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EFFECTS OF ORAL AND INTRAMUSCULAR VITAMIN K PROPHYLAXIS ON PIVKA-II ASSAY PARAMETERS IN BREASTFED INFANTS IN TURKEY*

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SUMMARY: Uluşahin N, Arsan S, Ertogan F. (Neonatal Intensive Care Unit, Department of Pediatrics, Ankara University Faculty of Medicine, Ankara, Turkey). Effects of oral and intramuscular vitamin K prophylaxis on PIVKA-II assay parameters in breastfed infants in Turkey. Turk J Pediatr 1996; 38: 295-300.

Protein induced by vitamin K absence (PIVKA-II) has been used for the evaluation of vitamin K deficiency in the newborn. Differing PIVKA-II detection rates in various studies on hemorrhagic disease of the newborn have not been explained satisfactorily. In this study we investigated the PIVKA-II values of 44 healthy breastfed infants, of whom 29 recevied vitamin K1 either orally (N=13) or intramuscularly (n=16), and the remaining 15 constituted the control group. PIVKA-II was detected in 15.3 percent (2/13) of the oral and 25 percent of the (4/16) intramuscular group on the third day of life. The detection rate was 93.3 percent (14/15) in the control group. However, at the one-month follow-up, there were no PIVKA-II positive infants. In conclusion, PIVKA-II positivity among breastfed Turkish infants on the third day of life was high compared to that in other studies, perhaps due to a delay in enzyme maturation related to racial, environmental and nutritional factors. *Key words: vitamin K1, neonate, PIVKA-II, hemorrhagic disease.*

"Hemorrhagic disease of the newborn" was first designed as a term in 1894, and its relation to vitamin K was discovered later[1]. Although considerable progress has been made in understanding the biological role of vitamin K in this disease, hemorrhagic disease of the newborn still remains a cause of infant morbidity and mortality[2-10]. It has also been documented that hemorrhagic disease of the newborn occurs more frequently in infants who are breastfed; thus, routine administration of vitamin K or requirements for fortification of all infant formulas with vitamin K has been proposed[10-12].

In order to assess the disease a method of detection of the protein induced by vitamin K absence (PIVKA-II) has been developed[13-15]. PIVKA-II is an abnormal protein (prothrombin) that is mainly induced in the absence of vitamin K. Various studies have been conducted for the detection of PIVKA prothrombin, though with some limitations, for the evaluation of vitamin K deficiency in the newborn.

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Since the first attempt to detect PIVKA-II in the cord blood of newborn infants[16], various reports have shown different detection rates depending on the method used; the differences in the reported incidences have not been explained satisfactorily[13]. In this small preliminary study, we investigated the effects of oral and intramuscular vitamin K1 on Turkish newborns by the PIVKA-II detection method.

Material and Methods

This study was conducted on newborn infants born at the Ankara University Obstetrics Department. Infants were enrolled in the study only if they were full-term and healthy at birth, had APGAR scores greater than six at five minutes and had mothers fully intending to breastfeed. Written informed consents were obtained prior to enrollment. The mothers had had uneventful pregnancies and were not on vitamin K1, anticoagulant, antiepileptic or antituberculosis treatment.

This two-phase study involved three groups of newborns (n=44), who were randomly allocated to one of the groups. These three groups consisted of infants given either oral (n=13) or intramuscular (n=16) treatment, the remainder (n=15) comprising the control group (Table I). Infants in the oral treatment group were given 2 mg vitamin K1 in the form of 1 mg/ml phytomenadione (Konakion, Hoffman-LaRoche) and the intramuscular treatment group received 1 mg vitamin K1 intramuscularly within six hours of birth. The control group was not treated after birth but received 1 mg vitamin K intramuscularly at the age of one month, just after the second blood samples were drawn.

In the first phase of the study a venous sample was drawn 68.8±19.9 hours after birth in all 44 subjects. In the second phase, venous samples were drawn again one month later. Unfortunately only nine subjects in the oral group, eight in the intramuscular group and eight in the control group came for this follow-up visit.

Venous blood samples for PIVKA-II assay were collected in citrated tubes (3.8% sodium citrate) and immediately centrifuged at 3000 g for 10 minutes. Supernatant plasma samples were stored at –30°C until PIVKA-II detection. PIVKA-II was

Table I: Demographic Comparison of the Three Study Groups

	Oral	Intramuscular	Control
Number of infants	13	16	15
Sex (F/M)	6 / 7	5 / 11	5 /1 0

Table II: Comparison of Gestational Age, Apgar Scores and Age at Vitamin K Administration for Orally and Intramuscularly Treated and Control Groups (Results given as mean ± SD).

	Oral	Intramuscular	Control	p
Gestational weeks	39.92 ± 1.04	39.19 ± 1.05	39.13 ± 1.13	0.79
Apgar Score (5 minutes)	9.23 ± 0.44	9.75 ± 0.45	9.27 ± 0.59	0.01
Age at Vitamin K administration (hours)	8.92 ± 13.6	12.13 ± 9.4		0.48

assayed by an enzyme-linked immunosorbent assay, implementing a monoclonal antibody method (Asserachrom PIVKA-II, Diagnostica Stago, France). With this methodology, PIVKA-II values were expressed in arbitrary units as AU/ml, where one AU was one microgram purified prothrombin per ml.

The normal detection limit was 0.13 AU/ml, which defined PIVKA-II concentrations as positive or negative when greater or less than this limit, respectively. The statistical evaluation of data was performed with the Statistical Package for Social Sciences Program (SPSS-PC, Version 4.01). For inter-group comparisons, analysis of variance, and for intra-group comparisons the student's t test was employed. Pearson's correlation coefficient was implemented for regression analyses.

Results

Selected patient characteristics are displayed in Tables I and II. No feature other then sex was found to be statistically different between the groups in either phase of the study.

All infants remained healthy during follow-up and no side effects were reported related to vitamin K administration. There were also no reports of bleeding diathesis during the study.

The presence of PIVKA-II was evaluated in all infants. Follow-up screening was performed no later than at the end of the first month. Patients' results are displayed in Table III.

Table III: Presence of PIVKA-II (Protein Induced by Vitamin K Absence) in Breastfed Infants at Different Ages In Controls and After Oral or Intramuscular Vitamin K Prophylaxis at Birth

	Time after birth	
	3 Days	1 Month
Oral group	2/13 (15.3%)	0/9
Intramuscular group	4/16 (25.0%)	0/8
Control group	14/15 (93.3%)	0/8

In the group treated orally, 15.3 percent of the infants were positive for PIVKA-II, while 25 percent of infants treated intramuscularly were positive. A much higher percentage of infants (93.3%) who had no vitamin K treatment had high PIVKA-II values. Although the number of infants who followed up at one month was much lower, none of them was PIVKA-II-positive.

The difference in percentages of PIVKA-II positive samples among orally treated patients compared with those treated intramuscularly was not statistically significant ($p=0.05$). However, there was a statistically significant difference between the control and treatment groups ($p=0.001$).

Discussion

In this preliminary study the detection of PIVKA-II was taken as an indication of vitamin K status in breastfed newborns in Turkey. Although further investigation is necessary before a possible correlation between PIVKA-II findings and early or late hemorrhagic disease of the newborn can be made our results show that PIVKA-II values were highly positive (93.3%) in control group infants who had not received vitamin K at birth. On the contrary, infants who were either orally or intramuscularly treated with vitamin K and breastfed had low PIVKA-II values.

To our knowledge, the PIVKA-II positivity rate found in this study is the highest reported. Motahara et al.[16] found levels of PIVKA-II in 21.5 percent of cord blood samples of 51 newborns; however, on the third and fifth days of his study these values had increased to 50 and 60 percent PIVKA-II positive, respectively. Hathaway et al.[7] also reported similar (27/51, 53%) PIVKA-II positivity on the fourth and fifth days of life without vitamin K supplementation.

There have also been controversial results from other studies. In the Widdershoven study[18], PIVKA-II was only found to be present in the cord blood of five newborns out of 16 (31.3%). However, on the fourth day of life, PIVKA was detected in the plasma of 12.9 percent of cases (12/93 cases).

A similar result was reported by Anai et al.[1], in whose study PIVKA II-positive infants represented only seven percent of cases (13/186) on the fifth day of life. According to Widdershoven[20], this difference in PIVKA-II values could only be attributed to the detectability of vitamin K in the breast milk of Japanese and Dutch women.

As the PIVKA-II results of several studies contradict each other, the cause of hemorrhagic disease of the newborn has not yet been explained clearly. In light of Widdershoven's attribution for the vitamin K content of breast milk in different races, limited transport of the vitamin to the fetus through the placenta or minute amounts of vitamin K production by the intestinal flora of newborns could be postulated. Although the data are inconclusive, the response to vitamin K administration is claimed to be limited by the immature liver's incapability to synthesize and carboxylate precursor proteins. Also, the difference in the capabilities of premature and full-term babies is unknown[20].

The PIVKA-II, which is considered an indicator of vitamin K levels, has no clinical consequences but indicates whether enough vitamin K has been available for carboxylation of vitamin K-dependent proteins[13]. High levels of PIVKA-II may be caused by a dysfunctioning liver or by environmental factors that may have an inhibitory effect on hepatic utilization of vitamin K.

The results of our preliminary study showed PIVKA-II positivity at levels higher than previously reported. These elevated levels of PIVKA-II may be evidence of a high prevalence of vitamin K deficiency among our breastfed infant population.

Another controversial finding of our study was the absence of PIVKA-II at the end of one month in all breastfed infants, whether or not they had vitamin K treatment. This inconclusive finding may be result of the limited number of follow-up infants. A prevalence study on hemorrhagic disease of the newborn in Turkey has not yet been performed. Our group is currently planning such a study as we believe that some racial factors may play an important role in the high incidence of elevated PIVKA-II levels we detected. Similarly, there is a high incidence of unexplained unconjugated jaundice among Turkish newborns. In conclusion, it would be worthwhile to conduct another study on Turkish mothers and newborns to detect the vitamin K content of breastmilk and the degree of immature reutilization of vitamin K epoxide by newborns, two possible etiological factors for the disease concerned.

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