

Vitamin A status of term and preterm infants delivered at Harare Central Hospital and fed exclusively on breast milk

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Abstract

Objective: To investigate the vitamin A status of pregnant mothers, lactating mothers, preterm and term infants who were being fed exclusively on breast milk.

Design: Systematic/cross sectional.

Setting: Vitamin A research laboratory, animal science research laboratory, University of Zimbabwe, and Harare Central Hospital.

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Subjects: 105 pregnant mothers attending the antenatal clinic at Harare Central Hospital for a routine check up were recruited for the study. Two groups of infants: those born at term and those with gestational age ≤ 36 weeks.

Main Outcome Measures: Serum retinol levels of infants/mothers pairs. Breast milk retinol levels.

Result: The serum retinol levels for the infants were similar irrespective of age with a mean of 26.15 ± 9.78 $\mu\text{g}/\text{dl}$. There was no statistically significant difference. The mean serum retinol levels of infants and mothers were significantly different. ($p = 0.001$). With mother:infant ratio of serum retinol concentration of 1.7:1. Maternal serum retinol levels correlated positively with infant serum retinol levels, $r = 0.728$. Forty four percent of the preterm and 17% of the term infants had serum retinol levels < 20 $\mu\text{g}/\text{dl}$, indicating deficiency, and only 20% of the infants had retinol levels > 40 $\mu\text{g}/\text{dl}$.

Conclusion: The majority of infants might be at risk of vitamin A deficiency. Increased intake of vitamin A in pregnant women is necessary, and direct vitamin A supplementation of infants should be considered.

Introduction

Vitamin A is an essential nutrient because it plays a critical role in reproduction, the immune system, and in the maintenance of cellular differentiation, such as that found in epithelium cells.¹ Prolonged vitamin A deficiency could cause acute and chronic inflammation, resulting in replacement of normal epithelium by squamous cells in different mucous membranes.² This could lower the epithelial barrier function and result in a higher risk of infection.² These roles were found to be particularly critical during the period of fast growth and tissue development as in pregnancy, infancy and early childhood.³ Towards the end of gestation, adequate maternal vitamin A status and dietary intakes are important to increase the vitamin A transferred to the foetus in preparation for parturition.³

Breast milk is the only source of vitamin A during the neonatal period for the exclusively breast fed infant and is the main source for the majority of infants from the developing world as long as breast feeding continues.¹ The ability to meet infant requirements depends on the vitamin A concentration and volume of milk consumed, parameters influenced by maternal vitamin A status and dietary intake.¹ Recent data indicated that the amount of vitamin A in breast milk was directly related to the maternal dietary intake, especially at the lower levels of intake.³ The mean level of vitamin A intake from food for most women from the developing world was found to be lower than the accepted safe level. Most often the body reserves of the vitamin were found to be deficient. Usually the diet of these women was not altered during pregnancy.¹ Lactating mothers from developing countries were reported to have mean serum retinol level in the deficient range of less than 20 $\mu\text{g}/\text{dl}$ or the marginal range of 20 to 30 $\mu\text{g}/\text{dl}$.³ A recent study found that the concentration of retinol in breast milk declined during lactation, and that maternal serum retinol levels correlated positively with milk retinol levels. It was also postulated that vitamin A was transferred to the foetus in the last trimester of pregnancy, and therefore the preterm newborn could have a low concentration of vitamin A.³ Basu, *et al.* found that serum concentrations of retinol in term newborns varied from between 13 and 46 $\mu\text{g}/\text{dl}$ while

in preterm they varied between 13 to 16 $\mu\text{g}/\text{dl}$ with a maternal/foetal ratio of 2:1.⁴

In regions of vitamin A deficiency, supplementation of lactating mothers has been proposed as a way to increase the breast milk vitamin A content to provide additional vitamin A to the breast fed infant.⁵ However, it was found that where maternal vitamin A status was deficient or marginal, foetal plasma concentration remained within normal limits or higher than that of the mother. Further, others who were supplemented with vitamin A gave birth to babies with a serum vitamin A concentration similar to those of unsupplemented mothers.⁴

The distribution of vitamin A in the body following supplementation possibly depended on initial vitamin A status, so that supplemented women showed a lower serum retinol level than their unsupplemented counterparts. Vitamin A deficiency is believed to be endemic in many developing countries.³ There are no published data on vitamin A status for infants in Zimbabwe. We hypothesised that:

1. There would be a significant difference in the mean serum retinol levels of the premature and the mature infant.
2. There would be no significant difference between maternal and infant mean serum retinol.

Materials and Methods

Area of study: The study was conducted at the Harare Central Hospital antenatal clinic (an urban referral Hospital).

Study population: Two groups of infants were studied:

1. Those delivered at term (mature) with a gestational age of 38 weeks and above (38 to 42 weeks) with birth weight 2.5 kg and above.
2. Those delivered at preterm (premature) with a gestational age of 36 weeks or less (26 to 36 weeks) with birth weight less than 2.5 kg.

Inclusion criteria: The infants to be recruited were to be delivered at Harare Central Hospital, with an adequate weight of not less than 2.5 kg and gestational age from 38 to 42 weeks for term infants. For preterm infants weight at

delivery was less than 2.5 kg, with gestational age from 26 to 36 weeks. Mothers had to be apparently healthy, and prepared to feed their infants exclusively on breast milk for at least three to four months. This implied that the infant was to be fed only on breast milk and nothing else, not even water. This study took place during routine clinical settings at the antenatal clinic, Harare Central Hospital. Permission for the study was granted by Harare Central Hospital Ethical Committee and the Medical Research Council of Zimbabwe.

Exclusion criteria: Mothers with systemic illness or with sick infants and infants whose mothers could not practice exclusive breast feeding were excluded from the study.

One hundred and five women who were seven to nine month's pregnant and attending Harare Central Hospital antenatal clinic were recruited for the study. The selection was systematic, with every third individual selected. Each selected pregnant woman had her hospital card tagged.

Methods: All data and samples were collected at the hospital's antenatal clinic. These included the mothers' height and weight as well as the infants' weights and lengths. Blood samples were taken from the pregnant women and they were advised to visit the clinic at six weeks and 12 weeks after delivery, the usual *post natal* check up period for mothers and their infants. Blood and breast milk specimens were taken from the mothers and infants on each visit to the clinic. Verbal consent was sought from the participants and samples were collected by doctors, trained research nursing sisters and midwives in a normal clinical setting. The study was carried out over a period of four months.

The sera and the breast milk were sampled simultaneously with venous blood samples of the infants at six weeks and 12 weeks after delivery. The blood samples were allowed to clot and centrifuged at 3 000 rpm for 10 minutes. The sera were divided into 2 ml and 1 ml aliquots and stored at -20°C for analysis. Breast milk samples were collected by manually expressing the milk into dark glass bottles, and 2 ml aliquots stored in brown polypropylene screw cap tubes at -20°C and subsequently at -80°C for analysis.² The vitamin A status was assessed by serum and breast milk retinol.⁵ The samples were analysed by thawing the frozen sera and adding all-trans retinyl acetate as internal standard. They were then mixed with ethanol, extracted into hexane and then dissolved in methanol. Retinol was determined by High Performance Liquid Chromatography, (HPLC) with methanol and dichloromethane as eluent.⁶ Milk samples were thawed, alkaline hydrolyzed at room temperature, washed with 100g /L sodium chloride, extracted into hexane and 3,4 di-dehydroretinyl acetate as internal standard was added. The samples were then washed with water, dissolved in methanol, and the retinol levels determined by HPLC.⁶

Analysis: Mean (\pm SD) and median values for all variables. Group comparisons for

levels of retinol for the mothers and their babies were calculated by paired t-test. Spearman's rank-order correlation was used to test relations among the variables. SPSS version 11 was used for the data analysis. Plasma retinol was used as a biochemical indicator for vitamin A and the following cut-off points were used as recommended by WHO/collaborative study: Deficiency < 10 μ g/dl, Marginal 10 to 20 μ g/dl, Satisfactory 20 to 50 μ g/dl, Excessive 50 to 100 μ g/dl, Toxic >100 μ g/dl. The mean concentration of retinol in breast milk was taken to be 28.6 μ g/d, and our cut-off point for sub-optimal breast milk retinol concentration was considered as < 30 μ g/dl.⁸

Results

Table I shows the baseline characteristics of the two groups. (term and preterm infants and the mothers). The term group consisted of 35 full term infants with a mean gestational age of 38.2 \pm 2.3 weeks, a mean birth weight of 3.058 \pm 0.465 kg and a mean length of 49.7 \pm 3.2 cm. The preterm group consisted of 70 premature infants with a mean gestational age of 30.01 \pm 4.4 weeks, a mean birth weight of 1.313 \pm 0.366 kg and a mean length of 39.4 \pm 5.2 cm.

Table I: Baseline characteristics of mothers and infants.

Groups	Term	Preterm
	Mean \pm SD	
Age of mothers/years	24.5 \pm 5.7	24.1 \pm 5.4
Weight of mothers/kg	70.5 \pm 10.8	58.5 \pm 8.5
Height of mothers/metres	1.61 \pm 0.08	1.57 \pm 5.3
Birth weight of infants/kg*	3.058 \pm 0.465	1.313 \pm 0.366
Length of infants/cm*	49.7 \pm 3.2	39.4 \pm 5.2
Gestational age/weeks*	38.2 \pm 2.3	30.1 \pm 4.4

* $p = 0.001$.

The mean serum retinol level in the term infants was 27.17 \pm 8.6 mg/dl, and for the preterm infants, the mean serum retinol level was 25.09 \pm 10.95. (Table II)

Table II: Comparison of maternal and infant serum retinol concentrations.

Groups	Mean \pm SD (retinol μ g/dl)	Minimum (retinol μ g/dl)	Maximum (retinol μ g/dl)
Control (non-lactating)			
non pregnant mother)	a 44.56 \pm 13.52	15.56	69.74
Preterm mother	a. 40.33 \pm 1.32	22.12	68.95
Term mother	a. 44.17 \pm 12.89	22.42	86.03
Pregnant mother	b. 24.83 \pm 8.97	8.35	57.75
Preterm infant	b. 25.09 \pm 10.95	6.17	49.09
Term infant	b. 27.17 \pm 8.59	10.80	59.00

Means marked with different letters show significant differences between mother and infant at $p = 0.001$.

Table III: Mean serum retinol levels of infants at six weeks and 12 weeks post partum.

Infant status	Mean \pm SD ($\mu\text{g/dl}$)	Minimum ($\mu\text{g/dl}$)	Maximum ($\mu\text{g/dl}$)
Term infant (6 weeks)	29.31 \pm 10.13	10.80	59.00
Preterm infant (6 weeks)	25.47 \pm 11.68	6.17	49.09
Term infant (12 weeks)	25.07 \pm 6.52	17.84	40.69
Preterm infant (12 weeks)	24.92 \pm 9.26	11.90	42.13

Differences observed in the mean serum retinol levels between term and preterm infants are statistically insignificant, p = 0.08.

The serum retinol levels for the 12 weeks old infants are generally lower than those of the 6 weeks old but the mean differences are statistically insignificant.

For the preterm infants p = 0.19, and for the term infants p = 0.16.

Significant differences were found between mean serum retinol levels of infants and the mothers, $p = 0.001$, (Table II). Positive correlation was also found between maternal and infant serum retinol levels ($r = 0.728$). The mean breast milk retinol levels measured at six weeks ($31.86 \pm 18.50 \mu\text{g/dl}$) and then at 12 weeks post partum ($20.20 \pm 10.55 \mu\text{g/dl}$) was significantly different ($p = 0.001$). There was no significant difference found in the preterm breast milk retinol level and the term breast milk retinol ($p = 0.632$). We found that the maternal mean serum retinol level decreased during pregnancy to the lowest level of $24.83 \pm 8.97 \mu\text{g/dl}$ and increased in the lactating mothers to the highest mean serum retinol value of $44.17 \pm 12.50 \mu\text{g/dl}$ (Table II) before decreasing slowly to the 12 weeks levels (Table IV).

Table IV: Maternal mean serum retinol levels at six and 12 weeks post partum.

Mothers status	Mean \pm SD retinol ($\mu\text{g/dl}$)	Minimum retinol ($\mu\text{g/dl}$)	Maximum retinol ($\mu\text{g/dl}$)
Control (non pregnant non-lactating mother)	44.56 \pm 13.52	15.56	69.74
Pregnant mother	24.83 \pm 8.97	8.35	57.75
Term lactating mother (6 weeks)	47.86 \pm 16.34	22.42	86.03
Term lactating mother (12 weeks)	40.13 \pm 6.06	30.17	49.66
Preterm lactating mother (6 weeks)	41.20 \pm 11.96	22.12	68.95
Preterm lactating mother (12 weeks)	37.65 \pm 8.51	24.07	55.22

Serum retinol levels decreased during pregnancy and increased to the non pregnancy levels after delivery, followed by a slow decrease during lactation.

Discussion

Cardona-Perez, *et al.* found lower levels of serum retinol in preterm infants compared to term infants.³ We found that the serum retinol levels of term and preterm infants were almost similar, $p = 0.08$. Our results, therefore, disagreed with Cardona-Perez, *et al.*³ but agreed with Moji, *et al.*¹¹ who found no significant difference in the mean serum retinol levels between term and preterm

infants. The statistically insignificant difference found in serum retinol levels for women at different stages of gestation agreed with the findings of Saskia de Pee,⁹ but was contrary to a published review article on breast milk vitamin A levels from developing countries.¹⁰ Vitamin A is usually transferred to the foetus in the last trimester of pregnancy, and therefore, the preterm infants would be expected to have lower concentrations of serum vitamin A compared to the term infants.¹²

The unexpected insignificant difference found in the mean serum vitamin A levels between term and preterm infants in our study could be due to supplementation of premature infants with vitamin A. It is the usual hospital practice for preterm infants who could not tolerate oral feeding to be routinely fed parenterally with amino acid dextrose mixture, and added multivitamin preparation containing retinol.¹³ A small sample size may also be to blame.

We found no correlation in infant serum retinol levels and breast milk retinol levels, as reported by Shenai, *et al.*¹³ Our results agreed with previous reports³ that maternal serum retinol levels correlated with infant serum retinol levels, with a high maternal/infant ratio of 1.7:1. However, since the mean serum retinol levels of the infants were low and within the suboptimal level, the results might indicate inadequate maternal transfer of vitamin A. It is probable that either exclusive breast feeding was not practised by the mothers or the volume of breast milk fed to the infants was inadequate.¹

Our results showed a decrease in retinol level during pregnancy and an increase to near non-pregnant non-lactating level during post partum, followed by a slow decrease during lactation. A similar finding was reported by Stotzlfus, *et al.*⁵ This phenomenon could be attributed to the transfer of vitamin A from the mother's storage to the infant through breast milk, coupled with insufficient intake of vitamin A in the diet during lactation.⁶ The low mean serum retinol levels reported for pregnant mothers could be due to the increased transplacental transfer of retinol to the foetus during the third trimester of pregnancy.^{12,3}

We found the maternal/infant ratio of serum retinol level to be 1.7:1. This agreed with reports where maternal/foetal ratio was 2:1.⁴ Where maternal vitamin A status was deficient or marginal, it was reported that foetal plasma concentration remained within the normal limit or could be higher than that of the mother,³ probably indicating transfer of vitamin A from mother to the foetus resulting in low maternal vitamin A status.

Conclusion

Our study indicated that 44 % of the premature infants evaluated had serum retinol levels of less than $20 \mu\text{g/dl}$, indicating a deficiency state, and 80% of the mature infants had a retinol level of 20 to $40 \mu\text{g/dl}$. This suggests that the majority of the infants were at high risk for vitamin A deficiency, and therefore towards the end of gestation, adequate maternal vitamin A status and dietary intakes

were necessary to increase the level of vitamin A transferred to the foetus. In our findings the mean breast milk vitamin A concentration of $31.86 \pm 18.50 \mu\text{g/dl}$ at six weeks might be adequate for the nursing infant but the concentration of $20.20 \pm 10.55 \mu\text{g/dl}$ at 12 weeks ($< 30 \mu\text{g/dl}$) would be inadequate to meet the physiological requirement in the latter half of infancy. Our results showed that breast milk vitamin A alone could not supply the requirement of the infants. Either exclusive breast feeding was not practised by the mothers or the volume of breast milk fed to the infants was inadequate. We, therefore, recommend direct vitamin A supplementation early in infancy to avoid a deficiency state.

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