Single megadose vitamin A supplementation of Indian mothers and morbidity in breastfed young infants

S Basu, B Sengupta, P K Roy Paladhi

Background and objective: In developing countries low maternal vitamin A stores combined with increased demands of pregnancy and lactation may lead to its deficiency in breastfed infants. This study evaluates the effects of maternal supplementation with a high dose of vitamin A on the serum retinol levels of exclusively breastfed infants, and their morbidity in the first six months of life.

Setting: Hospital based.

Study design: Randomised controlled trial.

Subjects and methods: Mothers in the test group (n=150) were orally supplemented with a single dose of retinol (209 µmol) soon after delivery and were advised exclusive breastfeeding for six months. Before supplementation retinol levels were estimated in the mothers’ and newborns’ blood, in colostrum. On follow up, breastmilk and infants’ serum retinol contents were assessed monthly for six months. Retinol level <0.7 µmol/l indicated vitamin A deficiency. Morbidity patterns like vitamin A deficiency, diarrhoea, febrile illnesses, acute respiratory infection, measles, and ear infection were also studied and compared between the two groups.

Results: Presupplement mean maternal serum retinol levels were 0.98 and 0.92 µmol/l and mean breastmilk levels were 3.85 and 3.92 µmol/l in the test and control groups respectively (p>0.05). Mean cord blood retinol levels were also comparable (0.68 v 0.64 µmol/l). After supplementation, the test group showed a rise in mean breastmilk retinol content (12.08 v 2.96 µmol/l) which remained significantly higher for four months. The infants’ mean serum retinol level, initially 322.06% of the baseline value, was significantly higher for five months. In the control group, significant numbers of mothers and infants showed deficient breastmilk and serum retinol throughout the follow up (p<0.01). Decreased incidence and duration of various diseases were also found in the test group suggesting lesser morbidity.

Conclusion: Maternal supplementation with single megadose vitamin A is an effective strategy for vitamin A prophylaxis of exclusively breastfed infants of 0–6 months.

Vitamin A supplementation and food fortification have beneficial effects on infant mortality and morbidity. The vitamin A status of most newborns is marginal and prevention from its deficiency may be ensured by breastfeeding, provided the retinol concentration in the breastmilk is adequate. In developing countries have an average daily intake of vitamin A of less than half and an average serum retinol concentration that is 70% of women in developed countries. In this situation even exclusive breastfeeding may not protect the newborn from vitamin A deficiency (VAD) and may lead to diseases, clinically overt (xerophthalmia, anaemia, growth retardation, increased infectious morbidity, and mortality) or occult (impaired iron metabolism, poor cellular differentiation, and depressed immune response). These result in increased rate and complications of respiratory tract infections, diarrhoeal diseases, and further micronutrient deficiency. VAD is also associated with increased incidence of measles. The anaemia of VAD is a subject of interest in the present day. Its pathogenesis is through diverse biological mechanisms such as effect on growth and differentiation of erythrocyte progenitor cells, immunity to infections, and mobilisation of iron stores from the tissues.

India’s national programme on VAD diseases ensures vitamin A supplementation of infants from the age of 6 months. Hence in the first six months of life the infant may remain unprotected if the vitamin A content of the mother’s breastmilk is low. However, there is a growing awareness that subclinical or overt deficiency can occur in infants less than 6 months of age. This study attempts to evaluate the effect of a single oral megadose of vitamin A on the breastmilk concentration, the infants’ serum level, and infant morbidity patterns during the first six months of life in an urban population of West Bengal, India.

SUBJECTS AND METHODS

A randomised controlled prospective study was conducted in the Department of Paediatrics, NRS Medical College and Hospital, Calcutta, India. It was approved by the hospital ethical committee. Apparently healthy pregnant women without any history of hypertension, diabetes, or any other chronic medical illness were recruited consecutively in their third trimester (beyond 34 weeks) from the antenatal clinic of the hospital. Inclusion criteria were at least two completed antenatal visits, tetanus immunisation in pregnancy, and term vaginal delivery. Those with stillbirth, an evenful peripartum period, or who received vitamin A supplementation in pregnancy were excluded. A total of 300 such mothers were finally recruited to the study; informed consent was received from all participants in the trial. The mothers along with their babies were randomised by systematic sampling technique to test and control groups of 150 each (alternate allocation to the test and control group). All were questioned about their detailed dietary habits from which an approximate daily intake of vitamin A was calculated by using the Indian food table. The newborns’ birth weights were recorded and the gestational ages were assessed using the Dubowitz criteria.

For estimation of serum retinol, 3–5 ml of cord blood from the newborns and 3–5 ml of venous blood from the mothers...
300 mother-newborn pairs

Inclusion criteria:
1. At least 2 antenatal visits
2. Tetanus immunisation in pregnancy
3. Term vaginal delivery
4. Intact cord

Exclusion criteria:
1. Stillbirth
2. Eventful peripartum period
3. Vitamin A supplementation in pregnancy
4. Chronic illnesses in the mother

Randomisation

Control group
(n = 150)

Test group
(n = 150)

Retinol content of mothers’ and infants’ serum, and in colostrum estimated in both the groups

Single oral dose of vitamin A (209 µmol) to the mothers of the test group soon after delivery

• Exclusive breastfeeding for 6 months
• Follow up monthly for 6 months
• Any illness in the babies, report immediately

At each follow up:

• Estimate retinol level in breast milk and infants’ serum
• Examine for any sign of VAD
• Record the total number of episodes of diarrhoea, ARI, measles, febrile illness, and ear infection, and compare between the two groups
• Examine the baby thoroughly
  • Any sign of VAD, withdraw the child from the study and supplement adequately
  • Treat infections accordingly

Calculate the mean (SD) and 95% confidence intervals

Analyze data by the Z test; p < 0.05 = significant

Figure 1 Protocol used for the study.

were collected, the serum separated, and transferred to stopped tubes. Colostrum was collected by manual breast pumps. Samples from both the breasts were mixed and 5 ml of this mixed sample was collected in a stopped tube. Both the serum and milk samples were protected from light, placed in a cool box at 4–8°C, and transferred within four hours to the central laboratory. Retinol concentrations were estimated by the modified method of Neeld and Pearson.

All neonates were examined thoroughly. A single oral dose of 209 µmol of retinol (200 000 IU of vitamin A) was given to each mother of the test group only within 24 hours of delivery and after the blood and breastmilk samples were collected. No prelacteal feeds were given to the neonates and all the mothers were strongly motivated for exclusive breastfeeding for six months. The process of motivation was initiated during the antenatal period and when they were admitted for childbirth. They were discharged from the hospital only after establishment of exclusive breastfeeding. Constant motivation for exclusive breastfeeding continued at all points of contact.

These mother-child pairs were called for monthly follow up for six months. However those who did not exclusively breastfeed their infants were not excluded from the study and were followed up like others for any sign of VAD. This was done on an “intention to treat” basis. In case of any illness of the infants during this period, mothers were instructed to contact the first author either in the paediatric outpatient department or in the ward and the nature, duration, and number of episodes of such diseases were recorded. At each follow up 3–5 ml of venous blood from the infants and 5 ml of breastmilk were collected from both the groups. Mothers were instructed not to breastfeed their infants after early morning on the following days. Breastmilk samples were collected between 9 am and 11 am. Method of sample collection, transport, and retinol estimation were the same as described above. The infants were thoroughly examined at each visit. The total number of episodes and duration of diarrhoeal disease, acute respiratory infection, febrile illness, measles, and ear infection of the infants in the last one month were asked about and recorded. For defining and diagnosing morbidity the disease definitions of Rahmathulla et al. (box 1) were followed. Lower respiratory infections and upper respiratory infections were both considered as acute respiratory infections. The infants who developed signs of VAD including those not exclusively breastfed were omitted from the study at the respective point of time and treated accordingly. The mean (SD) and 95% confidence intervals of birth weight, maternal age, body weight, dietary intake of retinol, serum and breastmilk retinol levels (predose and at each follow up), and morbidity data were calculated in both the groups. Statistical significance was assessed by the Z test and p value less than 0.05 was taken as statistically significant.

The flow diagram (fig 1) sums up the protocol used for the study.

RESULTS

Both the groups were comparable with respect to maternal age and body weight, birth weight and gestational age of the newborns, and mean maternal dietary intake of retinol (table 1). The presupplementation mean maternal serum retinol levels were 0.98 (0.93–1.03) µmol/l and 0.92 (0.86–0.98) µmol/l and the presupplementation mean breastmilk retinol levels were 3.85 (3.65–4.05) µmol/l and 3.92 (3.69–4.15) µmol/l in the test and control group respectively without any significant difference (table 2).

Out of 150 mothers in either group, 139 in the test and 132 in the control completed the six months’ follow up and exclusively breastfed their babies. Four in the test and 10 in the

Box 1: Definition of illness in infants

• Episode of diarrhoea: ≥ 1 day in which there are ≥3 stools of watery and loose consistency or any loose stool with blood.
• Episode of upper respiratory infection: ≥ 1 day of cough or nasal discharge or purulent ear discharge.
• Episode of lower respiratory infection: ≥ 1 day of combination of cough with indrawing of the lungs. Three or more symptom-free days differentiate episodes of diarrhoea or respiratory tract infections.
• Measles: Clinical signs of maculopapular skin rash with its typical distribution and fever with cough, coryza, or conjunctivitis was defined as measles.
• Fever: Rise of body temperature above 39°C axillary for a period of 48 hours or more was defined as fever.
• Vitamin A deficiency (VAD): VAD in mothers was considered with the presence of night blindness, Bitot’s spot, conjunctival xerosis, or corneal ulcers. In infants up to 9 months, conjunctival xerosis, Bitot’s spot, or corneal ulcers were considered as VAD.
• Ear infection: (A) Acute infection (serous/suppurative) was characterised by the presence of earache (or persistent crying on tugging at the ears) and examination with the otoscope reveals a hyperaemic, opaque bulging tympanic membrane or purulent discharge. (B) Chronic infection (serous/mucoid/purulent) was noted if there were symptoms of dull pain for a long period, fever with purulent discharging ears, or otoscopic examination reveals perforation of tympanic membrane with or without purulent discharge.
control group dropped out and were excluded from the study. Seven in the test and eight in the control did not exclusively breastfeed their babies. The breastmilk retinol concentration increased significantly in the test population within 24 hours of supplementation. On follow up, a gradual decline in the breastmilk retinol levels was observed in both the groups. However, the test population showed a significantly higher level up until four months of follow up (table 2). Before supplementation, the percentage of mothers below the cut off for deficient serum retinol were comparable in both the groups. On follow up the serum retinol levels of the test population remained significantly higher than the control group. The breastmilk retinol level was observed in both the groups. On follow up, a gradual decline in the breastmilk retinol levels was observed in both the groups. However, the test population showed a significantly higher level up until four months of follow up (table 2).

DISCUSSION

In the present study preterm infants were excluded as they might have poor storage of vitamin A at birth and hence develop VAD earlier. The study subjects represent a low income section of the urban population and the profile of such a target group in terms of maternal body weight, average daily retinol intake, baseline maternal serum, and breastmilk retinol levels has been taken into consideration. The vitamin A status of pregnant and lactating women tends to vary in different economic, ethnic, and regional groups. Most workers accept levels less than 0.7 μmol/l (20 μg/dl) as diagnostic of deficiency, though others use a higher level of 1.0 μmol/l (30 μg/dl) as the threshold. Vitamin A predominantly affects the immune system, and an increase in the incidence of ear infection between the two groups (table 5).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sample characteristics of the tests and the control groups; results are mean (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>Test group</td>
</tr>
<tr>
<td>Maternal body weight (kg)</td>
<td>Test group</td>
</tr>
<tr>
<td>Birth weight of newborn (kg)</td>
<td>Test group</td>
</tr>
<tr>
<td>Gestational age of newborn (weeks)</td>
<td>Test group</td>
</tr>
<tr>
<td>Maternal average daily intake of retinol (μmol)</td>
<td>Test group</td>
</tr>
<tr>
<td>Presupplementation maternal serum retinol level (μmol/l)</td>
<td>Test group</td>
</tr>
<tr>
<td>Presupplementation breastmilk retinol level (μmol/l)</td>
<td>Test group</td>
</tr>
</tbody>
</table>

*Statistically not significant (p>0.05).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Breastmilk retinol levels (μmol/l) after supplementation and on follow up; results are mean (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group</td>
<td>Control group</td>
</tr>
<tr>
<td>24 hours after supplementation</td>
<td>12.08 (11.45 to 12.71), n = 150</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>1.00 (0.90 to 1.10), n = 150</td>
</tr>
<tr>
<td>1</td>
<td>1.90 (1.79 to 2.01), n = 150</td>
</tr>
<tr>
<td>2</td>
<td>1.74 (1.66 to 1.82), n = 149</td>
</tr>
<tr>
<td>3</td>
<td>1.54 (1.46 to 1.62), n = 149</td>
</tr>
<tr>
<td>4</td>
<td>1.30 (1.11 to 1.29), n = 146</td>
</tr>
<tr>
<td>5</td>
<td>0.72 (0.62 to 0.82), n = 146</td>
</tr>
<tr>
<td>6</td>
<td>0.71 (0.63 to 0.79), n = 146</td>
</tr>
</tbody>
</table>

*Statistically significant (p<0.01).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Serum retinol levels (μmol/l) of infants at delivery and on follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group</td>
<td>Control group</td>
</tr>
<tr>
<td>At delivery (presupplementation baseline value)</td>
<td>0.68 (0.64 to 0.72), n = 150</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>Increase above baseline value (%)</td>
</tr>
<tr>
<td>1</td>
<td>2.87 (2.79 to 2.88), n = 150</td>
</tr>
<tr>
<td>2</td>
<td>2.17 (2.06 to 2.28), n = 149</td>
</tr>
<tr>
<td>3</td>
<td>1.60 (1.52 to 1.68), n = 149</td>
</tr>
<tr>
<td>4</td>
<td>1.41 (1.31 to 1.51), n = 146</td>
</tr>
<tr>
<td>5</td>
<td>1.06 (0.98 to 1.14), n = 146</td>
</tr>
<tr>
<td>6</td>
<td>0.72 (0.62 to 0.82), n = 146</td>
</tr>
</tbody>
</table>

*Statistically significant (p<0.01).
VAD with levels less that 0.35 µmol/l (10 µg/dl) closely correlating with inadequate stores of vitamin A and clinical manifestations of VAD. Similar values are used for defining deficient breastmilk retinol levels with a baseline deficiency being less than 1.05 µmol/l (30 µg/dl).

In this study we have taken a retinol level of 0.7 µmol/l as cut off for deficiency in serum and breastmilk. The mean baseline maternal serum retinol was lower than that reported by Roy et al (0.98 v 1.38 µmol/l) whereas the mean baseline breastmilk retinol levels were comparable (3.85 v 3.20 µmol/l). A high percentage of mothers in both the test and control groups (34.66% v 32.67%) were below the cut off level of baseline serum retinol which projects the vitamin A status in the postpartum mothers in this study. Despite such high values few mothers at delivery (before supplementation) had a breastmilk retinol content below the cut off value (10% and 8% in the test and control respectively). This is probably due to high levels of vitamin A in colostrum, which is almost uniform in postpartum mothers irrespective of serum retinol levels (table 4). The benefit of immediate postpartum supplementation of mothers with a single megadose was seen in the breastmilk of the test group for up to four months of follow up. The raised serum level found for up to five months of follow up has adequately protected the test group infants from VAD.

A variety of factors like recurrent infections, worm infestations, stress, variable dietary provitamin A intake, and lactational demands tend to affect vitamin A metabolism and stores in the mother and hence the breastmilk levels. Standardisation of all these variables is difficult and beyond the scope of this study. Ideally the hepatic retinol content best depicts the vitamin A status, the estimation of which was not practically feasible. We did not include serial maternal serum retinol estimation, which might reflect the vitamin A utilisation of the mothers after supplementation. Our main aim was to assess the effect of maternal supplementation on infant morbidity. Breastmilk retinol level is a better parameter as it directly attributes the availability of the vitamin to the infant. Moreover, its estimation is non-invasive, readily acceptable, and sample collection is relatively easy.

A number of previous studies have shown the benefits of postpartum vitamin A supplementation to mothers, either direct or through fortified foods. Food based interventions either use fortified foods like sugar, monosodium glutamate, or β-carotene rich fruits and vegetables. Questions remain on their efficacy and the ultimate aim of improving infant’s serum retinol level. Study using fortified monosodium glutamate showed an increase in vitamin A level in the mother but not in the infant. In another study it was shown that retinol levels in the maternal serum and breastmilk increased after consumption of synthetic supplements of β-carotene but not β-carotene in vegetables. Studies in which direct supplementation was done showed a satisfactory outcome. In a non-randomised study Thanangkul et al demonstrated a rise in maternal serum retinol levels for nine months, breastmilk levels for seven months, and a rise in the infants’ serum retinol at 1–7 months postpartum after a single dose of 312 µmol vitamin A to the mothers at delivery. A similar dose had been used by Stoltzfus et al in Indonesian mothers at 1–3 weeks postpartum. The breastmilk retinol content remained high up to eight months and also benefited the infants. In these studies, the higher dose of vitamin A was probably responsible for the high retinol levels for a longer period. Roy et al used a dose similar to ours and the effect achieved in the infants was for six months, the usual recommended period of breastfeeding. Vinutha et al used a similar dose but their follow up was for three months only.

Recently, in a multicentre trial, Bahl et al showed high breastmilk retinol levels at two months postpartum after supplementation at 18–42 days after delivery. However no significant effect was noted after six months. The infants were also supplemented at immunisation visits. This combined effect improved infants’ serum retinol at six months and decreased the proportion of infants with serum retinol less than 0.7 µmol/l. Rice et al showed that supplementation of lactating Bangladeshi women benefited mothers and infants but...
did not prevent subclinical deficiency.\textsuperscript{14} Irrespective of the treatment group, over 50% of women produced milk low in vitamin A concentration (≤1.05 µmol/l) and the overall proportion of infants with a serum retinol content less than 0.7 µmol/l was 33%. In another study, 56% of the breastfed infants who had received up to three doses of 50 000 IU of vitamin A still had serum retinol concentration less than 0.70 µmol/l at 25 weeks of age.\textsuperscript{15} We report a much lesser number (21.92% of the breastfed infants) at 6 months of age. In the study of Vinutha \textit{et al}, at the end of three months, 69.6% mothers in the control group had a breastmilk retinol level below 1.05 µmol/l against 36.1% in the experimental group. Forty five percent of infants in the control group had subclinical deficiency compared with none in the experimental group.\textsuperscript{16} In the present study 22.07% mothers in the control group had breastmilk retinol less than 0.70 µmol/l at the third month and 50.71% at the sixth month against 0.67% and 13.01% respectively in the test group. In the case of infants a much higher difference in their serum values was noted, 36.81% v 0% in the control and test groups respectively at third month (0.34 v 2.55) indicating that vitamin A supplementation to the mothers on the infants’ vitamin A levels is noted continuing optimally until 4–6 months postpartum. This is the period of exclusive breastfeeding and adequate protection of the infants during this period with vitamin A is important to decrease morbidity and hence this is emphasised. No case of VAD was registered in the test group, indicating a direct beneficial effect of the supplementation. Of the six cases of VAD in the control group only one showed the signs of deficiency before three months and five after three months, indicating that unsupplemented breastmilk cannot protect the infant in the later months of breastfeeding. Not only was there a significant difference in the number of episodes of acute respiratory infection (0.34 v 2.55) between the two groups, the duration of acute respiratory infection was also significantly longer in the control group (2.9 v 5.6 days). This is different from the study of Roy \textit{et al} where the duration of acute respiratory infection was shorter in the supplemented infants but the incidence was comparable in both groups.\textsuperscript{17, 18} Both the incidence and duration of diarrhoeal episodes were significantly less in the test group. A similar result was seen with febrile illnesses. Vitamin A supplementation of children and infants in the later months of breastfeeding is toxic and has shown side effects.\textsuperscript{19–21} This might explain the lesser infection rate and decreased duration of various diseases in the test group.

In a population in which breastfeeding rates are high, maternal supplementation offers several advantages. As the infant receives a daily low dose in a form that is well absorbed and utilised, it can be considered the safest way to deliver the vitamin to the newborn. Direct supplementation to the infant in the first six months of life is toxic and has shown side effects.\textsuperscript{22} Floretnito \textit{et al} has shown that when infants are given high dose vitamin A supplementation, transient side effect rates of 8.8% are attributable to the supplement itself.\textsuperscript{23} Secondly, maternal supplementation requires only one contact with the vitamin A delivery system, while that to the infant may require more than one. Lastly, both the mother and the infant benefit from the megadose of the vitamin.\textsuperscript{24}

In conclusion, low maternal stores cannot meet the lactational demand and directly contributes to infant morbidity. A single oral megadose of vitamin A is readily accepted by mothers, simply to administer, and optimum for meeting lactational demand in the first six months. It also decreases morbidity and provides adequate protection to infants against VAD. Hence, such supplementation in the immediate postpartum period is emphasised along with promotion of exclusive breastfeeding to realise the maximum benefit.

\textbf{References}


**IMAGES IN MEDICINE.**

An informative tattoo

A 28 year old man with type 1 diabetes had this tattoo inscribed a few weeks after starting insulin. He was worried about hypoglycaemia and was particularly concerned about this being unrecognised by others. He decided to have a tattoo rather than carry a Medic Alert bracelet.

Hypoglycaemia is the most common complication of insulin therapy and one that patients on insulin fear the most. Neuroglycopenic symptoms due to hypoglycaemia are not infrequently mistaken for alcohol intoxication. The frequency with which this occurs suggests that such accounts may be more than just anecdotal.

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