DISCUSSION

Vitamin K is an antihaemorrhagic factor needed for the γ carboxylation of prothrombin (factor II) and factors VII, IX and X in the liver. The consequences of vitamin K deficiency for haemostasis are an inability to synthesize functional molecules of factors II, VII, IX and X, resulting in a hypocoagulable state and a bleeding tendency that is individually unpredictable. Vitamin K deficiency bleeding (VKDB) in infants is a rare but serious worldwide problem with a high risk of mortality and permanent disability resulting from intracranial hemorrhage. [56, 64]

Inadequate colonic colonization with bacteria that synthesize vitamin K has long been presumed to be a significant cause of vitamin K deficiency in the breastfed newborn. However, more recent information concludes that most colonic bacterial synthesized pools of vitamin K is not likely the form of vitamin K essential for hemostasis and may not even be available for absorption in any significant quantity. Bacterial-synthesized vitamin K is largely vitamin K_2 not the vitamin K_1 essential for clotting. [87]

The needs of the human fetus and neonate are largely met by phylloquinone. This can be evidenced that long-chain MKs make up the majority of adult reserves (90%) but they are absent or very low at birth and build up slowly over several weeks. This means that total liver stores of VK at birth are very low compared to adults. [56]

The highest concentrations of VK in breast milk are provided by phylloquinone, followed by MK-4 and there are trace concentrations of some higher menaquinones (MKs 6–8). Average concentrations of phylloquinone in colostrums and mature human milk are now known to be about 2 μg/l and 1 μg/l, respectively. Concentrations of VK in cord blood are extremely low compared to those in adults. There is no accepted reference range, but best estimates suggest that cord concentrations are generally <50 ng/l. [56]

It is generally agreed that the vitamin K coagulation status of the healthy newborn infant is brittle. This conclusion is based on both tissue analyses of K vitamins and on sensitive functional assays such as PIVKA-II that can detect undercarboxylated species of factor II well before there are any changes in global coagulation assays such as the prothrombin time. [45]

Analysis of dietary intakes of vitamin K in a subgroup of 106 mothers showed that those mothers who had phylloquinone intakes below the adequate intake(AI) for pregnancy (<90 μg/d) had a higher prevalence of functional subclinical vitamin K deficiency as measured by a detectable PIVKA-II than mothers with adequate intakes. [88, 89]

The current study was designed to evaluate vitamin k level in breast fed and formula fed infants aged 2 month ± 1 week. PT and PA were also measured as potential indirect markers of vitamin k level.

Traditional screening tests for VK deficiency are based on global coagulation assays such as the prothrombin time (PT) and activity (PA). Thus while the PT is appropriate for diagnosis of overt VK deficiency, it is not useful for picking up subclinical deficiency because PT becomes prolonged only when the prothrombin concentration drops below about 50% of normal. The most useful confirmatory test is to show that the PT can be restored by VK administration. [56]

The most useful markers for assessment of neonatal vitamin K status are concentrations of vitamin K1 and PIVKA-II. Circulating vitamin K levels directly reflect its storage, intake, and transport. (76)

When vitamin K supply is insufficient, abnormal, functionally defective molecules of VK-coagulation factors are released into the bloodstream. These molecules comprise a heterogeneous spectrum of undercarboxylated molecules. The collective abbreviation for these undercarboxylated species is PIVKA (Proteins Induced by Vitamin K Absence or Antagonism).The development of assays to detect undercarboxylated prothrombin has proved to be extremely useful as a functional marker of subclinical VK deficiency in infants. (56)

Studying vitamin k level, the present work reported high prevalence of vitamin k deficiency among studied infants if compared to normal serum level of vitamin k which ranges from 200 to 1000 ng/l. (79)

In our study, the mean serum vitamin k levels were (125.62 ± 69.63 ng/l) among breast fed infants and (134.29 ± 59.27ng/l) among formula fed infants. No statistical significance was found between both groups.

Shearer et al., who studied vitamin k level and measured phylloquinone intakes in a North American cohort of exclusively breast-fed and formula fed infants, found that phylloquinone contents of milk formulae are typically 50-fold higher than human milk, providing average daily intakes of 50 µg of phylloquinone. This huge disparity in intakes was mirrored in plasma phylloquinone with concentrations ranging from (130–240 ng/l) in breast-fed infants and from (4400–6000 ng/l) in formula-fed infants. (45)

Widdershoven et al., studied breast fed infants and bottle fed infants not receiving vitamin k prophylaxis at birth and the results showed that plasma concentration of vitamin k among bottle fed were significantly higher than breast fed ones. These results are in contrast with findings from our study as well. (90)

There were no changes concerning the levels of prothrombin time and prothrombin activity among breast fed and formula fed infants in our present study. The mean prothrombin time was 12.28 ± 0.72 seconds among breast fed infants while it was 12.47 ± 0.82 seconds among formula fed infants.

Also the mean prothrombin activity was 90.16% ± 5.81 among breast fed infants and 87.88% ± 13.04 among formula fed ones. Statistically, no significant differences were shown between the two groups.

Nutritional lack of vitamin K, combined with pathological factors, results in a potentially life-threatening bleeding disorder known as vitamin K deficiency bleeding (VKDB). So many countries protect against VKDB by routinely supplementing all infants with vitamin K after delivery. (77)

Despite the high percentage of vitamin k deficiency, the studied infants didn't show any specific clinical symptoms or signs of vitamin k deficiency.

This finding is in contrast with Ozdemir et al., who evaluated 120 cases of late vitamin k deficiency, admitted at Erciyes University Medical Hospital. Infants showed

clinical signs as bulging fontanels(70%); irritability(50%); convulsions(49%); bleeding and ecchymosis(47%); feeding intolerance, poor sucking, and vomiting(46%); diarrhea (34%); jaundice(11%), and pallor (9%). [91]

In our study we will discuss some issues: Are breast-fed infants vitamins K deficient? Is VKDB a disease of breast-fed infants? Vitamin K prophylaxis is it really necessary for healthy newborns? Do Breast-fed infants benefit from increased maternal vitamin K intakes during lactation?

During the first 6 mo of life, breast-fed infants have an increased susceptibility of developing vitamin K deficiency. Reasons for this are multifactorial, but prime physiologic factors are:

1) Inefficient placental transport of vitamin K antenatal. The factors that govern placental transport of vitamin k are poorly understood but k_1 does not easily cross the placenta and the maternal cord concentration gradient is within the range of 20:1 to 40:1
2) Low concentrations of K_1 in human milk leading to daily intakes in breast-fed neonates of about 1–2 μg/d compared with 80–120 μg/d in adults.
3) Neonatal concentrations of the hepatic enzymes glutamyl carboxylase (GGCX) and vitamin K1 2,3-epoxide reductase (VKOR) may also be suboptimal thus preventing efficient recycling of vitamin K. This is supported by the known immaturity of other hepatic enzyme systems in this population.
4) Reduced production of vitamin K because of immature or altered gut flora. [77]

Late onset VKDB was first reported in 1977. It mainly occurs in breastfed infants but ¾ of cases have an underlying liver disorder or malabsorption syndrome, rather than insufficient dietary intake of vitamin K. [92]

This was evidenced by Schulte et al., who studied five cases of late VKDB occurring at a single tertiary care children's hospital between February and September 2013 where two infants had underlying hepatobiliary disease and the rest suffered of sepsis and failure to thrive.[93]

Shearer et al., studied Vitamin K deficiency bleeding (VKDB) in early infancy and stated that apart from breast feeding, the only other consistent factor is the growing evidence of an association of late VKDB with hepatobiliary dysfunction, which leads to an impaired secretion of bile salts and malabsorption of VK. Such high dependence of the intestinal absorption of VK on bile salts that any reduction in their production and/or luminal secretion will result in a degree of VK malabsorption. [56]

Van Hasselt, studied Vitamin K deficiency bleeding among Dutch and Danish infants where VKD was evident in all (30 of 30) Dutch breastfed infants but with underlying hepatbiliary problems and biliary atresia. [57]

On the contrary McNinch, who studied VKDB in united kingdom in the late 1980s found that 24/27 of the affected babies had been solely breast fed and 6/27 babies were found, after presenting with bleeding, to have unsuspected underlying liver disease. Screening for prolonged jaundice or failure to thrive might have allowed earlier detection of the liver diseases and treatment before bleeding occurred. [94]

Without any vitamin K prophylaxis the incidence of late VKDB per 100 000 births has been estimated to be 4.4 in the UK, 7.2 in Germany and as high as 72 in Thailand. VKDB represents a significant public health problem worldwide, it is mainly a problem in babies who are breastfed and have disorders of intestinal absorption and liver disease. One of the main reasons for this is because breastfed infants' vitamin K intake is approximately 1 µg/days where as formula fed babies typically receive 50 µg daily. However, there is agreement that both bottle and breastfed babies need extra vitamin K to prevent VKDB.[95,96]

In this study, maternal vitamin K supplementation during lactation reflects significantly on vitamin K level in breastfed infant. Infants of lactating mothers who received vitamin K show higher level of mean vitamin K than those of lactating mothers who didn't receive vitamin K (176.93 ± 68.23 vs. 76.54 ± 10.07). This difference shows statistical significance.

These results are in agreement with those reported by Greer et al., who studied improving the vitamin k status of breastfeeding infants with maternal vitamin k supplements. [97]

The mean prothrombin time was 12.19 ± 0.58 seconds among breast fed infants whose mothers were taking vitamin k supplementation during lactation while it was 12.37 ± 0.83 seconds among those who did not take any supplements. Also the mean prothrombin activity was 90.55 ± 4.71 and 89.78 ± 6.79 respectively. Statistically, no significant differences were shown between the two groups.These results are also in agreement with Greer et al., who studied improving the vitamin k status of breastfeeding infants with maternal vitamin k supplements where he found that PTs and PAs did not differ at any time point during the study. [97]

Interestingly, the current study showed a statistically significant relation between female sex of the infants and the mean serum vitamin K level especially among formula fed ones where mean serum vitamin k level was (174.69 ±72.07 in females vs. 104.77±17.24 ng/L in males). This difference is statistically significant. Thus formula fed males are predisposed to VKDB more than females. Ozdemir et al., studied late vitamin K deficiency bleeding among infants admitted in the pediatric ward of Erciyes University, reported that 83(69%) were males and 37(31%) were females among 120 infants who fulfilled the diagnostic criteria of late VKDB. [91] IJland et al., studied the incidence of late vitamin K deficiency bleeding in newborns in the Netherlands where he found that the boy: girl ratio was 2.5:1. Why late VKDB occurs more in boys than girls is unknown. [98]

Our study revealed also positive correlation between the birth weight of infants and serum level of vitamin k, these correlations are statistically significant. The results were in agreement to Chuansumrit et al., who studied the relationship to maternal phylloquinone intakes and delivery risk where the overall prevalence of vitamin K insufficiency were significantly more likely to occur in the 'high-risk' groups (p=0.006) such as prematurity, stressful deliveries or intrauterine growth retardation, low birth weight although this was found only in a minority of babies. [88]

As for maternal age, this work showed a statistical significant correlation between age of the mothers and serum vitamin k levels among breast fed and formula fed infants. A reasonable clarification for this is that old aged mother are supposed to have previous

multiple pregnancies and knowledge about importance of vitamin k supplementation during the nursing and lactation period.

In agreement to this result, a study done by Chuansumrit et al., who studied prevalence of subclinical vitamin K deficiency in Thailand and the relationship to maternal phylloquinone intakes and delivery risk, stated that infants with younger mothers, were more likely to have low vitamin k levels than infants with older mothers, a finding which could not be explained and required replication and further study. (88)

There has been some debate over the years whether or not HDN is actually caused by vitamin K deficiency. Certainly, giving vitamin K does arrest bleeding in the majority of cases, but this does not mean that vitamin K deficiency causes HDN. There is also no consensus to what level of vitamin K in plasma protects against HDN. (92)

Most of the reported cases of late onset HDN has presented with problems which affect the baby's ability to absorb or utilize vitamin K. These include: hepatitis, cystic fibrosis, chronic diarrhea, bile duct atresia, alpha-1-antitrypsin deficiency, celiac disease of insufficient plasma transport capacity. Subclinical cytomegalovirus has also been implicated. Vitamin K-responsive bleeding syndrome has been well documented after antibiotic therapy, especially with cyclosporins. (99, 100)

Other factors which place the newborn at higher risk include trauma caused by a difficult birth (forceps, vacuum extraction, bruising), limited early breastfeeding (thereby limiting oral vitamin K intake from high fat colostrum) and early infant circumcision all fall into this category. The newborn clotting system seems ideally designed for the complexities present during the first week of life. (66)

Some practitioners consider delayed cord clamping to be three minutes after birth. The effect on the newborn is dramatic where the baby receives a greater amount of clotting factors than in immediate cord clamping. (101,102)

Studies comparing breast milk with formula and cow's milk have shown that breast milk is lower in vitamin K. Breast milk substitutes are heavily supplemented with vitamin K however such high levels are not necessary. (92)

Plasma vitamin K levels in newborns reach 300 times normal adult levels for oral and almost 9000 times for IM vitamin K, some research needs to be done on the effects this may have. Studies have shown that physiological levels of vitamin K maintain a careful balance between coagulation and anti-coagulation and we have no idea what the effects of upsetting that delicate balance would be. (92)

Allowing access to the breast in the early days after birth is important, due to the higher levels of vitamin K in colostrum. Babies who have been fed within their first 24 hours have significantly better coagulation times than babies not fed until after 24 hours.(92)

When breastfeeding is initiated, the newborn initially receives only small amounts of colostrum. This reduced intake volume leads to slight physiological dehydration in the newborn, increasing blood viscosity even further for the newborn's first three to five days of life. While receiving this colostrum, the newborn also receives a small supplement of vitamin K. (103)

As the mother's milk comes in, dehydration slowly resolves while bacteria colonize the newborn's gut. Over the first week of life, the increase in vitamin K corresponds to the increase in the newborn's clotting factors. Depending on the mother's diet and supplements, varying amounts of vitamin K is contained in the mother's milk fat as well[97,104] during this same period, the newborn learns to thermoregulate. Heat loss through the classic four methods (evaporation, radiation, convection and conduction) can drop a newborn's temperature rapidly. Research has been done in adults, while being in normal room-temperature moving air, older adults experienced mild surface (skin) cooling that reduced core body temperature only 0.7° F while increasing the fraction of blood plasma containing platelets 15% and increasing blood viscosity. This rise in platelets was not immediate; most of it occurred after the first hour of cooling. [105,106]

The authors postulate that "increases in platelets, red cells and viscosity associated with normal thermoregulatory adjustments to mild surface cooling provide a probable explanation for rapid increases in coronary and cerebral thrombosis in cold weather so a physiologically born neonate who is learning to thermoregulate already having increased blood viscosity and platelets will be at risk for thrombosis if he had a full share of clotting factors. [105,106]

In clotting, vitamin K is required to activate prothrombin, leading to the blood's ability to form fibrin and use platelets to form clots. Without the necessary vitamin K and fibrin, clots would be unable to form no matter how many platelets were available in the blood. [106]

The amount of clotting factors in the newborn allows the body to deal with a slight injury from birth and prevents an embolus from forming in small blood vessels preventing thrombosis. By the time a breastfeeding newborn's blood viscosity returns to normal, levels of vitamin K would be increased gradually without supplementation at birth. [106]

Timing is everything. The neonate clotting system seems to be ideally designed for newborns' specific needs and low levels of vitamin k seems to be physiological at birth.[106]

SUMMARY

VKDB, formerly known as hemorrhagic disease of the newborn, is a bleeding disorder caused by low levels of vitamin K–dependent clotting factors. Although vitamin K deficiency can occur in older children and adults, it is most common in newborns who have limited stores of vitamin K and immature gastrointestinal tracts. Placental transfer of vitamin K is low, and the serum levels of vitamin K–dependent factors have been found to be as low as 50% those of adults. Diagnosis of VKDB can be made in infants younger than 6 months who have spontaneous bleeding, bruising, or intracranial hemorrhage with a prolonged clotting time but with a normal or elevated platelet count

The current study was designed to evaluate vitamin k level in breast fed and formula fed infants.

The study was conducted on ninety infants' aged 2 months ± 1week attending the outpatient clinics in Alexandria University Children's Hospital.

All infants were subjected to complete history taking, clinical examination and laboratory investigations (PT, PA, and Vitamin k).

Statistical analysis of data obtained from the present study revealed the following results:

Studying vitamin k level, the present work reported high prevalence of vitamin k deficiency among studied infants with a mean serum vitamin k level of (125.62 ± 69.63 ng/l) among breast fed infants and (134.29 ± 59.27ng/l) among formula fed infants.

There was significant relation between vitamin k level of breast fed infants and maternal vitamin k supplementation during lactation.($P<0.001$)

There were no changes concerning the levels of prothrombin time and prothrombin activity among breast fed and formula fed infants in our present study despite low levels of vitamin k.

There was a positive correlation between the birth weight of infants and serum level of vitamin k, these correlations are statistically significant. ($r = 0.450, 0.485$)

This work showed a statistical significant relation ($P<0.001$) between mothers aged (above 31 years) and serum vitamin k level among breast fed infants

CONCLUSIONS

From this study it was concluded that:

- No significant differences were found between vitamin k levels among formula fed infants and breast fed ones.
- There was significant relation between vitamin k level of breast fed infants and maternal vitamin k supplementation during lactation
- Breast feeding may not be responsible for late onset VKD as most of the reported cases of late onset VKDB have presented with problems which affect the baby's ability to absorb or utilize vitamin K.