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Original Article

Negative Impact of Prolonged Antibiotics or Persistent Diarrhea on Vitamin K1 Levels in 2-24 Weeks aged Egyptian Infants

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Abstract. ***Background:*** **To evaluate the hazard of prolonged antibiotic therapy and/or persistent diarrhea on vitamin K1 (VK1) level and bleeding profile in infants (2-24 weeks).**
Methods: **A one-year case-control study, conducted at Ain Shams University, Egypt. 338 infants (2-24 weeks) were recruited and divided into 3 groups (1:1:3 ratios); group A (n=67) patients who received antibiotics for ≥10 days, group B (n=67) who had persistent diarrhea ≥ 14 days and group C (n=204) age- and gender- matched infants who had not either received antibiotics nor had diarrhea. All subjected to clinical assessment, bleeding history and had their complete blood count (CBC), PT and PTT, liver transaminases and VK1 level assayed.**
Results: **There was a significant increase in frequency of VKDB (vitamin K deficiency bleeding) and abnormal bleeding profile in cases than control group. There was significant negative correlation between VK1 level and duration of diarrhea, length of antibiotics used and bleeding profile. Antibiotic usage has hazardous effect on VK1 level in those with diarrhea; more patients were receiving antibiotic in those with persistent diarrhea and VKDB (N=55) than those with persistent diarrhea and normal VK1 (N=12). The longer duration of antibiotic therapy the lower level of VK1. Combining cephalosporin/penicillin therapy and/or diarrhea, in particular, had an impact on VK1 level.**
Conclusion: **VKDB, a preventable cause of life-threatening hemorrhage, is still a major health problem in Egyptian infants, where persistent diarrhea and misuse of antibiotics are prevalent, necessitate a booster dose of VK in those high risk infants.**

Keywords: Vitamin K deficiency; Persistent diarrhea; Prolonged antibiotics therapy; Hemorrhagic disease of newborn.

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Introduction. Hemorrhagic disease of newborn (HDN) is one of the most common causes of acquired hemostatic disorder in early infancy.[1] It is categorized as early, classical and late depending on the time of onset.[2] The most common manifestation of late HDN reported are; intracranial hemorrhage, deep ecchymosis, bleeding from gastrointestinal tract and/or from the mucous membrane, skin punctures or surgical incisions.[3-4]

Vitamin K (VK) plays an integral role in the clotting cascade; its deficiency; specifically in

vulnerable neonates with insufficient stores, can lead to spontaneous bleeding and devastating effects. The American Academy of Paediatrics recommends intramuscular (IM) VK prophylaxis for all newborns to prevent early onset HDN.[5] Newborn babies are at particular risk of vitamin K deficiency (VKD), has only 20-50% of adult coagulation activity, as placental transfer is limited and human milk is a poor source. A minimal amount of VK passes through the placenta, and a negligible amount is also found in breast milk**.** Factors including failure to administer VK at birth, exclusive breastfeeding for a long period, prolonged or chronic diarrhea, and prolonged use of antibiotics could lead to vitamin K deficiency bleeding (VKDB).[1]

Data from a longitudinal household survey conducted in 1990–1991 in rural Egypt show that treatment of acute diarrheal episodes is still far from optimal, in particular, the prescription of antibiotics is still too frequent. Children taken to the government clinics, private physicians or pharmacies are more likely to be given antibiotics therapy.[6] Egypt is one of the developing countries where there are many mistakes from healthcare givers regarding management of diarrheal illness and early detection of VKDB disorders. Moreover, in a recent Egyptian study; exclusive breastfeeding, diarrhea lasting more than one week and antibiotic consumption were more common in intracranial hemorrhage than in the control group.[7] Consequently, the present study was intended to assess the impact of persistent diarrheal disease and prolonged use of antibiotics on the VK1 level in the infants aged from 2 to 24 weeks. By providing evidence of the unsafe impact of abuse of antibiotics on the VK1 level the study suggests the need for an additional dose of VK to patients on prolonged courses of antibiotics or persistent diarrhea.

Subjects and Methods. This study was a case-control study, conducted at Ain Shams University, Children Hospital throughout the period from May 2015 to May 2016. The infants included in our study were recruited from the emergency and outpatient clinic of Ain Shams University pediatrics hospital (tertiary care hospital) they were seeking medical advice for a variety of clinical symptoms.

A total number of 338 infants aged two to 24 weeks of both sexes were recruited. Exclusion criteria were infants or their mother who were on any treatment that will affect their bleeding profile, infants who had history of bleeding tendency or those who had an underlying condition that could affect their bleeding profile or those who had platelet count < 100,000/mm^3 or elevated liver enzymes or direct bilirubin > double high normal. They were categorized into three groups: group (A) included 67 patients who received antibiotics for ten days or more, group (B) included 67 had persistent diarrhea more than 14 days according to the World Health Organization (WHO).[8] Group (C) included 204 age- and gender-matched infants and were neither on antibiotics nor had diarrhea this group acted as control group.

A verbal informed consent was obtained from the guardian of each patient or control before participation. The procedures applied in this study were approved by the institutional, regulatory board Pediatric Hospital, Faculty of Medicine, Ain Shams University, on 23 March 2015, and are in accordance with the Helsinki Declaration of 1975.

All patients were subjected to thorough clinical assessment with special emphasis on antenatal history of any maternal illness or medication, mode and place of delivery, history of VK administration at birth, neonatal ICU admission, dietetic history, family history of consanguinity and bleeding tendency in siblings and relatives were inquired. In addition, a history of prolonged diarrhea or antibiotic use, or bleeding, liver or kidney diseases was stressed. They were also thoroughly examined for any signs of bleeding tendencies or liver disease to be excluded from the study.

Laboratory assessment: Six milliliters of venous blood was collected from the studied subjects under complete aseptic conditions for the evaluation of the following:

1. Complete blood count (CBC), samples were obtained on potassium-ethylene diamine acetic acid (K2EDTA) vacutainer and assessed using Beckman Coulter counter (Coulter Corporation, Florida, USA). Complete blood count was evaluated for all the recruited infants to exclude different causes of bleeding as thrombocytopenia.
2. Liver transaminases (ALT and AST): Liver enzymes were done to prohibit liver disease as a cause of VK insufficiency and all patients had ALT and AST inside of the ordinary levels.

3. Prothrombin time (PT) and partial thromboplastin time (PTT) assessment (using platelet poor plasma (PPP) preparation). Samples were obtained on Na citrated vacutainer (1 part citrated sodium (0.11 mol/L) with nine parts venous blood, (PPP) was collected by centrifugation at 3000 × g for 15 minutes. PT, PTT, and INR were done as determinants of the VK1 level of the included infants. According to our Ain Shams University, Children Hospital laboratory reference range; the control for PT was 14 seconds (s) classifying any result above 14 as drawn out, PTT control was 44s, and INR standard values were distributed within 1.4.
4. Vitamin K 1 level: samples were obtained on heparin, centrifuged for 30 minutes at 3000 rpm at 2-8°C and then stored at -20°C or -80°C for later assessment at national research centre by double antibody sandwich enzyme-linked immune-sorbent assay (ELISA) (Glory Science Co., Ltd, Del Rio, TX 78840, USA). According to manufacturer manual, standard and testing samples were singularly added to the conventional wells, then of HRP-conjugate reagent was also added to each well, covered and incubated and then washed. Next chromogen solution A and B were added, were gently mixed and were incubated. Afterward, stop solution was added to each well, the colour in the wells should then change from blue to yellow. Finally, the optical density (O.D.) was measured spectrophotometrically at a wavelength of 450 nm. The concentration of VK1 was determined by comparing the O.D. of the samples to the standard curve. Our cut-off, mean value of standards multiply by three, was 300 Pg/ml. The sensitivity of this assay is 1.0 pmol/mL.

Statistical analysis. Data were collected, coded and entered into the Statistical Package for Social Science (IBM SPSS). Qualitative data presented as number and percentages while quantitative data presented as mean, standard deviations and ranges when parametric and median with interquartile ranges (IQR) (IQR; 75^{th} and 25^{th} percentiles) when nonparametric. Kolmogorov Smirnov test was used for testing the distribution of normality. The comparison between two groups was performed by using Chi-square test and/or Fisher exact test (when the expected count in any cell was found less than five) when the data were qualitative, independent t-test when the data were quantitative with parametric distribution and Mann-Whitney test when data were non-parametric. The one-way analysis of variance (ANOVA) is used to test for differences among at least three groups; then a post hoc test was used whenever a significant difference between three or more sample means was revealed by ANOVA. The Kruskal–Wallis test is a non-parametric method for comparing two or more independent samples. Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, a Spearman correlation test was performed. Multivariable linear regression analysis was employed to determine the relation between VK1 level and other variables. The confidence interval was set to 95% and the margin of error accepted was set to 5%. Therefore, the p-value was considered significant < 0.05

Results. The study included 338 children with mean age of 11.69 ± 6.88 weeks (2 - 24), they were 221 males and 117 females with a ratio of 1.89:1. **Table 1** showed the demographic data of the studied infants; the three groups had comparable mean age and gender distribution. Some growth parameters, mean weight was 3.82 ± 1.11, showed delayed growth in the group with persistent diarrhea in comparison to control and those on antibiotics as shown in **Table 1**. Furthermore, mild anemia; statistically significant drop of hemoglobin level in the group on prolonged antibiotic use either due to underlying illness or prolonged antibiotic use.

To assess the cut-off level of VK1, a graph of sensitivity against 1 – specificity, a receiver operating characteristic (ROC) curve had been done. Although the ROC curve (**Figure 1**) showed that the best cut-off point between cases and control group according to vitamin k was ≤ 412 Pg/ml with sensitivity 95% (CI) of 92.54% and specificity 95% (CI) of 81.40% while area under the curve (AUC) 95% (CI) was 0.923, and +PV was 72.1 and –PV is 95.5, we used the cut-off of 300 Pg/ml which is the cut-off of the used kits in our study.

The mean VK1 (Pg/ml) level ± SD (Range) for the control group was 562.55 ± 196.44 (130 –

Table 1. Comparison between the studied groups as regards the demographic, clinical and laboratory characteristics.

Variables	Control group (N= 204)	Diarrhea > 14 days (N= 67)	Antibiotics for > 10 days (N= 67)	One Way ANOVA	
				F/K*/Z•	P-value
Age (weeks)	11.08 ± 6.95	13.27 ± 6.40	11.94 ± 6.98	2.624	0.074
Weight (kg)	4.52 ± 1.49	3.82 ± 1.11	4.87 ± 1.56	9.453	<0.001
Length (cm)	56.67 ± 9.17	54.17 ± 10.52	58.52 ± 8.44	3.701	0.026
Duration of diarrhea (weeks)*	–	3 (2 – 4)	1 (1 – 2)	-4.451•	<0.001
Duration of antibiotics (days)*	-	7 (5 – 14)	2 (1 – 13)	10.022*	0.007
Haemoglobin level (g/dl)	10.78 ± 1.46	10.12 ± 1.46	10.07 ± 0.82	10.277	<0.001
Platelet count (10^3/uL)	345.37 ± 115.23	307.03 ± 164.77	339.55 ± 114.58	2.342	0.098
Prothrombin time (seconds)	12.93 ± 1.17	15.96 ± 7.81	14.90 ± 1.89	19.922	<0.001
Prothrombin time (seconds)	12.93 ± 1.17	15.43 ± 5.69		-6.071	<0.001
Partial thromboplastin time (seconds)	39.81 ± 3.76	47.20 ± 15.23	44.28 ± 3.79	27.430	<0.001
Partial thromboplastin time (seconds)	39.81 ± 3.76	45.74 ± 11.16		-7.020	<0.001
International normalized ratio	1.10 ± 0.15	1.35 ± 0.41	1.44 ± 0.29	56.361	<0.001
International normalized ratio	1.10 ± 0.15	1.39 ± 0.36		-10.381	<0.001
Alanine aminotransferase (IU)*	12.5 (8 – 18)	20 (13 – 33)	9 (6 – 18)	44.591*	<0.001
Aspartate aminotransferase (IU)*	17 (11 – 23)	34 (22 – 44)	11 – (8 – 20)	90.142*	<0.001
Vitamin k (Pg/ml)	562.55 ± 196.44 (130 – 988)	206.03 ± 99.35 (45 – 547)	233.42 ± 114.47 (43 – 578)	170.741	<0.001

Data are expressed as mean± SD where t test was used unless specified as * median (inter-quartile range) where * Kruskal-wallis or • Mann-Whitney tests were used

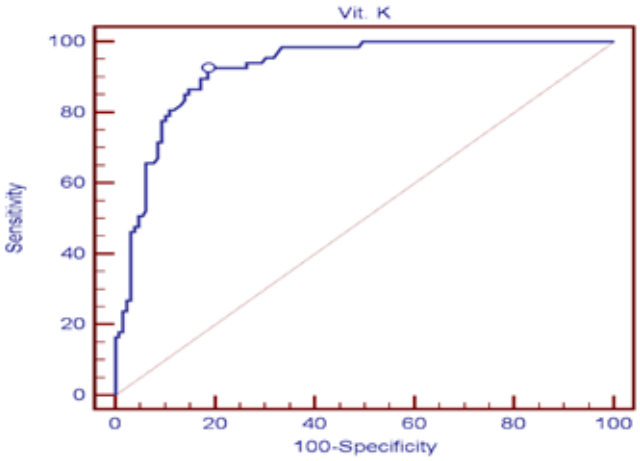

Figure 1. Receiver operating characteristic curve (ROC) for the differentiation between cases and controls according to vitamin K.

988), for those who suffer from diarrhea more than 14 days was 206.03 ± 99.35 (45 – 547), and for those receiving antibiotics therapy for more than ten days was 233.42 ± 114.47 (43 – 578). There was statistically significant lower VK1 level in both patients groups than control group as illustrated in **Table 1** and **2**.

Characteristics of patients who received antibiotics therapy for ≥ ten days (group A). Ninety-nine of the studied patients were on antibiotic treatment (74.6%) with median (IQR) of 7 (1 – 13.5); of them 28 (20.9%) were treated with cephalosporins, 26 (19.4%) were on penicillins, 12 (9%) were on metronidazole, 34 (25.4%) were treated with a combination. Sixty-seven patients received antibiotic therapy for ten days or more with a median duration of 12 days (10 – 18). Of them, 14 (21.2%) were treated with cephalosporins, 7 (9.8%) received penicillins, 3 (4.2%) metronidazole and 4 (2%) a combination.

Three main classes of antibiotics that were observed have indistinguishable impact on VK1. Thirty-five %, 32% and 40% of infants received cephalosporins, penicillins and metronidazole had serum VK1 below cut-off value respectively (but the aggregate sum of patients on metronidazole was only five infants). The group which received a combination of cephalosporins and penicillins had the most astounding occurrence of VK1 inadequacy (24 out of the 30 infants (80%).

Table 2. Post hoc analysis for the comparison between the studied groups as regards the studied variables.

Post hoc analysis			
Variable	**Control group VS Diarrhea >14 days**	**Control group VS Antibiotics for > 10 days**	**Antibiotics for > 10 days VS Antibiotics for > 10 days**
Age (weeks)	0.024	0.375	0.262
Weight (kg)	0.001	0.078	<0.001
Height (cm)	0.058	0.158	0.007
Antibiotic duration (days)	<0.001	0.749	0.083
Haemoglobin level (g/dl)	0.001	<0.001	0.843
Platelet count (10^3/uL)	0.032	0.744	0.137
Prothrombin time (seconds)	<0.001	<0.001	0.095
Partial thromboplastin time (seconds)	<0.001	<0.001	0.026
International normalized ratio	<0.001	<0.001	0.047
Alanine aminotransferase (IU)	<0.001	0.034	<0.001
Aspartate aminotransferase (IU)	<0.001	<0.001	<0.001
Vitamin k (Pg/ml)	<0.001	<0.001	0.343

The leading causes for the administration of the antibiotic treatment more than ten days were upper respiratory tract infection, pneumonia, and gastroenteritis. Twenty-one infants had gastroenteritis; 18 of them (85%) had low VK1 serum level, which was also found low in 76% of infants with pneumonia and in 71% of infants with upper respiratory tract infection (URTI).

Almost half of patients who received antibiotics for 10 days or more, 35 out of the 67 infants (52%) had abnormal coagulation profile in the form of prolonged PT, PTT and INR, which demonstrates the connection between prolonged usage of antibiotics and the variations from the norm in the coagulation profile and the bleeding that could ensue.

Fifty-two of the 67 (77%) who received antibiotics for ten days or more had lower serum level of VK1 than the predicted for age (300 Pg/ml). Duration of antibiotic utilization influenced VK1 level by a significant negative correlation as illustrated in **Figure 2**, and there was statistically significant negative correlation between VK1 and INR (r: -0.313, p: 0.001), and PT, PTT. Out of 18 children on 14 days or more of antibiotic therapy, 17 had levels underneath the cut off value (94%) (VKD), extremely low levels of VK1 were detected (<15% of the calculated low cutoff level), and one infant had a marginal value of 320 Pg/ml.

Characteristics of patients who received pronged use of diarrhea ≥14days (Group B). Sixty-seven patients had diarrhea for ≥14days with a median (IQR) 3 weeks (2 – 4). Fifty-five patients (82.1%) out of them had serum level of VK1 lower than predicted for age (300 Pg/ml), and 31.3% had abnormal coagulation profile in the form of prolonged PT, PTT, and INR.

Almost half of the infants who suffered from persistent diarrhea received antibiotics (n=33, 49.3%); ten of them was on cephalosporins, ten on metronidazole, five on penicillin, and eight on mixed antibiotics regimens. There is a significant difference between those on different type of antibiotics and those on none (F=4.868, p=0.002)

When patients having suffered from persistent diarrhea and having VKD (N=55) were compared to those who suffered from persistent diarrhea but had normal VK1 level (N=12), a statistically significant higher incidence of antibiotic therapy was found in patients with VK1 deficient (31 patients (93.9%)) than in those with normal VK1 (2 patients (6.1%)) [$x2$=6.211, p=0.012].

Patients who suffered from diarrhea for ≥14days have statistically significant negative correlation between VK1 and INR (r: -0.446, p: 0.001) and coagulation profile illustrated in **Figure 3** and statistically significant positive correlation with haemoglobin level (r: 0.292, p: 0.016).

Discussion. Vitamin K deficiency is an important cause of acquired bleeding diathesis in neonates and infants and its deficiency does not develop in healthy infants receiving a normal diet. However, in the presence of diarrhea and/or antibiotic usage leading to suppression of intestinal bacteria, and

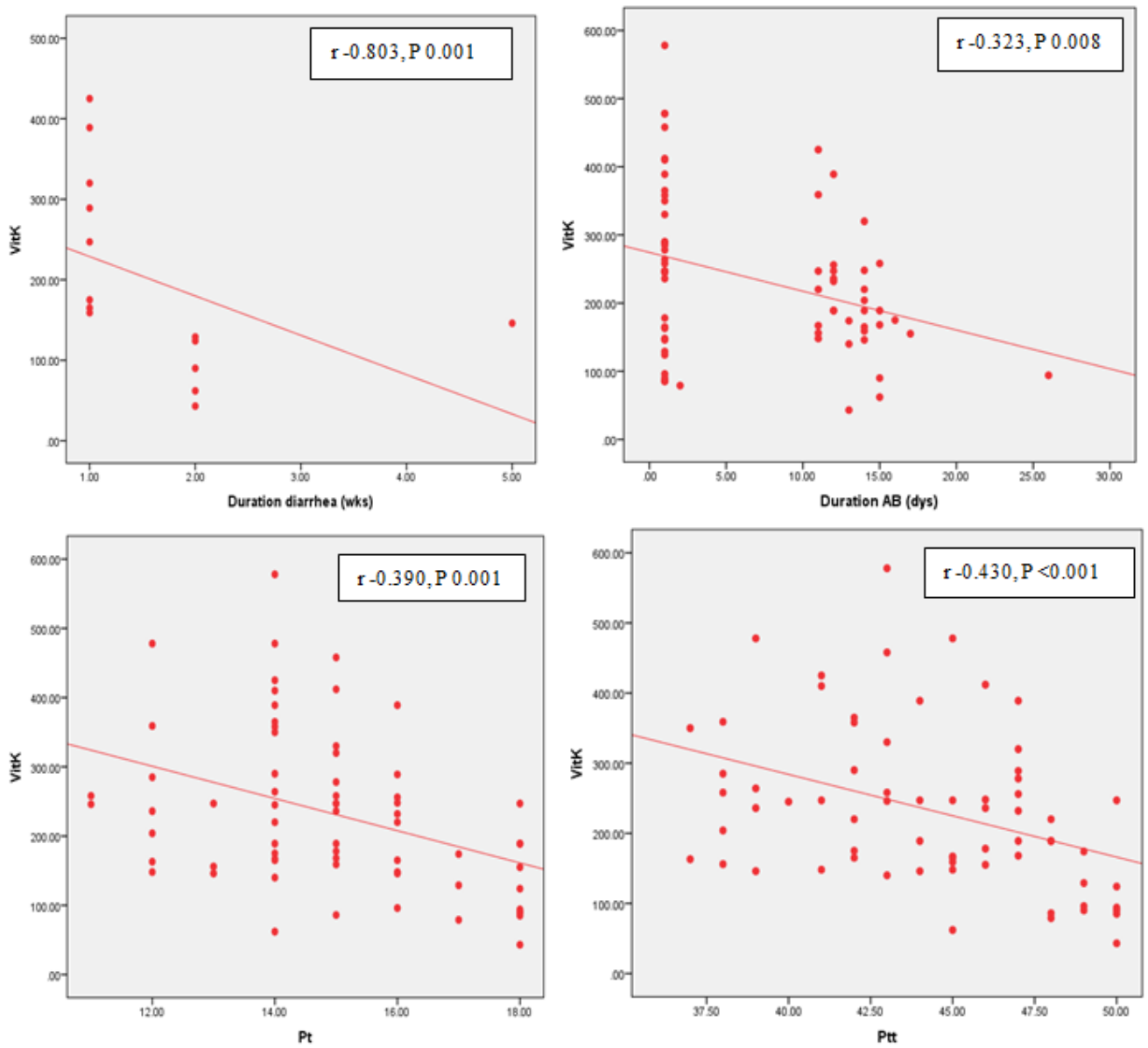

Figure 2. Correlation between vitamin K1 level and different studied variables in patients who received antibiotics therapy for ≥ 10 days.

consequently VKD may occur.[9] A systematic review reported that the median burden of late VKD bleeding was 35 per 100 000 live births in infants who had not received prophylaxis at birth; the burden was much higher in low- and middle-income countries as compared with high-income countries.[10]

The studied population in the present study was infants aged from 2 to 24 weeks; we were guided by the fact that more than 50% of the patients with low serum VK1 level was in the age group from one month to one year.[11] In addition, late HDN usually occurs between 2-12 weeks and manifests after the second week of life[12] up to 6 months of age.[13] Other causes of bleeding tendency like liver disease and thrombocytopenia or congenital clotting factors deficiency were ruled out by the simple investigations (liver function tests and platelet count). In addition, patients did not receive any dosages of VK in the preceding weeks to the study, and most parents of infants included did not recall if their infants received VK prophylaxis at birth.

It is important to highlight that VK occurred in two biologically active forms: phylloquinone (VK1), and menaquinones (VK2). The VK1, the type of plant origin, is predominantly transported to the liver and mediates in the maturation of several blood coagulation factors.[14] The VK2 is synthesized by bacteria in human and animal intestine.[15] It is the form almost exclusively stored in the liver in the neonates,[16] and it has a more widespread tissue distribution and is thus more specifically involved in the carboxylation of matrix Gla protein (MGP).[17] Vitamin K circulates in the human bloodstream at very low levels

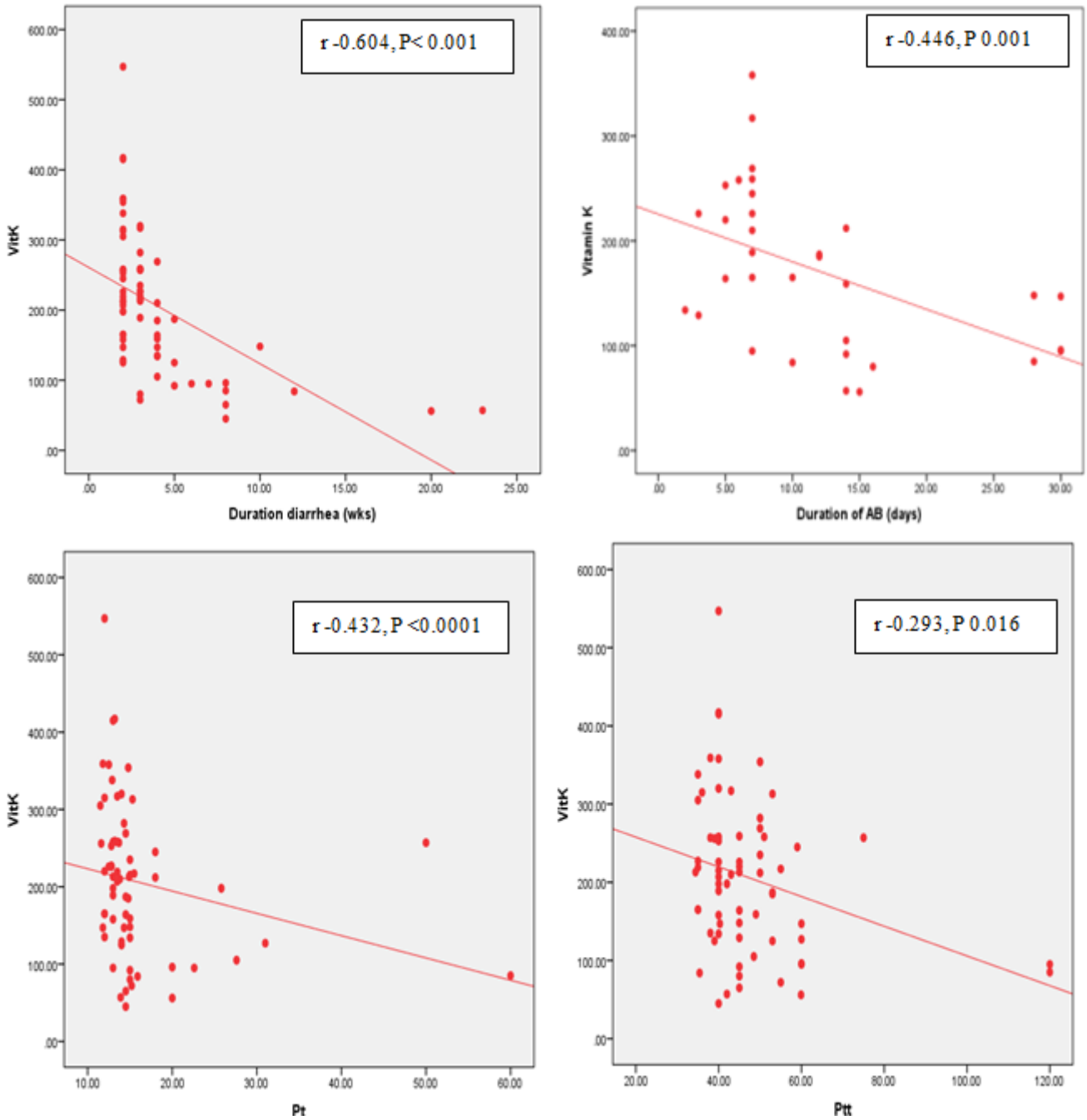

Figure 3. Correlation between vitamin K level and different studied variables in patients had diarrhoea ≥14 days.

because of a weak intake in the diet.[18] It could be detected in human plasma using high-performance liquid chromatography-tandem mass spectrometry.[19] In the current study, we measure VK1 directly using the ready available ELISA technique. This method is superior to measuring proteins induced by vitamin K absence (PIVKA) and synthesized as a result of vitamin K deficiency, whose level is related to the severity of the deficiency.[20]

The impact of diarrheal illnesses in elaborating VKD was related to insufficient intake; failure of synthesis owing to a shift in the bacterial flora caused by diarrhea and/or administration of antibiotics and decreased absorption from the intestinal tract.[21] In agreement with other studies,[22-24] we found that persistent diarrhea impairs VK1 level, and by that adversely affecting the coagulation profile of those infants, especially in the age less than 24 weeks old. Deficiency of VK in infants with diarrhea is likely to be more frequent and more severe in developing countries because of malnutrition and lack of vitamin K supplementation at birth.[1] Discovering of VKD in children with persistent diarrhea especially in those who sought medical advice shows that physicians lack the notion that correlates persistent diarrhea and VKD bleeding. Consequently, it is

highly advisable to monitor coagulation profile (VK testing) to identify risk factors related to VKD of patients in which the diarrheal process is perpetuating.

Also, it is to be mentioned that none of our patients suffered from any bleeding incidents, or had any hemorrhagic episodes during the treatment or afterward except a three months old male who suffered from intracranial hemorrhage after 20 days of intractable watery diarrhea with 10-15 motions per day. He has no history of VK supplementation at birth and has no bleeding history previously. A previous Egyptian study found that not only VKD bleeding is more prevalent than in developed countries (21 in 158,608 live births), but also it was the most common cause of intracranial hemorrhage in that age group, occurs in 50% of VKD cases.[7]

The role of antibiotics in the pathogenesis of VKD is produced by inhibition of intestinal microorganisms with loss of healthy bowel flora, which synthesizes the vitamin.[25] In addition, cephalosporins containing side chains of N-methylthiotetrazole are inhibitors of hepatic vitamin K epoxide reductase and that a lower nutritional-vitamin K status predisposes to hypoprothrombinemia.[26] In our study, we concluded that infants receiving antibiotics ≥ more than ten days showed the most astounding occurrence of VKD and the longer the antibiotic course was, the more affected the VK1 level. In agreement with other studies,[27-28] we found that treatment with combination of cephalosporins and penicillins showed the highest frequency of VKD and led to abnormal coagulation parameters. Furthermore, we found a higher incidence of VKD in those who had diarrhea and on antibiotic therapy (94%) than those who had diarrhea and not on antibiotics therapy (70%), this is showing the fact that both diarrhea and prolonged antibiotic usage have a double impact on VK1 levels. This association is crucial the antibiotic misuse alone can cause diarrhea further affecting the vitamin K status of the affected infant.

Our study spots the light on some health problems. The abuse of antibiotics seems most incriminated in the Arab region, and although not sufficiently investigated, studies performed in Jordan[29-30] revealed that just less than one-half of all dispensed antibacterial drugs were without a prescription (46%), either via self-medication (23.2%) or pharmacist recommendation (23.1%). The high prevalence of antibiotics misused in our cohort may be explained by the availability of over the counter antibiotics along with the low price of some antimicrobials encourage their consumption leading to numerous cases of antibiotic abuse and the prescription of antimicrobials for treatment of viral infections and inappropriate usage regarding duration of therapy.

Furthermore, although VK is obligatory administration to all newborns in our country yet the efficiency of VK prophylaxis program cannot be reliably assessed because the majority of the parents did not recall the information and also birth records are not sufficient to verify whether VK prophylaxis has been administrated or not. Similarly, in Turkey VKDB is one of the most common causes of acquired hemostatic disorder in early infancy. Although VK is practiced routinely after every birth, the compliance of prophylactic measures does not seem to be satisfactory.[31] As a further measure of tomorrow, we vigorously emphasize that a national surveillance program should be initiated. An additional intramuscular dose or oral supplementation of vitamin K especially for exclusively breastfed infants may reduce this catastrophic problem in our country. Thus, care providers need to give accurate information to families regarding the risks and benefits of VK prophylaxis and should be registered, and home births should be monitored for VK prophylaxis. Vitamin K prophylaxis status, date, dose, and route of administration should be documented on vaccination cards as it is being done for hypothyroid screening in our country. An inter-professional approach for education can be useful in increasing acceptance of VK prophylaxis and decreasing the incidence of VKD.

Limitations. In the study cohort, the area under the curve was chosen to determine VKD. The underlying condition of persistent diarrhea was not mentioned which could affect the VKD status. Including the data of an infant with ICH. The sample size is relatively small to use the data to be generalized to a population-based study.

Conclusions. Vitamin K deficiency, a preventable cause of life-threatening bleeding, is still a major health problem in Egyptian infants, where high incidence of persistent diarrhea and misuse of antibiotics is prevalent. Large-scale study to assess the value of booster prophylactic VK might avoid

a preventable cause of life-threatening hemorrhage.

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