Age and Sex are Important Factors in Determining Normal Retinol Levels

by Bo S. Lindblad,* Mahendra Patel,** Mustafa Hamadeh,** Nelly Helmy,** Ibrahim Ahmad,** Adekunle Dawodu,** *and* Shakila Zaman***

**Department of Paediatrics, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi-74800, Pakistan and Karolinska Institutet, Stockholm, Sweden*

***Department of Paediatrics, United Arab Emirates University, Al Ain, United Arab Emirates*

****Department of Preventive Paediatrics, King Edward Medical College, Lahore, Pakistan*

Summary

Cut-off levels for serum retinol levels of 20 μg/dl for marginal and 10 μg/dl for definite deficiency have been advocated and extensively used in population studies. However, the blood serum levels of retinol of the newborn are known to be very low and although the age dependency of the retinol binding protein has been described, the normal levels of serum retinol at different ages have not been reported from larger series. While studying poor populations of young infants in Lahore, Pakistan, we thought it necessary to try to achieve appropriate reference values by analysing the levels of serum retinol of expatriates from the Indian subcontinent who live in the affluent United Arab Emirates, where retinol deficiency is not seen either at the hospital or the community level. We have studied maternal, cord blood, infantile and adult levels of retinol and found a highly significant age relationship of serum retinol levels. During very early infancy the 'normal' mean is below what has been considered deficiency. This is new information and important in the evaluation of retinol status of individuals as well as populations. In addition, we found lower levels in women, pregnant or non-pregnant, than those in adult men. This sex difference in adults was not seen in infants. We recommend a cut off level for deficiency of 10 μg/dl, but only for those above 1 month of postnatal age.

Introduction

Retinol was described already at the turn of this century as a growth factor. The recent discovery of a cellular receptor of retinoic acid and this retinol metabolite's influence on cellular differentiation and immunity has stimulated intensive research on the molecular biological mechanisms of retinol and its metabolites.

Findings from a large number of field interventions were recently submitted to a comprehensive meta-analysis on the effectiveness of vitamin A supplementation in reducing child morbidity and mortality.[1] A 23 per cent reduction in mortality (95 per cent CI: 15–29 per cent) for children 6–72 months of age was obtained. The importance of diagnosing a subclinical vitamin A deficiency state in child populations has been stressed, as eye symptoms and immunological deficiency are earlier manifestations of vitamin A deficiency than serum retinol levels. The advantages of using the relative dose-response, or the modified relative dose response assays have been put forward and training manuals have been circulated.[2] However, the simpler method of determining the level of serum retinol can still give valuable information on retinol status.[3]

Cut-off serum levels for retinol deficiency state of below 20 μg/dl for marginal and below 10 μg/dl for definite deficiency have been used in several large field studies. However, the serum levels of newborn premature babies are known to be very low[4] and although the age dependency of the retinol binding protein has been described,[5] the normal levels of serum retinol at different ages have not been published from any larger series.

While studying poor populations in Lahore, Pakistan[6] we thought it appropriate for a comparison to analyse the normal levels of serum retinol of expatriates from the Indian subcontinent in the affluent UAE, where clinical retinol deficiency is not seen. The results are of general interest for population surveys, as well as individual estimations of retinol sufficiency.

Subjects and Methods

Twenty-four non-pregnant women of fertile age, 16 third trimester pregnant healthy women, and 19 adult men of Indian or Pakistani origin were studied. Twenty

Acknowledgements

The authors thank the personnel of the obstetrical units and the paediatric out-patient units of Tawam and Al Ain Hospitals, UAE, for their collaboration. The study was made possible through grants from the UAE University Faculty of Medicine Research Grants and the Swedish Agency for Research Collaboration with Developing Countries (SAREC).

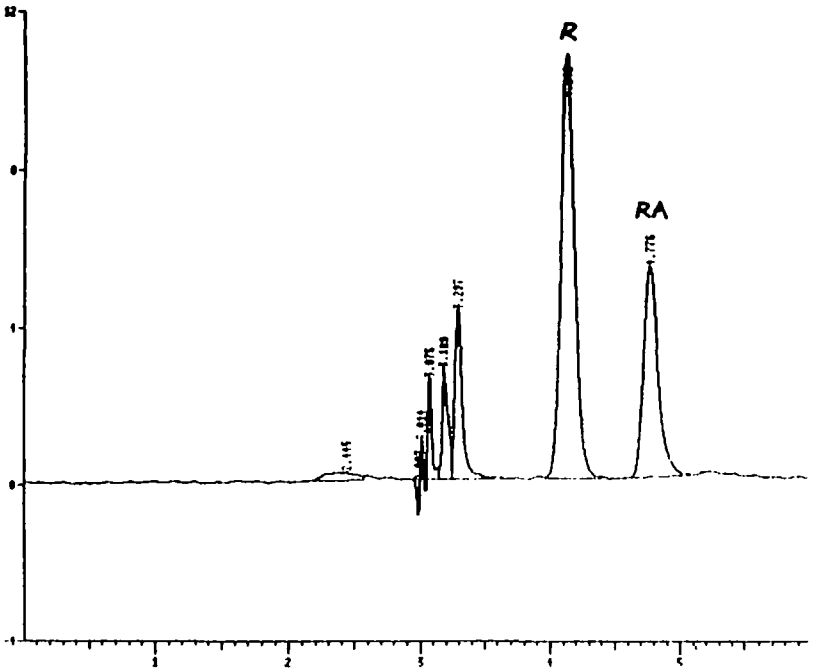

FIG. 1. The absorbance curve of the retinol assay. The *Y*-axis is showing the absorbance of the retinol (R) and the internal standard retinol acetate (RA). The *X*-axis shows the time in minutes of the HPLC assay.

cord blood samples from normal deliveries in the same ethnic group were collected from the obstetrical units of Tawam and Al Ain Hospitals, UAE. Sixty-five normal term newborn babies without signs or suspicion of infections were studied during the neonatal period while performing routine analyses such as determining haemoglobin levels. A total of 105 infants of different ages seen in the nurseries and out-patient departments of the two hospitals, likewise without signs of infection or growth deficiency, were also studied while drawing blood for the sake of other investigations. Blood was drawn from 44 newborn infants on the first day of life, from 20 during 2–21 days, 23 1–2 months, 34 3–6 months, 24 7–12 months and 25 13–21 months of age.

The blood was drawn from a cubital vein and the heparinized tube immediately covered with aluminium foil, centrifuged and the plasma stored in −70°C until analysed.

The analyses were performed according to Shearer[7] on a 1090 Packard HPLC system. The column was a Whatman No 4228-001 Partisil 5 ODS-3, 25 cm × 4.6 mm, fully silanized reversed phase C18 groups Si–O–Si bonded to partisil; particle size 5 μm; mobile phase 100 per cent methanol; flow rate 1.00 ml/min and detection by UV variable wavelength absorbance detector set at 325 nm, referencer at 550 nm. Standard was all trans retinol from Sigma Chemical nr R7632 and internal standard retinol acetate nr R4632. An absorbance curve is shown in Fig. 1. Plasma (100 μl) was used for the preparation of the sample, 20 μl of the extraction was put on the column.

Statistical analysis of the data obtained was carried out by presenting individual retinol levels for each age and sex group included. Regression analysis was performed for the infants from ages 0 days–21 months. The coefficient of determination (R^2) is presented.

TABLE 1

Mean serum retinol levels (μg/dl) ± SEM in cord blood, neonates (age in days), young infants (age in months), and young adult non-pregnant women and adult males of a healthy population

Age	*n*	Serum retinol Mean (μg/dl) ± SEM
Cord blood	20	25.43 ± 1.23
Day 1	44	19.06 ± 1.13
Day 2	12	22.39 ± 2.30
Day 3–21	8	26.97 ± 4.61
Day 28–2 months	23	31.37 ± 1.66
3–6 months	29	37.60 ± 2.27
7–12 months	28	45.10 ± 2.23
13–21 months	28	46.59 ± 2.32
Adult males	19	69.07 ± 3.41
Adult females*	79	48.77 ± 1.56

n = Number of individuals in each group; SEM = standard error of the mean.

*Non-pregnant women of child bearing age.

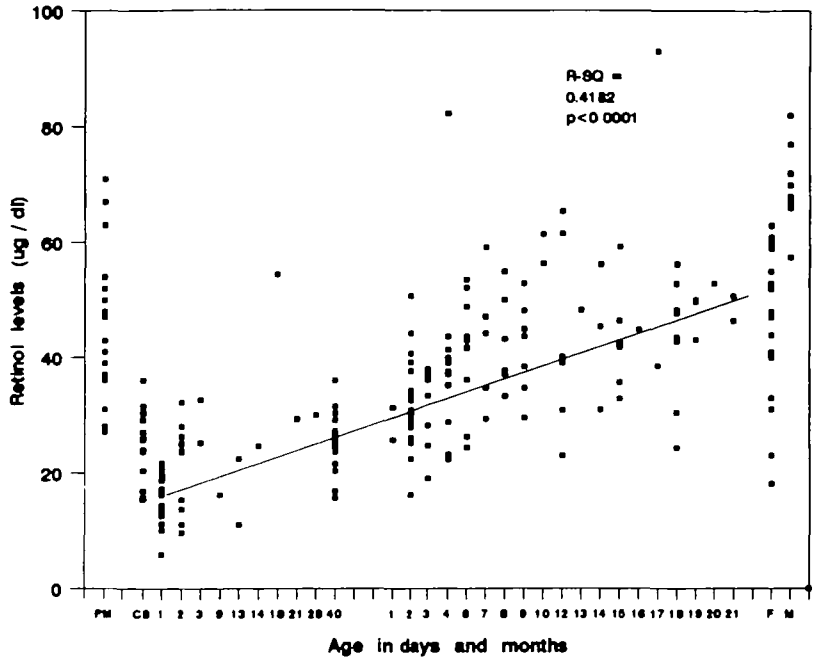

FIG. 2. Normal serum retinol levels. The serum concentrations are given in μg/dl; in pregnant women during the last trimester (PM); in cord blood (CB); during the first 40 days and first 21 months of extra-uterine life; in non-pregnant women of fertile age (F); in adult men (M). Each point represents the individual retinol values. More than one similar values may be overlapping. The line of regression is for the infants only.

Results

Women had lower levels than men, around 40 against 70 μg/dl, while the pregnant women had similar levels when compared with non-pregnant women of similar age. We found no sex difference amongst the infants studied. The cord blood levels were considerably lower than the maternal levels, around 25 μg/dl, whereas the neonatal levels decrease from those levels to well below 20 μg/dl, after which they show an increase towards normal levels for non-pregnant women and adult men which were reached at around 1 year of age (Table 1). The age correlation was highly significant ($R^2 = 0.4182$, $P < 0.0001$) (Fig. 2).

Discussion

This study showed a highly significant age relationship of serum retinol levels, which is of importance in evaluating retinol levels of individuals as well as child populations. Although it is well known that premature infants are born with very low liver reserves and low retinol levels in peripheral blood,[4] the continued low levels of term newborn and young infants has not been shown previously. The only comparable study known to the investigators (R. L. Tilden, personal communication) showed a mean of 20.9 μg/dl at 0–12 months of age with a progressive increase up to 27.4 μg/dl at 108–120 months. These levels are even lower than ours, which is not surprising as the sampling was from a seemingly normal population in Nepal, where subclinical deficiency and/or infections is more likely to have been present. A normal material must, as far as possible, exclude infections, especially measles, pneumonia and persistent diarrhoea, during which retinol levels are well known temporarily to decline. These infections were not present in the actual subjects. We recognize that the levels given here for women and for young infants are lower than those previously described. Thus, this should be taken into account when evaluating field surveys. We recommend a cut off level for deficiency of below 10 μg/dl after 1 month of postnatal age.

References

1. Beaton GH, Martorell R, L'Abbe KA, *et al.* Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries: summary report. University of Toronto, 1992.
2. Furr HC, Tanumihardjo SA, Olson JA. Training manual for assessing vitamin A status by use of the modified relative dose response and the relative dose response assays. Office of Nutrition, Bureau for Research and Development, US Agency for International Development Project no. 936-5116, Washington DC 20523.
3. Temple VJ, Ighogboja IS, Okonji MC. Serum vitamin A and

beta-carotene levels in pre-school age children. J Trop Pediat 1994; 40: 374–5.
4. Shenai JP. Vitamin A. In: Tsang R, Lucas A, Vany R, Zlotkin SH, *et al.* (eds) Nutritional needs of the preterm infant. Williams and Williams, Baltimore 1993.
5. Vahlquist A, Rask L, Peterson PA, Berg T. The concentrations of retinol-binding protein, prealbumin, and transferrin in the sera of newly delivered mothers and children of various ages. Scand J Clin Lab Invest 1975; 35: 569–75.
6. Jalil F, Lindblad BS, Hanson LÅ, *et al.* Early child health in Lahore, Pakistan: I. Study design. Acta Paediat 1993; 82, Suppl 390: 3–16.
7. Shearer MJ. Vitamins. In: Lim CK (ed.) HPLC of small molecules—a practical approach. Oxford: IRL Press, 1986: 157–218.