The Cytokines IL-4 and IL-5 in Pre-Term vs Full-Term Infants: Effect of Retinol Supplementation

Ehab K Emam, Amany El-Wakkad, Mamdouh A. Mohamed and Howaida El-Gebally

The aim of this research was to study the effect of intramuscular supplementation of 5000 IU of vitamin A three times per week for 28th days on the serum levels of interferons 4 and 5 and on the incidence of chronic lung disease in premature and low birth weight infants. This study was conducted on 30 premature and low birth weight infants admitted to Neonatal Intensive Care Unit (NICU) of the Pediatric Hospital of Ain Shams University. They were 14 males and 16 females with a mean gestational age of 31.88±2.17 weeks and a mean birth weight of 1.723±0.372 kg. Critically ill newborns were excluded from the study. The patients included in this study were randomly divided into 2 groups; Group 1 comprised 16 premature infants who received vitamin A supplementation in a dose of 5000 IU intramuscular (IM) 3 times per week starting from the 1st day of admission till the 28th day and Group 2 comprised 14 premature infants who did not receive vitamin A supplementation. Ten apparently healthy full-term newborns appropriate for gestational age served as controls. All premature and full-term infants included in the study were subjected at birth to measurement of serum levels of retinol, IL-4 and IL-5. On 28th day, levels of serum retinol, IL-4 and IL-5 were reassessed for the premature infants only. The mean serum levels of retinol, IL-4 and IL-5 were significantly lower in both group 1 and 2 infants at birth compared to those of controls. After vitamin A supplementation to group 1 infants, the mean serum levels of retinol, IL-4 and IL-5 as well as the rates of change of serum retinol, IL-4 and IL-5 levels were significantly higher in group 1 infants compared to those of group 2 after 28 th days of life. However, the mean levels of serum retinol in group 1 infants was still significantly lower in group 1 infants compared to controls. Further, the serum IL-4 and IL-5 levels were significantly correlated positively with the serum retinol levels on 28th day after birth in group 1 infants. The preterm infants of group 1 had significantly lower incidence of chronic lung disease than infants of group 2. Meanwhile, gestational age, birth weight, the mean serum levels of retinol, IL-4 and IL-5 as well as the mean rate of change of serum retinol were significantly lower in infants who developed CLD on 28th day. Finally, we can come to the conclusion that vitamin A supplementation to premature and low birth weight infants appears to increase serum levels of IL-4 and IL-5 and may be behind the decrease in the incidence of chronic lung disease. Thus, it is recommended to supplement all premature and low birth weight infants with vitamin A.

Key words: Cytokines, preterm, retinol, supplementation

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INTRODUCTION

The premature infants face a variety of physiologic handicaps. The major causes of death in premature infants are Respiratory Distress Syndrome (RDS), Bronchopulmonary Dysplasia (BPD), severe immaturity, intraventricular hemorrhage, congenital anomalies, infections and necrotizing enterocolitis. These are related to the difficulty of extrauterine adaptation due to immaturity of organ systems (Hohlagschwandner et al., 2001).

Premature and low birth weight infants have reduced hepatic stores of retinyl ester and low retinol/RBP (retinol binding protein) ratios, suggesting that they are vitamin A deficient (Shenai et al., 2000).

Vitamin A, or retinol, is an important nutrient needed for normal body functions including growth, sight, reproduction and immunity. It is also necessary for differentiation and proliferation of epithelial cells especially in the respiratory tract and it has a unique role in the photochemical basis of vision (Greser, 1997). Vitamin A and its metabolites potentiate the antibody response to T-cells-dependant antigens, increase lymphocyte proliferation and cytokine production, restore mucosal function and maintain mucosal integrity (Semb, 1994). Moreover, supplementation with vitamin A in laboratory animals induces the resistance against infection through the effect of vitamin A on interleukin-4 (IL-4) and interleukin-5 (IL-5) (Nikaw et al., 1999).

IL-4 and IL-5 have various effects on the immunologic system. IL-4 causes differentiation of T and B cells, macrophages and mast cells. It also promotes synthesis of IgG and IgE antibodies. On the other hand, IL-5 induces B cells differentiation and IgM and IgA antibody synthesis and promotes cytotoxic T-cell production (Penak et al., 2001).

Vitamin A deficiency in laboratory animals produces a sequence of histopathological changes in the respiratory epithelium including necrotizing tracheobroncholitis and squamous metaplasia which can be reversed by restoration of adequate vitamin A status. Similar changes are observed in ventilated preterm infants with chronic lung disease; suggesting that vitamin A deficiency may contribute to this disease (Woodle et al., 2001).

Vitamin A supplementation in Very Low Birth Weight (VLBW) infants may be associated with 24% reduction in mortality and morbidity and is suggested to reduce the incidence of Chronic Lung Disease (CLD) (Tyson et al., 1999; Rahmatallah et al., 2003).

Hence, the aim of this study was to study the effect of intramuscular supplementation of 5000 IU of vitamin A three times per week for 28th days on the serum levels of interleukins 4 and 5 and on the incidence of Chronic Lung Disease (CLD) in premature and low birth weight infants.

MATERIALS AND METHODS

This study was conducted after taking the approval of the National Research Center and the vernal approval of the infants parents.

This study was conducted on 30 premature and Low Birth Weight (LBW) infants admitted to Neonatal Intensive Care Unit (NICU) of the Pediatric Hospital, Ain Shams University in the period between 1st January to 30th September 2006. They were 14 males and 16 females with a mean Gestational Age (GA) of 31.88±2.17 weeks and a mean Birth Weight (BW) of 1.723±0.372 kg. These patients were receiving management according to the guidelines of the NICU of the Pediatric Hospital of Ain Shams University.

Critically ill newborns with any of the major causes of neonatal mortality were excluded from the study. This included any newborn who had severe immaturity, severe respiratory distress syndrome, sepsis, congenital anomalies, necrotizing enterocolitis, birth asphyxia or trauma, meconium aspiration pneumonia as well as those thought to have a terminal illness (as indicated by a pH below 6.8 or by the presence of hypoxia with bradycardia for more than 2 h) (Tyson et al., 1999). Moreover, newborns who received steroids during the course of the study were also excluded (Demissie et al., 2001).

The patients included in this study were randomly divided into 2 groups:

**Group 1:** Included 16 premature infants (6 males and 10 females) with a mean gestational age 32.69±2.15 weeks and a mean birth weight 1.838±0.462 kg. These infants received vitamin A supplementation in a dose of 5000 IU intramuscular (IM) 3 times per week starting from the 1st day of admission till the 28th day of life (Rahmatallah et al., 2003).

**Group 2:** Included 14 premature infants (8 males and 6 females) with a mean gestational age was 31.07±2.2 weeks and a mean birth weight was 1.608±0.282 kg. These infants did not receive vitamin A supplementation.

Ten apparently healthy full-term newborns who were appropriate for gestational age served as controls. They were 5 males and 5 females with a mean gestational age 38.47±0.72 weeks and a mean birth weight 3.048±0.27 kg (Group 3).

All premature and full-term infants included in the study were subjected to:
Full history taking:
- Sex, gestational age (in weeks), order of birth.
- Maternal medical history including cardiopulmonary disorders, infectious diseases, collagen, immune or genetic disorders, anemia, jaundice or diabetes mellitus.
- Previous maternal obstetric problems (abortion, stillbirth, prematurity, blood group sensitization).
- History of present pregnancy (vaginal bleeding, medications including steroids, acute illness).
- Details of the delivery (vaginal or cesarean section, anesthesia or sedation, use of forceps, Apgar score and the need for resuscitation).

Thorough clinical examination:
- Vital data including pulse, respiratory rate, temperature.
- Anthropometric measurements including weight, length and head circumference.
- Abdominal, chest, cardiac, neurological and other systems examination to exclude the possibility of birth trauma, congenital anomalies, sepsis and other major problems.
- Assessment of gestational age using new Ballard score (Wariyar et al., 1997).

Laboratory investigation:
- Routine investigations including:
  - Complete Blood Picture (CBC) by Coulter method.
  - C-Reactive Protein (CRP) by AVITEX CRP which is a rapid test kit for the detection of CRP in human serum.
- Assessment of serum retinol levels.
- Measurements of serum interleukin-4 and interleukin-5 levels.

On 28th day:
- Levels of serum retinol, IL-4 and IL-5 were reassessed for the premature infants only (groups 1 and 2).
- The premature infants (groups 1 and 2) were assessed for the development of chronic lung disease which was diagnosed in infants who continue to require supplemental oxygen, have abnormal physical findings with tachypnea, retractions, crackles or wheezes and have abnormal chest radiographs (Vibhuti and Arne, 2001).

Biochemical analysis and techniques: Human Interleukin-4 (hIL-4) was assayed by using Cyt ELISA hIL-4 enzyme immunoassay for the detection of free hIL-4 from Cyt Immune Sciences Inc. Maryland, USA according to the manufacture instructions. Sensitivity 0.195 pg mL⁻¹. Intraassay coefficient of variation 7.7%. Interassay coefficient of variation 10.9%.

Human Interleukin-5 (hIL-5) was assayed by using Cyt ELISA hIL-5 enzyme immunoassay for the detection of free hIL-5 from Cyt Immune Sciences Inc. Maryland, USA according to the manufacture instructions. Sensitivity 0.92 pg mL⁻¹. Intraassay coefficient of variation 8.3%. Interassay coefficient of variation 10%.

The vitamin A content of plasma was determined according to the method reported by Neeld and Pearson (1963) as follows:

Reagents: Petroleum ether (40-60°C), ethanol (95%), chloroform, trifluoroacetic acid reagent.
- Vitamin A stock standard: Retinol acetate (52.1 mg) were dissolved in 100 mL Chloroform for vitamin A working standard; the sub dilutions of vitamin A standard were prepared in concentrations of 0.113, 0.226, 0.45 and 0.679 µg mL⁻¹.
- Procedure: Petroleum ether (3 mL) was added to each tube and shaken to extract vitamin A and B-carotene into petroleum ether phase. The tubes were centrifuged for 10 min at 3000 rpm. Two milliliters of the petroleum ether phase were carefully withdrawn (upper layer) and transferred into a dry cuvette. The absorbance at 450 nm was read against a petroleum ether blank without delay to prevent evaporation of solvent and destruction of carotenoids by light. The contents of the cuvette were evaporated to dryness in 35-40°C water bath with the aid of a stream of nitrogen then 0.1 mL of chloroform was added to each cuvette and mixed, then 1 mL trifluoroacetic acid reagent was added and the intensity of the developed color was measured against chloroform using a Spikol spectrophotometer at 620 nm. The concentration of vitamin A and B-carotene were calculated from the standard curves previously prepared.

RESULTS AND DISCUSSION

The mean serum levels of retinol, IL-4 and IL-5 were significantly lower in both group 1 and 2 infants at birth compared to those of controls. However, these values were not significantly different between groups 1 and 2 infants (Table 1 and Fig. 1-3).
Table 1: Comparison between infants of groups 1, 2 and controls (group 3) at birth as regards the laboratory data

<table>
<thead>
<tr>
<th>Serum levels</th>
<th>Group 1 (n = 16)</th>
<th>Group 2 (n = 14)</th>
<th>Group 3 (n = 10)</th>
<th>1 Vs 2</th>
<th>1 Vs 3</th>
<th>2 Vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (µg/dL⁻¹)</td>
<td>14.0±4.75</td>
<td>11.89±3.31</td>
<td>23.4±2.08</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4 (U/L⁻¹)</td>
<td>0.14±0.11</td>
<td>0.17±0.14</td>
<td>0.88±0.62</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-5 (U/L⁻¹)</td>
<td>4.85±7.83</td>
<td>3.75±5.12</td>
<td>16.8±10.03</td>
<td>&gt;0.05</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
</tbody>
</table>

Table 2: Comparison between infants of groups 1 and 2 on 28th day of life as regards the laboratory data

<table>
<thead>
<tr>
<th>Serum levels</th>
<th>Group 1 (n = 16)</th>
<th>Group 2 (n = 14)</th>
<th>t/s*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (µg/dL⁻¹)</td>
<td>21.05±2.61</td>
<td>11.27±1.69</td>
<td>2.56</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>IL-4 (U/L⁻¹)</td>
<td>6.57±0.11</td>
<td>0.26±0.12</td>
<td>3.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-5 (U/L⁻¹)</td>
<td>47.06±8.45</td>
<td>4.00±5.41</td>
<td>4.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol (Rate of change)</td>
<td>63.94±26.78</td>
<td>9.47±8.66</td>
<td>4.57*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4 (Rate of change)</td>
<td>3387.0±483.98</td>
<td>50.00±72.01</td>
<td>3.73*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-5 (Rate of change)</td>
<td>21280.5±2160.35</td>
<td>282.7±40.50</td>
<td>3.24*</td>
<td>&lt;0.010</td>
</tr>
</tbody>
</table>

*: Mann Whitney test

Fig. 1: The serum levels of IL-4 (U/L⁻¹) at birth and on the 28th day after birth in the 3 studied groups

After vitamin A supplementation to group 1 infants, the mean serum levels of retinol, IL-4 and IL-5 as well as the rates of change of serum retinol, IL-4 and IL-5 levels were significantly higher in group 1 infants compared to those of group 2 on 28th day of life. However, the mean serum level of retinol was still significantly lower in group 1 infants compared to those of controls (Table 2 and Fig. 1-3).

The serum IL-4 and IL-5 levels were significantly correlated positively with the serum retinol levels on 28th day after birth in group 1 infants (Fig. 4). The preterm infants of group 1 who received vitamin A supplementation had significantly lower incidence of chronic lung disease (CLD) (3 out of 16 = 18.75%) than infants of group 2 (10 out of 14 = 71.4%) with p<0.05 (Fig. 5). Meanwhile, the mean rate of change of serum retinol level was significantly lower in infants who developed CLD on 28th day (Fig. 6).

Fig. 2: The serum levels of IL-5 (U/L⁻¹) at birth and on the 28th day after birth in the 3 studied groups

Gestational age, birth weight as well as the mean serum levels of retinol, IL-4 and IL-5 after 28th day of life were significantly lower in infants who developed CLD compared to those who did not develop CLD (Table 3).

In this study, we found significantly lower mean plasma retinol concentration at birth in premature (groups 1 and 2) than in full term infants (controls). This agrees with the study done by Shenai et al. (2000) who stated that the fatus accumulates vitamin A in the third trimester, thus, premature infants have reduced hepatic stores of retinol. Moreover, Darlow and Graham (2003) found that premature infants have lower concentrations of plasma Retinol Binding Protein (RBP), which is a specific carrier protein of vitamin A, than term infants and most preterm infants have both low plasma vitamin A concentrations and low plasma retinol/RBP molar ratios, suggesting that they are vitamin A deficient.
Fig. 3: The serum levels of retinol (µg dL⁻¹) at birth and on the 28th day after birth in the 3 studied groups.

Fig. 4: The positive correlation between the serum levels of retinol and IL-4 on the 28th day after birth in group 1 infants.

As regards the mean plasma retinol concentration after 28th day, we found that the preterm infants of group 1, who received intramuscular vitamin A supplementation in the dose of 5000 IU 3 times per week for 28th days, had significantly higher mean plasma retinol concentration as well as higher rate of change of plasma retinol than the preterm infants of group 2 who did not receive vitamin A supplementation. This agrees with the study done by Tyson et al. (1999) who found that intramuscular administration of 5000 IU of vitamin A in preterm and LBW newborn infants 3 times per week for 4 weeks reduced biochemical evidence of vitamin A deficiency. Also, Wardle et al. (2001) found that vitamin A supplementation of preterm and LBW newborns in a dose of 5000 IU day⁻¹ orally results in an increase in plasma retinol concentrations in preterm and LBW infants after 28th days. However, there was still significantly lower mean plasma retinol concentration in group 1 infants after 28th days of vitamin A supplementation compared to controls. These findings are supported by the study of Ambalavanan et al. (2003) who also found that supplemental vitamin A may be used to produce clinical benefit without necessarily improving plasma concentration or body stores. This could be attributed to the low carrier protein RBP in preterm infants as Perrotta et al. (2003) stated that the pharmacokinetics of vitamin A are likely to vary between infants and that plasma retinol may reflect the availability of its carrier protein RBP, which is typically low in the preterm infant.

At birth, there was significantly lower mean serum levels of IL-4 and IL-5 among both group 1 and 2 infants compared to those of controls. Meanwhile in group 1 infants, after vitamin A supplementation, there was a positive correlation between serum retinol level and both serum IL-4 and IL-5 levels. These findings are supported by the studies done by Nikawa et al. (1999), Hoag et al. (2002) and Albers et al. (2003) who also found that low serum retinol significantly decreased the secretion of both IL-4 and IL-5. On the 28th day of life in group 1 infants, there was significantly higher mean serum levels and rate of change of both IL-4 and IL-5 compared to those of group 2. This could be attributed to vitamin A supplementation. These findings are supported by the studies done by Semba (1994) who also found that vitamin A supplementation increases the production of Th2 type cytokines.

Present study proved that in pre-term infants, there was a negative correlation between serum IL-4 level and both gestational age and birth weight. This could be attributed to inadequate body stores of vitamin A and low serum retinol levels as well as the immaturity of the immune system in premature infants. These findings are in agreements with the studies done by Shenai (1993) and Darkow and Graham (2003).

In this study, the preterm infants of group 1 who received vitamin A supplementation had significantly lower incidence of chronic lung disease (3 out of 16 = 18.75%) than infants of group 2 (10 out of 14 = 71.4%) with p<0.05. Meanwhile, the study showed that the mean plasma retinol concentration after 28th day, as well as the rate of change of plasma retinol levels, were significantly lower in infants who developed CLD than those who did not. This favors the hypothesis we aimed to prove by this study that increasing the mean plasma retinol concentration by IM vitamin A supplementation in the
Table 3: Comparison between infants who developed CLD and those who did not develop CLD on 28th day of life regarding gestational age, birth weight and serum retinol levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>-ve CLD</th>
<th>+ve CLD</th>
<th>t/z*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>33.2000±1.14000</td>
<td>31.3000±2.47000</td>
<td>-2.236</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1.9222±0.48740</td>
<td>1.4250±0.36562</td>
<td>-2.252*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-4 on 28th day (IU L⁻¹)</td>
<td>5.2800±0.15000</td>
<td>4.2500±0.10000</td>
<td>-2.321*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-5 on 28th day (IU L⁻¹)</td>
<td>46.2000±6.32000</td>
<td>42.500±3.21000</td>
<td>4.311*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Retinol on 28th day (µg dl⁻¹)</td>
<td>24.7000±2.89800</td>
<td>21.2600±2.55600</td>
<td>2.404</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

= Mann Whitney test*

Fig. 5: The percentage frequency of infants who developed CLD and those who did not develop CLD among infants of groups 2 and 3

Fig. 6: The mean rate of change of retinol among infants who developed CLD and those without CLD on 28th day after birth

dose of 5000 IU 3 times per week for 28th days will decrease the incidence of CLD. This result agrees with that of Darlow and Graham (2003) who said that supplementing very low birth weight infants with vitamin A is associated with benefit in terms of reducing death or oxygen requirements at one month of age. Moreover, Stoll and Kliegman (2004) found that vitamin A supplementation (5000 IU intramuscularly three times per week for 4 weeks) in ELBW infants reduces the risk of BPD. However, the study done by Wardle et al. (2001) was in contrast with our study as they stated that oral supplementation of vitamin A in a dose of 5000 IU/day for 28th days resulted in an increase in the retinol concentration but did not decrease the incidence of CLD in preterm and LBW infants.

A large bolus of vitamin A early in the neonatal period may provide a stimulus to rapid maturation of both gut and respiratory epithelium. This matured epithelium may be more resistant to invasion by pathogens or may clear organisms much efficiently (West et al., 1999, 2002). Retinol helps to maintain the surface lining and integrity of mucous membrane lining the respiratory tract and vitamin A deficiency appears first in the tissues that have rapid turnover rate such as the epithelial lining of the respiratory tract (Semba, 1998). Moreover, levels of vitamin A determine whether mucin or keratin will be synthesized in the epithelial cells. So, adequacy of vitamin A allows mucin production which lines and protects all mucosal surfaces, while, lack of vitamin A results in hyperkeratinization of mucous membranes including that of the respiratory tract (Murray, 1996). Also, vitamin A deficiency in experimental animals leads to loss of ciliated epithelium and squamous metaplasia in the airway; changes similar to those observed in CLD (Darlow and Graham, 2003).

The study also proved that the mean serum levels of both IL-4 and IL-5 were significantly lower in infants who developed CLD than those who did not. There are no previous studies that assessed serum IL-4 and IL-5 levels in prematurity or LBW infants who developed CLD. However, this could be explained by that IL-4 may be of clinical importance in the prevention and treatment of inflammatory diseases since it inhibits the production of inflammatory cytokines such as IL-1, IL-6 and TNF-α by monocytes and T-cells. Also, IL-5 appears to induce the differentiation of activated conventional B-2 cells into Ig-secreting cells and to induce the growth of B-1 progenitors as well as the production of IgM, IgA, IgE and promotes cytotoxic T-cell production (Peckman et al., 2001). Thus the significantly higher levels of IL-4 and IL-5 after vitamin A supplementation may be a contributing factor for decreasing the incidence of CLD.
In this study, both gestational age and birth weight were significantly lower in infants who developed CLD than those who did not. This is in accordance with the study of Kotecha (2002) who stated that the occurrence of Bronchopulmonary Dysplasia (BPD) is inversely related to gestational age and that BPD is a disease primarily of Extremely Low Birth Weight (ELBW) infants who are usually under 1000 g at birth, many of whom have little or no lung disease at birth but in whom progressive disease progresses by time. The explanation for the development of CLD in preterm and LBW infants is that they have few lipid-soluble antioxidant vitamins, such as vitamin A, in their blood compared to term infants. Therefore, it is possible to postulate that preterm and LBW infants are more susceptible to oxidative stress (Giyasettin et al., 2002). Oxygen radical injury is thought to be one of the common mechanisms of many neonatal diseases including CLD (Sullivan, 1998).

CONCLUSIONS

Finally we can come to the conclusion that the levels of serum retinol, IL-4 and IL-5 are significantly lower in premature and low birth weight than full term infants at birth and that vitamin A supplementation to premature and low birth weight infants appears to increase serum levels of retinol, IL-4 and IL-5 that might help to decrease in the incidence of chronic lung disease. Thus, it is recommended to supplement all premature and low birth weight infants with vitamin A in a dose of 5000 IU intramuscular (IM) 3 times per week starting from the 1st day of life till the 28th day of life, so as to decrease the risk of developing CLD.

REFERENCES


