesterol levels in newborn infants (sample from 1 male baby was lost).

## Discussion

The plasma vitamin E levels found in newborn infants in this study of the urbanized Coloured population of Cape Town are comparable with results of similar studies performed elsewhere (Table I).

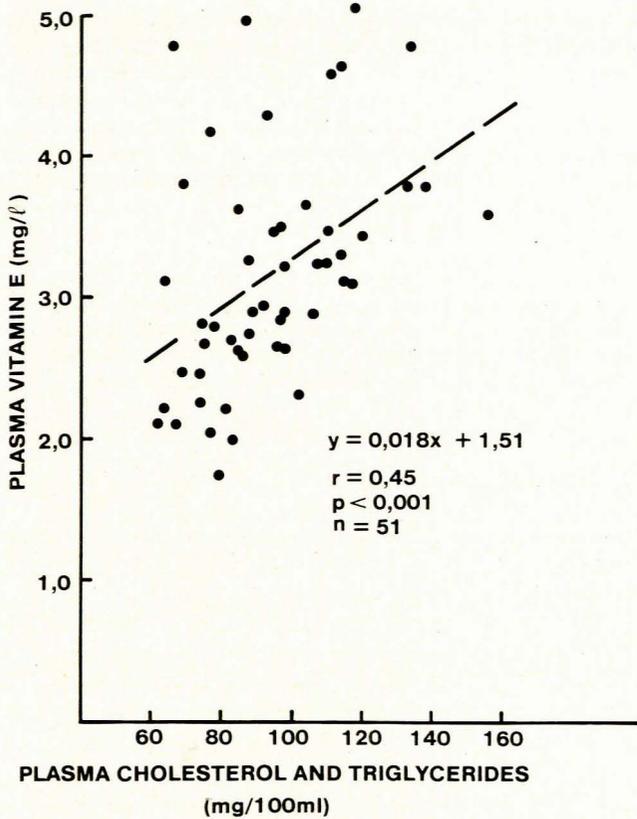


Fig. 3. Correlation between plasma vitamin E levels and the sum of the plasma cholesterol and triglyceride levels in newborn infants (sample from 1 male baby was lost).

TABLE I. VITAMIN E STATUS (MEAN  $\pm$  SD) IN THE NEWBORN

Reference	Vitamin E (mg/l)
This study	3,2 $\pm$ 0,8
Martinez <i>et al.</i> <sup>9</sup>	5,8 $\pm$ 3,8
Leonard <i>et al.</i> <sup>10</sup>	2,4 $\pm$ 0,2
Baker <i>et al.</i> <sup>11</sup>	4,0*
Hashim and Schuttringer <sup>12</sup>	2,3 $\pm$ 0,7
Haga and Lunde <sup>13</sup>	3,8
Haga <i>et al.</i> <sup>14</sup>	3,9

\*Ninety-five per cent confidence limits, 1-6 mg/l.

The results emphasize the reported low absolute levels of vitamin E in the newborn as opposed to the higher corresponding levels in their mothers. Indeed, the mean ratio of maternal plasma vitamin E/neonatal plasma vitamin E was  $5,3 \pm 0,3$  (mean  $\pm$  1 SEM), although individual cases varied from a maximum of 13,1 to a minimum of 2,5. No relationship was found between maternal and neonatal plasma vitamin E levels; this is in agreement with the findings of Haga *et al.*<sup>14</sup> and Haga and Lunde<sup>13</sup> in a Norwegian population, but contradicts the results of Leonard *et al.*<sup>10</sup> in an Irish population and of Martinez *et al.*<sup>9</sup> in a Brazilian working-class population. Both these latter workers found correlations between the two values. No dietary information is given for these patients, except that they were not receiving vitamin E supplements. Martinez *et al.*<sup>9</sup> gave no statistics for the correlation. Leonard *et al.*<sup>10</sup> divided their population into four groups according to the maternal plasma vitamin E level (< 5 mg/l, 5 - 7 mg/l, 7 - 10 mg/l, and > 10 mg/l), and found highly significant ( $P < 0,001$ ) differences between the upper three groups with regard to neonatal vitamin E levels.

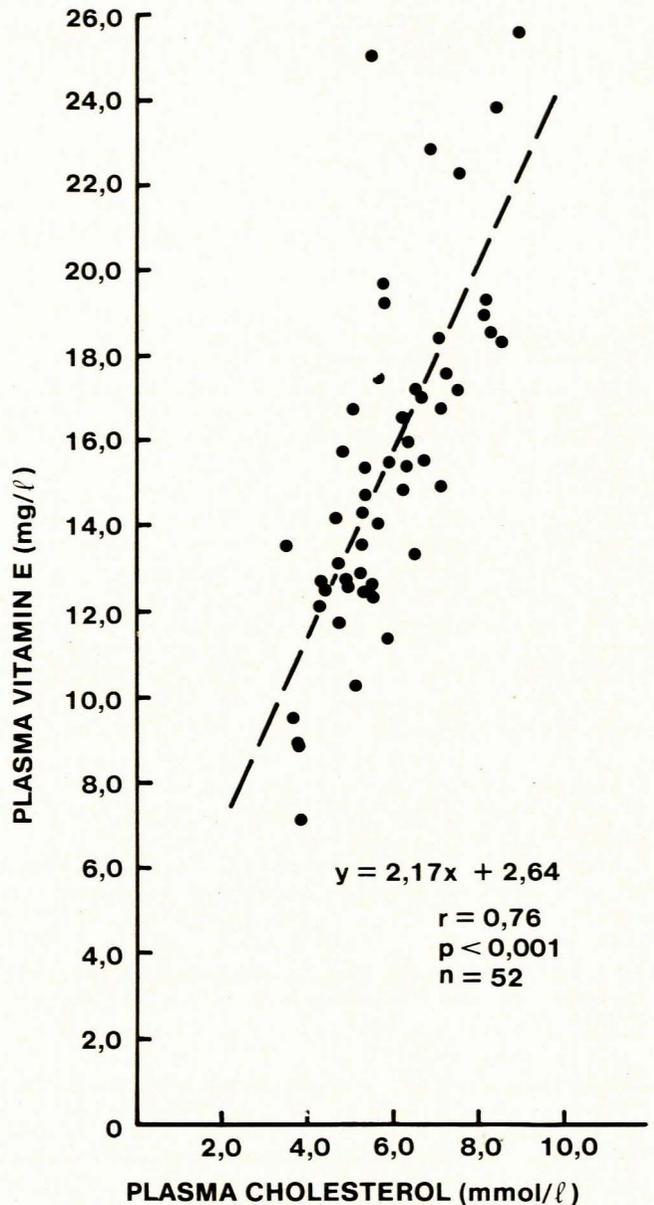


Fig. 4. Correlation between plasma vitamin E levels and plasma cholesterol levels in the mothers of newborn infants.

The lower vitamin E levels in the newborn than in normal adults have been postulated to arise from a placental block.<sup>3</sup> The low values have been used to ascribe vitamin E deficiency to this group of the population<sup>15</sup> since a level of 5 mg/l is regarded as the lower limit of normal in the adult population.<sup>1</sup> However, neonatal blood is also low in lipid content, and a direct correlation has been shown between the plasma vitamin E level and total lipid content, both in this population (Figs 3 and 5) and in other populations.<sup>16-18</sup> This direct correlation of plasma vitamin E and total lipids arises because vitamin E is transported in the plasma by various lipoproteins, there being no specific lipoprotein carrier for the vitamin. In normal adult subjects 65% of the plasma vitamin E was found in low-density lipoproteins and 24% in high-density lipoproteins.<sup>1</sup> Vitamin E correlates well with the total lipid content (Figs 3 and 5), and since the total lipid content itself correlates strongly with component lipid classes, correlations of vitamin E with other lipid classes such as cholesterol<sup>16</sup> (Figs 2 and 4) are not unexpected.

It has been suggested that a more accurate assessment of nutritional status of vitamin E may be gained by quoting the ratio

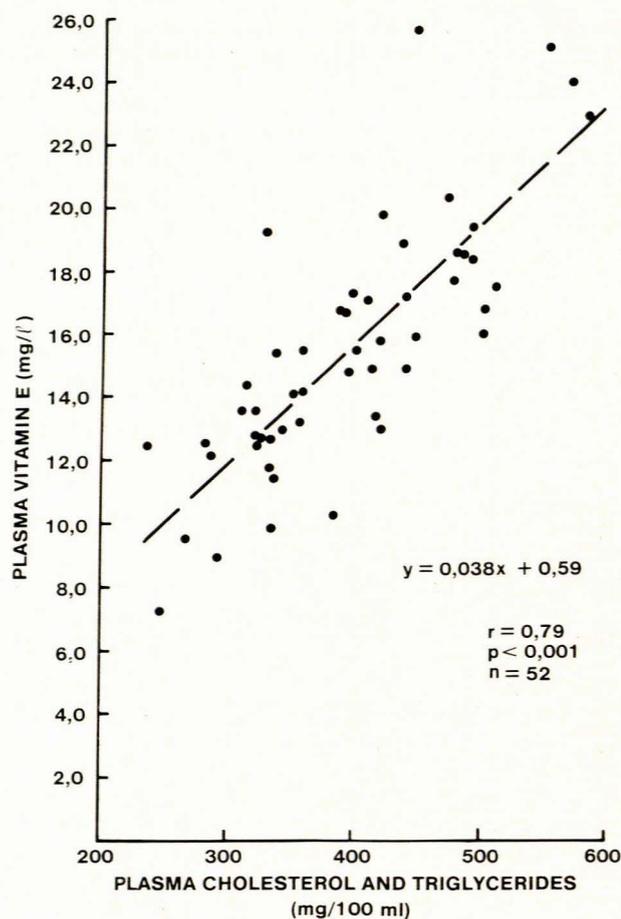


Fig. 5. Correlation between plasma vitamin E levels and the sum of the plasma cholesterol and triglyceride levels in the mothers of newborn infants.

of vitamin E to total lipid.<sup>19,20</sup> In this study, results for mothers were  $3,9 \pm 0,1$  mg vitamin E/g total lipid (cholesterol + triglyceride) and for neonates  $3,4 \pm 0,1$  mg vitamin E/g total lipid (mean  $\pm$  1 SEM). Although these results are statistically significantly different ( $P < 0,005$ ) from each other, they suggest that the very large absolute difference between maternal and neonatal vitamin E when expressed in mg/l is largely due to differences in blood lipid concentration. If the criterion of vitamin E/lipid ratio is used to define normal levels, then newborn infants are not vitamin E-deficient. Although similar findings have been reported,<sup>20</sup> it should be borne in mind that the use of the ratio merely indicates the degree of saturation of the carrier lipoprotein and does not address the question of whether blood of low lipid content, as in neonates, has adequate transport capacity to satisfy body requirements in which the site of action of the vitamin is in the cellular and subcellular membranes. As circulating lipid levels fall, so plasma  $\alpha$ -tocopherol levels will also decrease and, in extreme cases, vitamin E deficiency has been reported in patients with abetalipoproteinaemia.<sup>21</sup>

Vitamin E supplementation has been proposed for premature infants and for the treatment of, among other conditions, retrolental fibroplasia,<sup>1,2,3,24</sup> haemolytic anaemia and hyperbilirubinaemia.<sup>1,6</sup> The work reported here has defined normal vitamin E levels in a group of South African infants and will thus enable the vitamin E status of such patients to be determined and the need for and efficacy of supplementation to be assessed.

The expert and kind assistance of the sisters of the maternity services, paediatric outpatient department and labour ward is hereby acknowledged. Thanks are also due to the staff of the Department of Obstetrics and Gynaecology for their excellent co-operation, and the Cape Provincial Administration for transport arrangements.

#### REFERENCES

- Bieri JG, Farrell PM. Vitamin E. *Vitam Horm* 1976; **34**: 31-75.
- Anderson L, Dibble MV, Turkki PR, Mitchell HS, Rynbergen HJ. *Nutrition in Health and Disease*. 17th ed. Philadelphia: JB Lippincott, 1982: 119-124.
- Jansson L, Holmberg L, Nilsson B, Johansson B. Vitamin E requirements of preterm infants. *Acta Paediatr Scand* 1978; **67**: 459-463.
- Hassan H, Hashim H, Van Itallie TB, Sebrell WH. Syndrome in premature infants associated with low plasma vitamin E levels and high polyunsaturated fatty acid diet. *Am J Clin Nutr* 1966; **19**: 147-157.
- Oski FA, Barness LA. Vitamin E deficiency: a previously unrecognized cause of hemolytic anemia in the premature infant. *J Pediatr* 1967; **70**: 211-220.
- Gross SJ, Landaw SA. The effect of vitamin E on red cell hemolysis and bilirubinemia. *Ann NY Acad Sci* 1982; **393**: 315-322.
- Dubowitz LMS, Dubowitz V, Goldberg C. Clinical assessment of gestational age in the newborn infant. *J Pediatr* 1970; **77**: 1-10.
- De Leenheer AP, De Bevere VO, Cruyl AA, Claeys AE. Determination of serum  $\alpha$ -tocopherol (vitamin E) by high performance liquid chromatography. *Clin Chem* 1978; **24**: 585-590.
- Martinez FE, Goncalves AL, Jorge SM, Desai ID. Vitamin E in placental blood and its interrelationship to maternal and newborn levels of vitamin E. *J Pediatr* 1981; **99**: 298-300.
- Leonard PK, Doyle E, Harrington W. Levels of vitamin E in the plasma of newborn infants and their mothers. *Am J Clin Nutr* 1972; **25**: 480-484.
- Baker H, Frank O, Thomson AD *et al*. Vitamin profile of 174 mothers and newborns at parturition. *Am J Clin Nutr* 1975; **28**: 59-65.
- Hashim SA, Schuttlinger GR. Rapid determination of tocopherol in macro- and micro-quantities of plasma. *Am J Clin Nutr* 1966; **19**: 137-145.
- Haga P, Lunde G. Selenium and vitamin E in cord blood from preterm and full term infants. *Acta Paediatr Scand* 1978; **67**: 735-739.
- Haga P, Ek J, Kran S. Plasma tocopherol levels and vitamin E/ $\beta$ -lipoprotein relationships during pregnancy and in cord blood. *Am J Clin Nutr* 1982; **36**: 1200-1204.
- Ehrenkranz RA. Vitamin E and the neonate. *Am J Dis Child* 1980; **134**: 1157-1166.
- Rubinstein HM, Dietz AA, Srinivasan R. Relation of vitamin E and serum lipids. *Clin Chim Acta* 1969; **23**: 1-6.
- Kater RMH, Unterecker WJ, Kim CY, Davidson CS. Relationship of serum tocopherol to beta-lipoprotein concentrations in liver disease. *Am J Clin Nutr* 1970; **23**: 913-918.
- Davies T, Kelleher J, Losowsky MS. Interrelation of serum lipoprotein and tocopherol levels. *Clin Chim Acta* 1969; **24**: 431-436.
- Horwitt MK, Harvey CC, Dahm CH, Seany MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann NY Acad Sci* 1972; **203**: 223-236.
- Farrell PM, Levine SL, Murphy MD, Adam AJ. Plasma tocopherol levels and tocopherol lipid relationship in a normal population of children as compared to healthy adults. *Am J Clin Nutr* 1978; **31**: 1720-1726.
- Bieri JG, Poukka RKH. Red cell content of vitamin E and fatty acids in normal subjects and patients with abnormal lipid metabolism. *Int J Vit Nutr Res* 1970; **40**: 344-350.
- Oski FA, Stockman JA. *The Year Book of Pediatrics*. 1st ed. Chicago: Year Book Medical Publishers, 1983: 406-410.
- Kretzer FL, Hittner HM, Johnson AT, Mehta RS, Godio LB. Vitamin E and retrolental fibroplasia: ultrastructural support of clinical efficacy. *Ann NY Acad Sci* 1982; **393**: 145-166.
- Johnson L, Schaffer D, Quinn G *et al*. Vitamin E supplementation and the retinopathy of prematurity. *Ann NY Acad Sci* 1982; **393**: 473-495.