RESEARCH PAPER

Prophylactic Vitamin K Administration in Neonates on Prolonged Antibiotic Therapy: *A Randomized Controlled Trial*

Amanpreet Sethi[1], M Jeeva Sankar[1], Anu Thukral[1], Renu Saxena[2], Suman Chaurasia[1] and Ramesh Agarwal[1]

From Departments of [1]Pediatrics and [2]Hematology, All India Institute of Medical Sciences, New Delhi, India.
Correspondence to: Dr Ramesh Agarwal, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi 110 029, India. ra.aiims@gmail.com
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Objective: To compare the prevalence of vitamin K deficiency after intramuscular vitamin K or no treatment in neonates with sepsis on prolonged (>7 days) antibiotic therapy.

Study Design: Open label randomized controlled trial.

Setting: Level 3 Neonatal Intensive Care Unit (NICU).

Participants: Neonates with first episode of sepsis on antibiotics for ≥7 days were included. Neonates with clinical bleeding, vitamin K prior to start of antibiotic therapy (except the birth dose), cholestasis or prenatally diagnosed bleeding disorder were excluded.

Intervention: Randomized to receive 1 mg vitamin K (*n*=41) or no vitamin K (*n*=39) on the 7th day of antibiotic therapy.

Main outcome measure: Vitamin K deficiency defined as Protein Induced by Vitamin K Absence (PIVKA-II) >2 ng/mL after 7 ± 2 days of enrolment.

Results: The prevalence of vitamin K deficiency was 100% (*n*=80) at enrolment and it remained 100% even after 7 ± 2 days of enrolment in both the groups.

Conclusion: Neonates receiving prolonged antibiotics have universal biochemical vitamin K deficiency despite vitamin K administration on 7th day of antibiotic therapy.

Keywords: *Antibiotics, Neonatal sepsis, PIVKA.*

Trial registration: Clinical Trial Registry of India (CTRI/2017/02/007776).

Prolonged antibiotic therapy can lead to deficiency of vitamin K either by eradication of gut flora, a common source of mena-quinones or by direct inhibition of the vitamin K dependent step in clotting factor synthesis by some antibiotics containing 1-N-methyl-5-thiotetrazole (NMTT) side group [1]. Vitamin K deficiency is a frequent complication in patients admitted in adult intensive care units (ICU) with incidence as high as 25% [2]. One of the important implicated risk factors is prolonged antibiotic usage, a common scenario in neonatal intensive care units (NICU).

Prolonged antibiotic usage has been found to result in vitamin K-related coagulopathy in about one-third of children with higher incidence in malnourished children and infants [3]. Extrapolating the evidence from adult and limited pediatric studies, most of the NICUs in India prefer to administer vitamin K every 7 days to neonates on antibiotic therapy. However, evidence for this practice is lacking in the neonatal population. Studies on preterm neonates and neonates in surgical ICU on prolonged antibiotic therapy have demonstrated high levels of vitamin K after its single parenteral birth dose [4,5]. Therefore, this study was planned to revisit the practice of administering vitamin K during prolonged antibiotic therapy amongst neonates.

Accompanying Editorial: Pages 459-60.

Methods

This open-label randomized clinical trial was conducted in a level-3 neonatal unit in Northern India, between July 2015 and August 2016. Neonates with first episode of symptomatic sepsis on antibiotics for 7 or more days were included. Neonates who received vitamin K prior to start of antibiotic therapy (except the birth dose), who had any clinical bleeding, cholestasis or prenatally diagnosed bleeding disorder were excluded. Eligible neonates were enrolled on the seventh day of antibiotic therapy after obtaining written informed consent from the parents. The study was approved by the Institute Ethics Committee, All India Institute of Medical Sciences, New Delhi, India.

Enrolled neonates were randomized at seventh day of antibiotic therapy to receive single dose of 1 mg intramuscular vitamin K or no vitamin K. Both vitamin

K1 (Inj. Kenadione; Samarth Pharma Private Ltd., Mumbai) and K3 (Inj. Reokay; Rathi Laboratories Private Ltd, Patna) were used in the study as per their availability in the central drug store of the hospital. All neonates received intramuscular Vitamin K1 at birth as per the unit protocol (Weight ≥1000 g: 1 mg and <1000 g: 0.5 mg). Computer generated random numbers with variable block size (2 to 8) were used to allot the neonates into the two groups. The randomization sequence was prepared by another investigator who was not involved in collecting baseline variables, applying the intervention and measuring the outcomes. To ensure allocation concealment, random treatment assignment was placed in serially numbered opaque and sealed envelopes. Blinding of the intervention was not possible because of the nature of the intervention. Vitamin K dose was administered by the nurse on duty who was not involved in group allocation.

Primary outcome was the proportion of neonates with PIVKA-II levels more than 2 ng/mL after 7±2 days of enrolment. Secondary outcome was the proportion of neonates with coagulation abnormalities (prothrombin time (PT): 2 seconds more than upper limit as per gestational age and post-natal age cut-off [6]) after 7±2 days of enrolment.

At enrolment, 1 mL of blood sample was drawn by venepuncture from all the neonates and sent for PIVKA II estimation in the coagulation laboratory of the institute. Second sample was taken after 7±2 days of enrolment. All the samples were centrifuged at 2500 rpm for 10 minutes and supernatant serum was decanted and stored at -70°C. All the samples were analyzed together. For PIVKA II estimation, samples were thawed in the water bath at 37ºC for 15 minutes. PIVKA-II estimation was done using specialized kits with sandwich ELISA technique (Flarebio Biotech, USA & Elabscience Biotechnology, China) and PT estimation was done using an automated ACL Elite Pro system (Instrumentation Laboratory, USA). During analysis, all the serum samples tested above the highest detectable range of the kit (10 ng/mL). So, standardization of the ELISA assay took extra efforts. In the next step, six of the randomly selected samples were run in serial dilutions. Eventually, at 1:128 dilution of the serum with buffer, the PIVKA-II levels were in the detectable range. We used healthy adult blood samples as control.

There was no published study that had used PIVKA-II levels as surrogate marker for vitamin K deficiency in neonates on prolonged antibiotics. So, sample size calculation was based on a study in patients aged 3 months to 23 years, suffering from cystic fibrosis and on prolonged antibiotic therapy where abnormal coagulation and raised PIVKA-II levels were found in 33% of cases [7]. Thus, a total of 94 neonates (47 in each group) were required to have 80% chance of detecting with significance at 5% level, a decrease in vitamin K deficiency from 33% in the control group to 10% in the intervention group.

Statistical analysis: Statistical analysis was performed using Stata 11.2 (Stata-Corp, College station, Texas, US). Categorical variables were compared by Chi square/ Fisher Exact test. Continuous variables were compared by Student t-test (if normally distributed) or Wilcoxson rank sum Test (if skewed).

RESULTS

A total of 80 neonates were enrolled out of 120 neonates with sepsis during the study period. Among enrolled neonates, 41 were allocated to vitamin K group and 39 were allocated to 'no vitamin K' group (***Fig.* 1**). Two neonates in the vitamin K group died before outcome

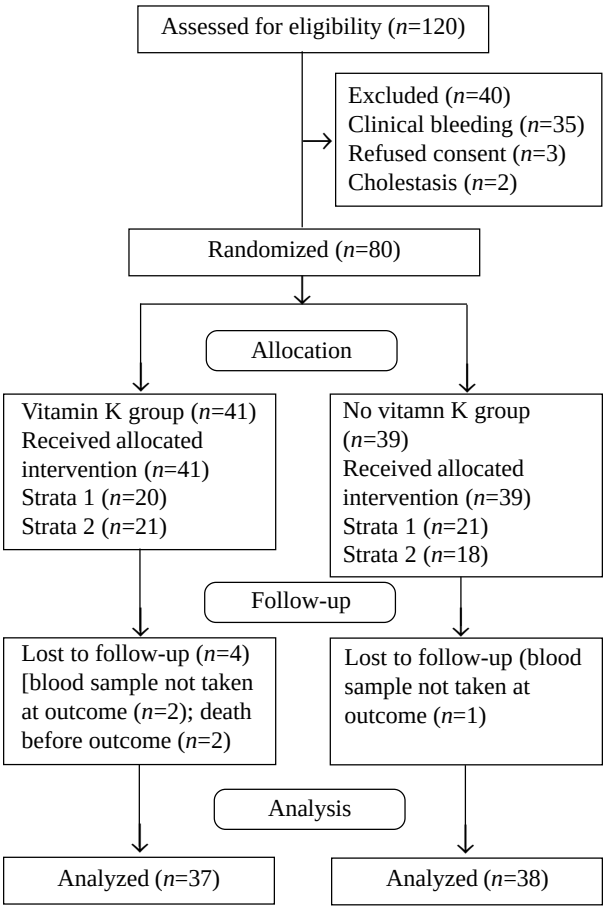

FIG. 1 *Trial Flow.*

TABLE I BASELINE DEMOGRAPHIC VARIABLES AND POSTNATAL MORBIDITIES

Variable	*Vitamin K group (n=41) (%)*	*No Vitamin K group (n=39) (%)*
Gestation (wks)*	31.4 (4.0)	32.5 (4.1)
Birth weight (g)#	1380 (961-2005)	1394 (955-2043)
Appropriate for gestational age	29 (70.7)	26 (66.6)
Male	19 (46.3)	23 (58.9)
Preterm premature rupture of membrane (> 24 h)	7 (17)	6 (15.3)
Mother on drugs causing vitamin K deficiency	3 (7.3)	2 (5.1)
Intrapartum antibiotics	7 (17)	7 (17.9)
Clinical chorioamnionitis	2 (4.8)	0
Apgar at 5 min#	8 (7-9)	8 (7-9)
Birth trauma	1 (2.4)	1 (2.5)
Prophylactic vitamin K at birth	41 (100)	39 (100)
Exclusive breast milk at enrolment	34 (82.9)	36 (92.3)
Respiratory distress syndrome	19 (46.3)	11(28.2)
Hyperbilirubinemia requiring phototherapy	13(31.8)	7 (18)
Intraventricular hemorrhage	4 (9.7)	5 (12.8)
Early onset sepsis (d" 72 h)	24 (58.5)	16 (41.0)
Post-natal age at enrolment # (d)	10.5 (9 to 18.2)	10 (9 to 18)
Blood culture positive sepsis	3 (7.3)	5 (12.8)
Hypotension requiring inotropes	11(26.8)	9 (23.1)
Total parenteral nutrition (TPN) at enrolment	4 (9.7)	2 (5.1)
Duration of antibiotics during the sepsis episode* (d)	10.8 (5.4)	12.1 (6.3)

*Values in n (%) except *Mean (SD) and #Median (IQR).*

TABLE II PREVALENCE OF VITAMIN K DEFICIENCY AND PIVKA II LEVELS AT ENROLMENT

Variable	*Vitamin K group*	*Control group*
PIVKA-II >2 ng/mL, *n* (%)	(*n*=41) 41 (100)	(*n*=37) 37 (100)
PIVKA-II levels, mean (SD)	(*n*= 20) 992 (215)	(*n*= 17) 929 (263)

assessment because of severe sepsis. In three neonates (2 in vitamin K group and 1 in no vitamin K group), second blood sample could not be collected after 7±2 days of enrolment. Thus, 37 neonates were analyzed in the vitamin K group and 38 in 'no vitamin K' group. Baseline demographic variables and postnatal morbidities were comparable between the two groups (***Table* I**).

The baseline prevalence of vitamin K deficiency was 100% in both the groups. Quantitative PIVKA-II levels could be done in only 37 neonates (20 in the vitamin K group and 17 in the no vitamin K group). Mean (SD) PIVKA-II levels were very high in both the groups with no significant difference between the two groups (***Table* II**).

The prevalence of vitamin K deficiency after 7±2 days of enrolment was 100% in both the groups. Quantitative PIVKA-II levels could be done in only 35 neonates (18 in the vitamin K group and 17 in the no vitamin K group). Mean (SD) PIVKA-II levels were comparable between the two groups (***Table* III**). There was no difference in the prevalence of coagulation abnormalities as assessed by deranged PT or clinical bleeding between the two groups.

DISCUSSION

This open label randomized controlled trial, compared the effect of 1 mg parenteral vitamin K administration on seventh day of antibiotic therapy *versus* no vitamin K on the prevalence of vitamin K deficiency in neonates with sepsis. All neonates in both groups were deficient in vitamin K when assessed for outcome.

TABLE III PREVALENCE OF VITAMIN K DEFICIENCY AND PIVKA II LEVELS AFTER 7 ± 2 DAYS OF ENROLMENT

Variable	*Vitamin K group*	*Control group*	*Relative risk/Mean difference (95% CI)*	*P value*
PIVKA-II >2 ng/mL, *n* (%)	(*n*=37)37 (100)	(*n*=38)38 (100)	–	1.00
PIVKA-II levels, mean (SD)	(*n*= 18)946 (153)*	(*n*= 17)959 (280) *	-13	(-167 to 140) 0.86
Deranged PT, *n* (%)	(*n*=31)3 (9.6)	(*n*=34)1 (2.9)	1.63(0.8 to 3.1)	0.27
Clinical bleeding, *n* (%)	(*n*=41)4(9.6)	(*n*=39)1 (2.6)	1.62(0.9 to 2.6)	0.11

WHAT IS ALREADY KNOWN?

- Coagulopathy due to vitamin K deficiency is reported to be a frequent complication in adult patients in the medical/ surgical intensive care unit as a result of prolonged antibiotic therapy.

WHAT THIS STUDY ADDS?

- Deficiency of vitamin K (as assessed by PIVKA-II levels >2 ng/mL) persists even after intramuscular administration of 1 mg vitamin K in neonates with sepsis on prolonged antibiotic therapy.

The strength of our study is that PIVKA-II levels were assessed by an independent clinician, blinded to the group allocation, thus minimizing bias. The major limitation of our study was that only 80 neonates out of the total planned 94 neonates could be enrolled because of limited budget and time factors. Blinding could not be done due to nature of the intervention. Both vitamin K1 and K3 were utilized in the intervention group, as per availability in the drug store of the institute. However, this is not likely to affect the outcome as both vitamin K1 and K3 have equal efficacy in preventing vitamin K deficiency [8]. Further, PIVKA-II level assessment, ELISA kits from two different manufacturers were used due to financial constraints and quantitative PIVKA-II levels could not be done in all the enrolled neonates due to non-availability of blood serum samples.

High levels of PIVKA-II of fetal origins may persist upto first 48 to 72 hours after birth in the peripheral blood so, PIVKA-II levels were evaluated only on the 7th day of antibiotic therapy to avoid false positive results [8]. Such high proportion of neonates with abnormal PIVKA-II levels has not been reported in the published literature. In the prospective observational study by Najmaldin, *et al*. [4], 49 infants (<6 weeks) admitted in surgical ICU on antibiotics were enrolled. They observed detectable PIVKA-II levels in 41% of the study population but the method of PIVKA-II estimation and exact cut-off followed is not mentioned. De Montalbert, *et al*. [7] enrolled 43 patients (3 months-23 years) with cystic fibrosis on antibiotic therapy. They found abnormal PIVKA-II concentrations in 33% of the patients but the method for PIVKA-II estimation was not ELISA-based, and the cut-off used was also different. In a subset of neonates, where quantitative estimation was possible, we noticed PIVKA-II levels in the range of 900-1000 ng/ml. Such high levels have not been previously described in the literature. In a previous study from our center including term healthy neonates, median PIVKA-II levels were in the range of 1.9-2 ng/mL at 72 hours of age [8]. Possible reasons for this difference could be use of kits from a different manufacturer, and that majority of patients in our study population were born preterm. The overall prevalence of coagulopathy was only 6% in the study population, with no difference between the two groups. In the previous studies by Aziz. *et al*. [9] and Bhat, *et al*. [3] in the older children, the prevalence of coagulopathy at 10 days of antibiotic therapy was 15% and 33%, respectively. The overall low prevalence of coagulopathy in our study could be due to the fact that all our neonates received prophylactic vitamin K at birth and all neonates with clinical bleeding before 7th day of antibiotic therapy were excluded.

We conclude that neonates with sepsis receiving antibiotics for 7 or more days have universal biochemical vitamin K deficiency even after vitamin K administration, and question the utility of this practice in neonates on prolonged antibiotic therapy. However, it also raise questions about the usefulness of PIVKA-II measurement for assessing vitamin K deficiency in neonates. There is a need to establish normal values and standards of PIVKA-II measurements for assessing subclinical vitamin K deficiency in the newborn infant.

Contributors: AS: study conception and implementation, data management and writing the manuscript; MJS,AT,RS,SC,RA: supervised implementation of the study and contributed to writing of the manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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