Plasma concentrations of vitamin K1 and PIVKA-II in bottle-fed and breast-fed infants with and without vitamin K prophylaxis at birth

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Abstract. Plasma vitamin K1 and proteins induced by vitamin K absence (PIVKA) were assayed simultaneously 1-4 days and 29-35 days after delivery in three groups of infants: breast-fed not receiving vitamin K at birth (n = 12), bottle-fed without vitamin K administration at birth (n = 7) and breast-fed receiving 1 mg vitamin K1 administered by intramuscular injection at birth (n = 13). The bottle-fed infants had a significantly higher vitamin K1 plasma level than breast-fed infants who did not receive vitamin K1 at birth. Extremely high levels of vitamin K were obtained 1-4 days after intramuscular administration. At the age of 1 month, breast-fed infants had the same plasma vitamin K1 concentration whether or not they had received vitamin K1 supplements. Decarboxy prothrombin (PIVKA-II) a reliable indicator of biochemical vitamin K deficiency, was found in 5 out of 12 breast-fed and in 2 out of 6 bottle-fed infants who had not received supplemental vitamin K1 after birth. In a separate study, we followed up to 90 days after birth a larger group of infants. PIVKA-II was found with significantly greater frequency in breast-fed infants receiving no vitamin K than in breast-fed infants receiving 1 mg vitamin K intramuscularly at birth, or in bottle-fed infants without extra vitamin K1. These data form a strong argument for routine vitamin K prophylaxis after birth for all breast-fed infants. The optimum dose and manner of administration require further study.

Key words: Vitamin K deficiency – Breast-fed infants – Vitamin K prophylaxis

Introduction

Vitamin K is essential to the function of prothrombin, factor VII, IX, X, protein C and protein S. In the presence of vitamin K, glutamic acid residues at the amino-terminal end of the precursors of these coagulation proteins are carboxylated to gamma-carboxyglutamic acid in a post-translational reaction. The adjacent carboxyl groups of gamma-carboxyglutamic acid residues provide the vitamin-K-dependent proteins with characteristic calcium and phospholipid binding properties. In vitamin K deficiency, abnormal decarboxylated coagulation factors appear; these are known as proteins induced by vitamin K absence (PIVKAs). Vitamin K-dependent bleeding has been reported in young infants. These bleedings are subdivided into early (within 24 h), classic (between 2 and 5 days after birth) and late-onset (after 1 month of age) haemorrhagic disease of the newborn. Breast-feeding has a role in classic and late-onset haemorrhagic disease [6].

Human milk contains a mean value of 2.1 µg/l vitamin K1 [3], while the formula most frequently used in the Netherlands (Almiron) contains 50 µg/l. In an earlier study, decarboxy prothrombin (PIVKA-II) could be demonstrated in 9 out of 62 breast-fed infants after the age of 1 month. In none of the bottle-fed infants was PIVKA-II found over the same period [16].

The aim of this study was to compare the levels of vitamin K1 and PIVKA-II in cord blood in three groups of infants, bottle-fed and breast-fed with and without vitamin K prophylaxis at birth, at the age of 4 days and after 1 month. The prophylactic effect of intramuscular injection of 1 mg vitamin K after birth, as recommended by the American Academy of Pediatrics [1], was evaluated in breast-fed infants up until the age of 3 months.

Subjects and methods

Vitamin K1 levels were measured in three groups of infants. The first group (n = 12) was breast-fed and did not receive vitamin K at birth. The second group was bottle-fed (n = 7), and similarly did not receive vitamin K at birth. The third group was breast-fed (n = 13) and received 1 mg vitamin K (Konakion) by intramuscular injection at birth. All infants were delivered vaginally after uneventful pregnancies. Blood for vitamin K1 determination was collected on days 1-4 and 29-35 days after delivery. In addition cord blood was collected (n = 16). Five millilitres of blood was drawn by venipuncture, centrifuged and stored at -20°C in the dark. Vitamin K1 levels were measured using high-performance liquid chromatography with post-column reaction and fluorescence detection as previously described [5].
In all samples assayed for vitamin K\textsubscript{1}, PIVKA-II was also measured by an enzyme-linked immunosorbent assay using a monoclonal antibody as described previously [11]. This antibody reacts quantitatively with abnormal prothrombin (decarboxylated prothrombin) and does not cross-react with native prothrombin. PIVKA-II was expressed in arbitrary units (AU)/ml, 1 AU corresponding to 1 pg prothrombin. PIVKA-II was undetectable in healthy adults (< 0.13 AU/ml). In severe vitamin K deficiency as observed in Japan, PIVKA-II was above 20 AU/ml.

In a separate study PIVKA-II determinations were also performed in blood collected from breast-fed infants (n = 61), receiving 1 mg vitamin K by intramuscular injection at birth. This group was compared with bottle-fed (n = 52) and with breast-fed (n = 93) infants without vitamin K prophylaxis at birth, extending an earlier study [16]. Citrated blood was drawn by venipuncture 4, 30, 60 and 90 days after birth.

For statistical calculations Fisher's exact test was used. Informed consent was obtained from all parents and from the adult patients. The study was approved by the local ethical committee.

**Results**

The results of vitamin K\textsubscript{1} measurement are presented in Fig. 1. In cord blood vitamin K\textsubscript{1} levels were below the detection limit (< 70 pg/ml). In bottle-fed infants, already in the first few days after birth, a steep rise in vitamin K\textsubscript{1} levels related to the increase in feeding volume was recorded (Fig. 1a), resulting in a mean value of 1595 ± 1269 pg/ml (n = 7). After 1 month the vitamin K levels in this group were 2890 ± 633 pg/ml (n = 6). Breast-fed infants, however, had a rather low level in the first few days of life (378 ± 370 pg/ml, n = 12), with a moderate rise in most cases at 1 month of age (Fig. 1b), when a mean value of 707 ± 747 pg/ml was reached (n = 12). Comparison of Fig. 1a and b shows that bottle-fed infants have higher vitamin K levels in the first few days of life (P = 0.07) and even significantly higher levels after 1 month (P = 0.0017), than breast-fed infants not given vitamin K prophylaxis at birth. Breast-fed infants receiving 1 mg vitamin K\textsubscript{1} by intramuscular injection at birth have levels as high as 3271 ± 11977 pg/ml (n = 13) shortly after birth (Fig. 1c). At 1 month of age the vitamin K\textsubscript{1} levels of these infants were down to 698 ± 536 (n = 9) and this is in the range found in breast-fed infants not receiving vitamin K prophylaxis.

PIVKA-II levels were measured in the samples in which vitamin K\textsubscript{1} was determined. In cord blood PIVKA-II was detected in 5 out of 16 samples with a range of 0.22–3.57 AU/ml, while in all samples, as mentioned before, vitamin K\textsubscript{1} was below the detection limit.

In bottle-fed infants aged 1–3 days, PIVKA-II was detected in 2 out of 7 infants, (0.23 AU/ml in one and 0.86 AU/ml in the other) while 1 had an undetectable vitamin K level and the other 927 pg/ml. After 1 month PIVKA-II was not detected in this small group (n = 6). In breast-fed infants not receiving vitamin K prophylaxis, PIVKA-II was detected in 5 out of 12 samples, drawn 3–5 days after birth, with a range of 0.37–13.94 AU/ml, while the vitamin K\textsubscript{1} levels varied from undetectable to 431 pg/ml. After 1 month no PIVKA-II was found.

### Table 1. Frequency of PIVKA-II detection (> 0.13 AU/ml) in bottle-fed and in breast-fed infants with and without vitamin K prophylaxis at birth.

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<td><strong>4 (n = 43)</strong></td>
<td><strong>11 (n = 93)</strong></td>
<td><strong>5 (n = 61)</strong></td>
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<td>4</td>
<td>[0.23/0.23/0.35/0.86]</td>
<td>[0.25/0.30/0.35/0.45/0.90/1.50/6.14/7.64/13.94]</td>
<td>[0.23/0.43/0.45/0.48/1.46]</td>
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Number of infants in parentheses; the levels of PIVKA-II are given in square brackets. Fisher's exact test: *P = 0.005 when compared with bottle-fed infants. **P = 0.10 when compared with breast-fed infants without vitamin K.