

INTRODUCTION

Hemorrhagic disease of newborn (HDN) is one of the most common causes of acquired haemostatic disorder in early infancy (*Pooni et al., 2003*).

It is a form of bleeding that is caused by reduced activity of vitamin K dependant coagulation factors (II, VII, IX, X), has normal or even increased activity of vitamin K independent coagulation factors and responds to vitamin K (*Sutor, 2007*).

Newborns have only 20-50% of adult coagulation activity. Lack of vitamin K administration at birth, exclusive breast feeding, chronic or persistent diarrhea and prolonged use of antibiotics make them more prone to vitamin K deficiency bleeding (*Pooni et al., 2003*).

It is categorized as early, classical and late depending on the time of onset. Late HDN usually occurs between 2-12 weeks. Rare cases occur also after week 15; therefore the upper age limit should be 6 months and not 3 months (*Sutor, 2007*).

The common manifestation of late HDN reported are evidence of intracranial hemorrhage, deep ecchymosis, bleeding from gastrointestinal tract and/or bleeding from mucus membrane, skin punctures or surgical incisions (*Bor et al., 2000*).

Important issues to be considered in prevention of VKDB are administration of vitamin K prophylaxis to all newborns, alertness about the symptoms of any disease that

would predispose to bleeding (prolonged jaundice, growth retardation and chronic diarrhea) and any warning bleeds (nose, umbilicus and skin) and consideration of repeated vitamin K doses especially if hepatic or intestinal disease is present (*Cekinmez et al., 2008*).

A study done on Egyptian infants revealed higher prevalence of late onset vitamin K deficiency bleeding than the international rate, the serum level of vitamin K was thought to be responsible, with the two main risk factors being the misuse of antibiotics and persistent diarrhea (*Elalfy et al., 2014*).

Newborn babies are at particular risk of vitamin K deficiency, as placental transfer is limited and human milk is a poor source. A minimal amount of vitamin K passes through the placenta, and a negligible amount is also found in breast milk (*Vermer et al., 2000*).

Deficiency of vitamin K in infants with diarrhea is probably more frequent and more severe in developing countries because of the increased likelihood of malnutrition and lack of vitamin K supplementation at birth (*Pooni et al., 2003*).

Children with acute and intractable diarrhea have been shown to have increased prothrombin time (PT) and higher rates of clinically significant hemorrhage (*Kumar et al., 2001*).

These symptoms may be related to inadequate intake, insufficient absorption and/or decreased synthesis due to changes in gut flora (*Firkin et al., 1989*).

AIM OF THE WORK

We are a developing country where there are a lot of mistakes from healthcare givers regarding management of diarrheal illness and early detection of vitamin K bleeding disorders, consequently our research aiming to:

- 1- Provide a proof that persistent diarrhea is an important risk factor for VKDB
- 2- Provide proof for importance of vitamin K supplementation for certain population of children with persistent diarrhea more than two weeks.

VITAMIN K AND ITS ROLE IN COAGULATION

Introduction

Vitamin K was first discovered in the early 1930s by the Danish biochemist Henrik Dam who observed while studying cholesterol metabolism in chickens that chicks fed with a diet free of sterols and low in fat tended to develop subcutaneous and intramuscular haemorrhages (*Henrik, 1935*).

Further studies on different foods led to the discovery of an "anti-haemorrhagic factor", which was designated vitamin K (with the "K" standing for "Koagulations-Vitamin") given that it was essential for normal haemostasis (*Dam, 1929*).

Vitamin K is synthesized by intestinal bacteria. This fat-soluble vitamin plays an important role in the synthesis of factors II, VII, IX and X in the liver. Vitamin K present in plants as phylloquinone and produced by bacteria as menaquinone. It is acting as a cofactor for γ -glutamyl carboxylase. This enzyme is responsible for post-translational modification of some glutamate side chains to γ -carboxyglutamate in hepatic and extra-hepatic proteins involved in blood coagulation and preventing cartilage and vasculature calcification (*Shearer, 1992*).

Vitamin K differs from other fat-soluble vitamins (A, D, and E) in that it functions as a cofactor for a single microsomal enzyme, namely γ -carboxyglutamyl carboxylase (GGCX). GGCX is needed to catalyze a reaction in which

specific peptide-bound glutamate residues found in certain specialized proteins are converted to γ -carboxyglutamate (Gla) as illustrated in Figure (1). This posttranslational protein modification, that was first discovered in 1974 (*Stenflo et al., 1974*) is the only firmly established biochemical function of vitamin K. The resultant vitamin K-dependent (VKD) proteins, or Gla proteins, are diverse in both structure and function and are found in many cell and tissue types. It is this very diversity of Gla proteins that makes vitamin K a truly multi-functional vitamin (*Booth, 2009*).

The central role, that vitamin K plays in homeostasis, is the only health role for vitamin K that is supported by incontrovertible evidence. The functions of many Gla proteins remain uncertain, but are suspected to play roles in processes as diverse as bone and cardiovascular mineralization, vascular hemostasis, energy metabolism, immune response, brain metabolism, and in cellular growth, survival, and signaling (*Booth et al., 2013*).

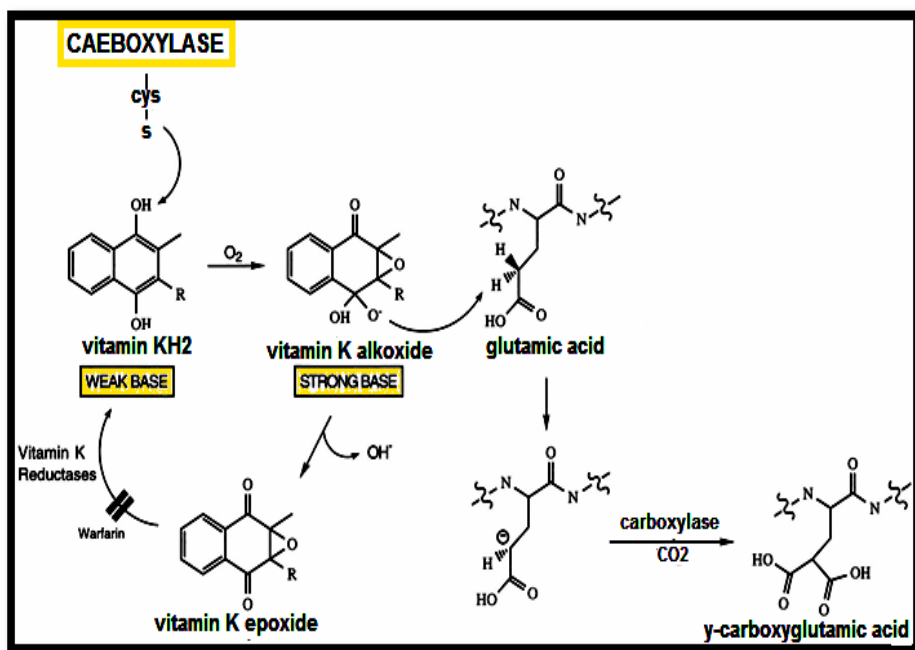


Figure (1): Biosynthetic pathway for vitamin K-dependent production of γ -carboxyglutamic acid

Vitamin K also differs from other fat-soluble vitamins in that there are naturally occurring and synthetic antagonists that block the synthesis of fully γ -carboxylated Gla proteins. Apart from their well-known use as clinical anticoagulants, coumarin vitamin K antagonists (VKAs) offer a powerful laboratory tool for investigating the metabolism of vitamin K, and the roles of Gla proteins in putative physiological processes. They also pose interesting questions as to whether they impair the non-coagulation functions of vitamin K and hence the health of people taking them (*Shearer and Newman, 2008*).

Structure and function of vitamin K:

Vitamin K is the common denominator of several molecular forms, all sharing a 2-methyl-1,4-naphthoquinone ring but differing regarding the structures of the side chain at the 3-position. Chemical structures of vitamin K compounds are illustrated in Figure 2 (*Shearer, 1995*).

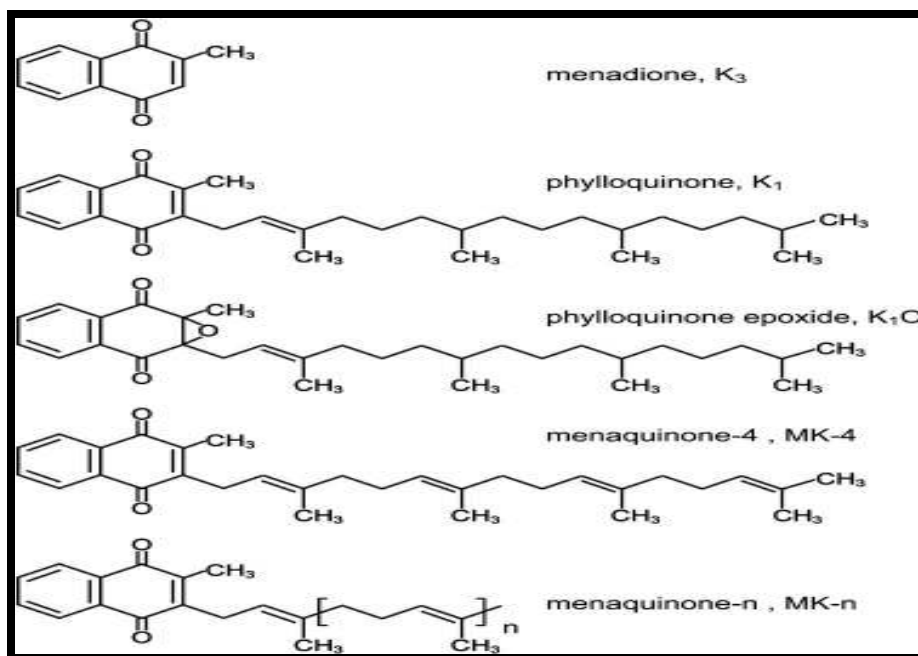


Figure (2): Chemical structures of vitamin K compounds

Phylloquinone (or vitamin K₁) is the only important form from plant origin that has a phytyl side chain. The group of menaquinones (or vitamin K₂) differs in the number of isoprenyl units in the side chain and is synthesized by bacteria in human and animal intestine. Finally, menadion (or vitamin K₃) is a synthetic and water-soluble vitamin K without a side chain

(*Shearer, 1995*). Use of menadion or vitamin K3 has been abandoned after reports showing that its use in high doses was associated with haemolytic anaemia, indirect hyperbilirubinemia and kernicterus (*Hey, 2003*).

Vitamin K acts as a cofactor for γ -glutamyl carboxylase (GGCX), serving as an electron donor for the posttranslational conversion of protein-bound glutamate into γ -carboxyglutamate. During this process, it is oxidized to vitamin K2, 3-epoxide. Gla residues are calcium binding groups which are essential for the biological activity of the proteins in which they are found. Gla containing proteins are the vitamin K-dependent coagulation factors II, VII, IX and X, protein C, protein S, protein Z, osteocalcin, and others. They are found in a variety of tissues. The function of some of them is still unknown (*Nantel and Tontisirin, 2002*).

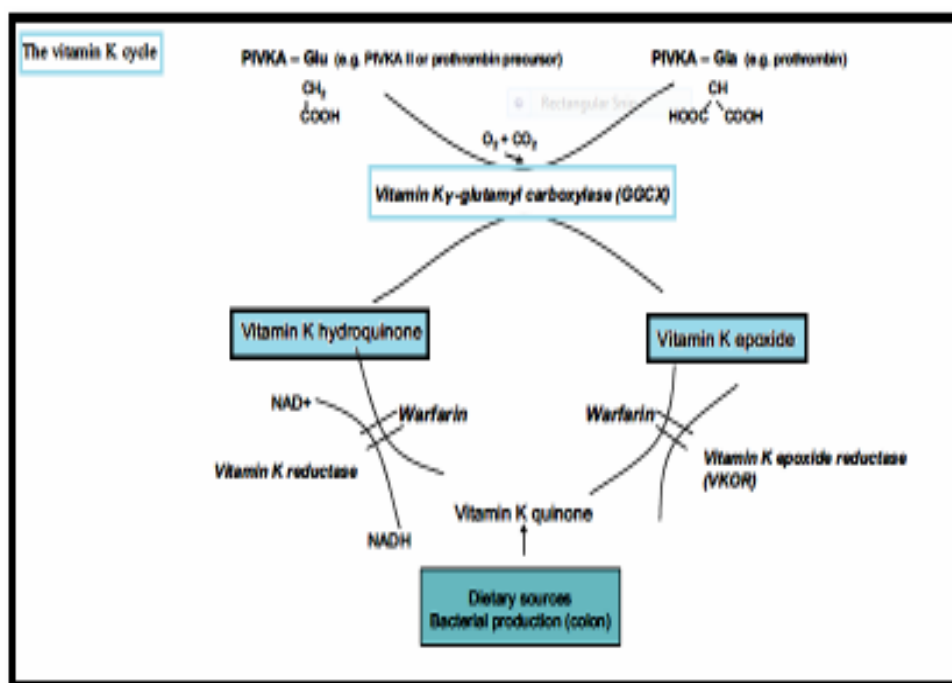


Figure (3): Vitamin K cycle proteins induced by vitamin K absence (PIVKA)

Vitamin K deficiency leads to the synthesis of under carboxylated proteins unable to bind calcium and hence inactive. These under carboxylated forms of vitamin K-dependent coagulation proteins (proteins induced by vitamin K absence (PIVKA) are released from the liver into the blood, where they can be measured. Their level increases with the severity of the deficiency. PIVKAII or under carboxylated prothrombin is a marker of subclinical vitamin K deficiency and becomes measurable before the development of abnormal coagulation tests (*Widdershoven et al., 1987*).

Vitamin K-epoxide is recycled to vitamin K by vitamin K-epoxide reductase (VKOR). This recycling process, as illustrated in Figure (3), is inhibited by coumarin and warfarin, explaining their anticoagulant activity which can be antagonized by high doses of vitamin K (*Oldenburg et al., 2008*).

Autosomal recessive vitamin K dependent clotting factor deficiencies (VKCFD) have been described either caused by a mutation in γ -glutamylcarboxylase (VKCFD1) or in vitamin K-epoxide reductase (VKCFD2). Both coumarin sensitivity and coumarin resistance have been linked to mutant alleles of these enzymes (*Shearer, 1992*).

Requirements and sources of vitamin K

The recommended dietary intake of vitamin K is 1 ug/kg/day (*Natel and Tontisirin, 2002*). This means a daily requirement for infants of 5–10 ug/day, for children of 15–30 ug/day, for adolescents of 55–65 ug/day, for adult women of 90 ug/day and for adult males of 120 ug/day (*National Academy of Science, 2001*) Although these intakes are sufficient to maintain normal plasma prothrombin levels in healthy subjects, it has been suggested that they may be suboptimal for adult bone health (*Cashman, 2005*).

Phylloquinone (vitamin K1) is present primarily in green leafy vegetables and is the main dietary form of vitamin K (*National academy press, 2001*). Menaquinones, which are predominantly of bacterial origin, are present in modest amounts in various animal based and fermented foods (*Elder et*

al., 2006). Almost all menaquinones, in particular the long chain menaquinones, are also produced by bacteria in the human gut (*Suttie et al., 2010*). MK-4 is a unique in that it is produced by the body from phylloquinone via conversion process, which does not involve bacterial origin (*Suttie et al., 2014*). Most of the vitamin K directly measured in plasma is phylloquinone, whereas more than 90% of the human liver content of vitamin K consists of menaquinone (*Shearer, 1995*).

Since the 1980s, the availability of specific and sensitive assays based on high performance liquid chromatography has enabled reliable measurement of the phylloquinone content of foods, including human milk (*Booth et al., 1998*).

Dietary sources

The best sources of vitamin K are green leafy vegetables, certain legumes and some vegetable oils such as rapeseed, soybean and olive oils. Other vegetable oils such as corn, peanut, sunflower and safflower oil have much lower phylloquinone content. Human milk is a poor source of vitamin K, containing 1– 4 ug/l, with high intra- and interindividual variability and the average concentrations near the lower end of this range (*Nantel and Tontisirin, 2002*).

A small placebo-controlled trial has shown that supplementing lactating mothers with high-dose vitamin K (5 mg/day) increases the level in their breast milk and is associated with lower PIVKAI levels in their infants (*Greer et al., 1997*).

Infant formulas are fortified and contain 50 ug/l. Little is known about the bioavailability of phyloquinone from different food sources. The phyloquinone content in different dietary sources is illustrated in Table1 (*National Nutrient Database for Standard Reference, 2008*), and vitamin K content is illustrated in Table 2. Bioavailability from the same food source (e.g. spinach) is better if ingested together with fat (*Booth and Suttie, 1998*).

Table (1): The phyloquinone content of different food

Phyloquinone content (µg/100 g edible portion)	
800–900	Kale, parsley
600–700	Collards
400–500	Spinach
100–200	Sprouts, broccoli, onions, lettuce, cabbage, endives
50–100	Asparagus, olive oil
20–50	Peas, kiwi, blackberries, nuts, soy oil
10–20	Carrot, cucumber, grapes
5–10	Tomato, whole bread
1–5	Potato, eggplant, apricot, peach, apple, pear, strawberries, white bread, meat, egg, cheese
<1	Rice, fish, milk

Table (2): Vitamin k content of diary and egg products

Table 2. Vitamin K content of dairy and egg products (mcg/100g)										
	K1	mk-4	mk-5	mk-6	mk-7	mk-8	mk-9	mk-10	Country	Reference
1% milk	-	0.4	-	-	-	-	-	-	U.S	(Elder 2006)
1% milk	-	-	-	-	-	-	-	-	Netherlands	(Schurgers 2000)
2% milk	-	-	-	-	-	0.1	-	-	Netherlands	(Schurgers 2000)
2% milk	0.2	1	-	-	-	-	-	-	U.S	(Elder 2006)
whole milk	0.3	1	-	-	-	-	-	-	U.S	(Elder 2006)
Whole milk	1	2	-	-	-	-	-	-	Japan	(Kamao 2007)
Whole milk	1	1	0.1	-	-	-	-	-	Netherlands	(Schurgers 2000)
Butter	2	21	-	-	-	-	-	-	Japan	(Kamao 2007)
Butter	15	15	-	-	-	-	-	-	Netherlands	(Schurgers 2000)
Ice cream	2	3	-	-	-	-	-	-	U.S	(Elder 2006)
Cream	1	8	-	-	-	-	-	-	Japan	(Kamao 2007)
Cream, whipping	5	5	-	-	-	-	-	-	Netherlands	(Schurgers 2000)
Buttermilk	-	0.2	0.1	0.1	0.1	1	1	-	Netherlands	(Schurgers 2000)
Yogurt, whole	0.4	1	0.1	-	-	1	-	-	Netherlands	(Schurgers 2000)
Milk, soured										
whole	0.2	1	0.3	0.2	0.4	2	5	-	Finland	(Tikkanen 2000)
Yogurt, plain	0.2	0.4	0.1	-	-	-	-	-	Finland	(Tikkanen 2000)
Egg, white raw	-	0.4	-	-	-	-	-	-	U.S	(Elder 2006)
Egg, white raw	-	1	-	-	-	-	-	-	Japan	(Kamao 2007)
Egg white	-	1	-	-	-	-	-	-	Netherlands	(Schurgers 2000)
Egg, yolk raw	1	16	-	-	-	-	-	-	U.S	(Elder 2006)
Egg, yolk raw	7	64	-	-	-	-	-	-	Japan	(Kamao 2007)
Egg yolk	2	31	-	1	-	-	-	-	Netherlands	(Schurgers 2000)
Eggs, whole raw	0.3	6	-	-	-	-	-	-	U.S	(Elder 2006)
Egg, whole raw	1	7	-	-	-	-	-	-	Japan	(Kamao 2007)
Egg, whole fried	1	9	-	-	-	-	-	-	U.S	(Elder 2006)
Egg, whole boiled	0.4	7	-	-	-	-	-	-	U.S	(Elder 2006)

(-) not measured. () not detected. (K1) phylloquinone. (mk) menaquinone.

Non dietary sources

Controversy persists regarding the contribution of bacterial production of menaquinones in the intestine to the requirements of vitamin K. Menaquinone content of foods, with the exception of animal liver and some fermented products is negligible (*Booth, 2012*).

Despite the fact that more than 90% of vitamin K store in the liver consists of menaquinones, it is unclear what their relative contribution is to overall vitamin K status, and their absorption from the colon is probably very limited (*Suttie, 1995*).

Bacterial numbers and composition vary considerably along the human gastrointestinal tract. In the small intestine growth is limited by the rapid transit times and secretion of bile and pancreatic juice. In humans, by far the largest reservoir of bacteria occupies the large intestine where the majority of species are strict anaerobes. Quantitatively, the most important genera of intestinal flora are the bacteroids and bifidobacteria, which together can account for over half of the total anerobic bacterial population; they differ, however, in that only the bacteroids synthesize menaquinones (*Salminen et al., 1998*).

Placental transfer of vitamin K is limited, and phylloquinone levels in cord blood are very low, about 1/30 of the levels in maternal plasma. Liver reserve also is substantially lower than in adults, estimated at 1/5. Menaquinones are not present in the liver of infants before the age of 2 to 3 months (*Shearer, 1995*).