Vitamin K Insufficiency in an India Population: A Pilot Study

Sidney Stohs, Ashok DB Vaidya, Jayesh Sheth, Shashank Jadhav, Umakant Mahale, Dilip Mehta, Vladimir Badmaev

Submitted to: JMIR Public Health and Surveillance
on: July 13, 2021

Table of Contents

Vitamin K Insufficiency in an India Population: A Pilot Study

Sidney Stohs[1, 2] BSc, MS, PhD; Ashok DB Vaidya[3] BSc, MD; Jayesh Sheth[4] BA, DPhil; Shashank Jadhav[5] BA, MD; Umakant Mahale[5] BSc, DPhil; Dilip Mehta[5] BA, MD; Vladimir Badmaev[5] BA, MD

[1]Creighton University Medical Center Frisco US
[2]Kitsto Consulting LLC Frisco US
[3]Medical and Research Centre, Kasturba Health Society Mumbai IN
[4]Foundation for Research in Genetics and Endocrinology Ahmedabad, Gujarat IN
[5]Synergia Life Sciences Pvt. Ltd. Mumbai, Maharashtra IN

Corresponding Author:
Sidney Stohs BSc, MS, PhD
Creighton University Medical Center
2500 California Plaza
Frisco
US

Abstract

Background: The fat-soluble K vitamins K1 and K2 are well recognized and exert an essential biological role in the blood coagulation cascade. However, vitamin K is less known for its non-essential roles in participating in the growing family of vitamin K-dependent proteins that promote various functions of organs and systems in the body, sustaining health and preventing disease. These less well-known actions depend on the availability of vitamin K for non-essential multi-tasking functions. This fat-soluble vitamin is available predominantly through selective dietary intakes, and its presence is absent or very low in many diets. The lack of vitamin K for non-essential biological functions exemplifies vitamin/nutritional element insufficiency, which is different from a clinically apparent vitamin deficiency.

Objective: The current epidemiological study evaluated the nutritional status of vitamin K in a sample of the Indian population and its content in staple Indian foods.

Methods: Serum levels of vitamin K1 and vitamin K2 in the form of MK-7 were assessed by high-performance liquid chromatography (HPLC) coupled with a fluorescence detector in 209 patients with Type 2 diabetes and 50 healthy volunteers and in common, staple foods in India.

Results: The results indicate that in comparison to populations with high and low serum levels in various geographical regions, the sample of India population of apparently healthy individuals and Type 2 diabetes patients showed low (insufficient) levels of vitamin K2 (menaquinone-7). The staple, commonly consumed Indian foods that were tested in the study showed undetectable content of vitamin K2.

Conclusions: The general population could benefit from the consumption of vitamin K2 in the form of MK-7 supplements, with emphasis on patients with diabetes, elevated blood pressure, a harbinger of cardiovascular disease, and compromised immune systems. The results can be extrapolated world-wide. Clinical Trial: CTRI 2019/05/014246

(JMIR Preprints 13/07/2021:31941)
DOI: https://doi.org/10.2196/preprints.31941

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✓ **Please make my preprint PDF available to anyone at any time (recommended).**
Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.
No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✓ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**

Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v
Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http

Original Manuscript

07-08-21

VITAMIN K INSUFFICIENCY IN AN INDIA POPULATION: A PILOT STUDY

Rama Vaidya[1], Ashok DB Vaidya[2], Jayesh Sheth[3], Shashank Jadhav[4], Umakant Mahale[4], Dilip Mehta[4], Vladimir Badmaev[5], Sidney J. Stohs[6]

[1]Rama Vaidya, Director, Division of Endocrine and Metabolic Disorders, Medical and Research Centre, Kasturba Health Society, Mumbai, Maharashtra, India. vaidya.rama@gmail.com. Sthanakwasi Jain Aradhana Dham, 17, Khandubhai Desai Rd, Vile Parle West. Mumbai, India. Pin 400 056

[2]Ashok DB Vaidya, Research Director, Medical and Research Centre, Kasturba Health Society, Mumbai, Maharashtra, India. ashokdbv@gmail.com. Sthanakwasi Jain Aradhana Dham, 17, Khandubhai Desai Rd, Vile Parle West. Mumbai, Maharashtra, India. Pin 400 056

[3]Jayesh Sheth, Chairman at Foundation for Research in Genetics and Endocrinology, Ahmedabad, Gujarat, India. jayesh.sheth@frige.co.in. Street Address: FRIGE House, Jodhpur Village Road, Satellite, Ahmedabad, Gujarat, India. Pin 380 015

[4]Shashank Jadhav, Med. Director, Synergia Life Sciences Pvt. Ltd., Mumbai, Maharashtra, India. shashank@viridisbiopharma.com. Street Address**:** 6/312, Jogani Industrial Complex, V N Purav Marg, Chunabhatti. Mumbai, India. Pin 400 022

[4]Umakant Mahale, Manager, Analytical (R&D) Department, Synergia Life Sciences Pvt. Ltd., Mumbai, Maharashtra, India. umakant@viridisbiopharma.com. Street Address: 6/312, Jogani Industrial Complex, V N Purav Marg, Chunabhatti. Mumbai, India. Pin 400 022

[4]Dilip Mehta CEO, Synergia Life Sciences Pvt. Ltd., Mumbai, Maharashtra, India. dilipmehta39@hotmail.com. Street Address: 6/312, Jogani Industrial Complex, V N Purav Marg, Chunabhatti. Mumbai, India. Pin 400 022

[5]Vladimir Badmaev, Advisory board of Synergia Life Sciences Pvt. Ltd. Forest Hill Rd., Staten Island, NY 10314, USA; vebadmaev@attglobal.net. Tel./text: 347 439-6237

[6]School of Pharmacy and Health Professions, Creighton University, Omaha, Nebraska 68178 USA. sid.stohs9@gmail.com. Tel: 1-214-215-6655.

[*]Correspondence: S.J.Stohs, 7068 Maumee Valley Court, Frisco, TX 76036 USA. Tel: 1-214-215-6655. Email: sid.stohs9@gmail.com

Running title: VITAMIN K INSUFFICIENCY

ABSTRACT

Background: The fat-soluble K vitamins K1 and K2 are well recognized and exert an essential biological role in the blood coagulation cascade. However, vitamin K is less known for its non-essential roles in participating in the growing family of vitamin K-dependent proteins that promote various functions of organs and systems in the body, sustaining health and preventing disease. These less well-known actions depend on the availability of vitamin K for non-essential multi-tasking functions. This fat-soluble vitamin is available predominantly through selective dietary intakes, and its presence is absent or very low in many diets. The lack of vitamin K for non-essential biological functions exemplifies vitamin/ nutritional element insufficiency, which is different from a clinically apparent vitamin deficiency.

Objective: The current epidemiological study evaluated the nutritional status of vitamin K in a sample of the Indian population and its content in staple Indian foods.

Methods: Serum levels of vitamin K1 and vitamin K2 in the form of MK-7 were assessed by high-performance liquid chromatography (HPLC) coupled with a fluorescence detector in 209 patients with Type 2 diabetes and 50 healthy volunteers and in common, staple foods in India.

Results: The results indicate that in comparison to populations with high and low serum levels in various geographical regions, the sample of India population of apparently healthy individuals and Type 2 diabetes patients showed low (insufficient) levels of vitamin K2 (menaquinone-7). The staple, commonly consumed Indian foods that were tested in the study showed undetectable content of vitamin K2.

KEYWORDS: Phylloquinone, menaquinone-7, vitamin K1, vitamin K2, MK-7, insufficiency, deficiency, Indian population, diabetes, healthy people

INTRODUCTION

Clinically apparent nutritional deficiency diseases as scurvy (vitamin C), rickets (vitamin D), beriberi (vitamin B1) or pellagra (vitamin B3) differ from nutritional insufficiency, a relatively new finding. The effects of vitamin insufficiency for a number of vitamins are well known. However, the roles of vitamin K exemplified by the insufficiency of this vitamin are much less well known [1,2].

Nutritional insufficiency has been recently explained with a triage (sort and select) mechanism, and can be exemplified by the nutritional insufficiency of vitamin K. The term "triage" is used on the battlefield to prioritize treatments for the survival of the wounded. In a similar way, the human body selects micronutrients, nutrients and vitamins for an immediate need (survival) over long-term needs (sustaining life) by prioritizing nutrient use and borrowing nutrients from less critical depots in the body, always securing the emergency requirements.

In healthy people consuming a varied diet, the clinical deficiency of vitamin K is practically not encountered because the body prioritizes available vitamin for essential life-sustaining K-dependent proteins which include securing carboxylation and activation of the vitamin K-dependent blood coagulation proteins factors II (thrombin), VII, IX, and X. However, vitamin K may be insufficient for at least 18 vitamin K-dependent nonessential calcium-dependent proteins that are responsible for healthy cardiovascular, immune, skeletal, and neuro-muscular systems [1,3].

With the recent discovery of its many biological functions, vitamin K is a "multi-tasking" vitamin. In the long term, the insufficient status of this multi-tasking vitamin may prevent optimal functioning of K-dependent nonessential proteins and result in the development of chronic degenerative conditions, such as osteoporosis, cardiovascular disease, metabolic (diabetes) and neurodegenerative conditions that ultimately diminish the quality of life and shorten life span [1].

With the growing awareness of the importance of vitamin K nutritional status, the objective of this epidemiological study was to determine serum levels of vitamin K in healthy and diabetic men and women selected from an Indian population, and assess the average vitamin K content in the indigenous diet of India. The study evaluated two fat-soluble vitamins, namely, vitamin K1 or phylloquinone, commonly found in a diet of green vegetables and plant margarine; and K2 or menaquinone, specifically menaquinone-7 or MK-7, derived mainly from meat, liver, butter, egg yolk, fermentation foods (e.g., cheese, curd) and indigenous India dietary products including dosa, dhokla, and yogurt.

Besides food sources, vitamin K2 is also synthesized by the human gut microbiome, predominantly *Bacteroides* and *Veillonella* bacteria [4]. However, gut bacteria that generate menaquinones reside mostly in the large intestine where they are less bioavailable since their absorption occurs predominantly in the small intestine. Therefore, serum levels of menaquinones depend largely on dietary sources [4].

METHODS

The Department of Medicine, Kokan Hospital, Mumbai, India, selected the sample of the native population with the following admission criteria for the study:

Inclusion Criteria

1. Male and female healthy subjects 28 to 45 years old.
2. Body Mass Index (BMI) 18.5 and 24.9.
3. Male and female with Type 2 Diabetes mellitus ≥25 years old.
4. Duration of diabetes ≥ six months from the date of diagnosis.
5. Fasting plasma glucose levels ≥ 126.0 mg/dl.
6. Willing to sign the informed consent.

Exclusion Criteria

1. People suffering from systemic and chronic illness.
2.Subjects on corticosteroids and oral contraceptives.

3. People on antibiotics within last week.

4. Pregnant and lactating women.

5. Participation in clinical trials evaluating investigational pharmaceuticals or biologics within three months or devices within 30 days of admission to the study.

6. People who are on coumarin analogs or quinine hydrochloride.

7. A history of smoking, alcohol or substance abuse.

8. Subjects with Type 1 diabetes.

The Inter System BioMedica Ethics Committee (ISBEC), an independent ethics committee, approved the protocol of the study and registered the study with the Clinical Trial Registry of India (CTRI) ---- CTRI 2019/05/014246 for healthy and CTRI 2019/03/018278 for diabetic population, respectively, (www.ctri.nic.in the website and the WHO portal). The Department of Medicine, Kokan Hospital, Mumbai, India, approved the study protocol according to International Conference on Harmonization of Good Clinical Practice (ICH-GCP E6) Guidelines.

The participants agreed and signed the informed consent prior to admission to the study groups. The blood samples were collected from 209 patients with Type 2 diabetes mellitus and from 50 healthy volunteers. Baseline demographic and clinical data are presented in Table 1 for male and female subjects in each group.

Table 1. Baseline demographic and clinical data of the study population

	Study 1		**Study 2**	
	Type 2 diabetes mellitus patients		Healthy volunteers	
No. of volunteers	209		50	
	Male	Female	Male	Female
No. of patients (N)	100	109	25	25
Age (years)	50.3 ± 13.1	48.6 ± 14.0	41.6 ± 12.8	36.0 ± 5.4

BMI (kg/m^2)	25.4 ± 4.2	26.9 ± 5.4	23.8 ± 2.3	22.7 ± 1.9
HbA1c (%)	7.6 ± 1.7[a]	7.2 ± 1.7[a]	4.9 ± 0.4[b]	4.7 ± 0.4[b]
Systolic Blood Pressure (mm Hg)	128.6 ± 15.6	126.8 ± 16.7	114.6 ± 10.4	122.6 ± 12.9
Diastolic Blood Pressure (mm Hg)	89.8 ± 21.5	86.4 ± 22.9	80.0 ± 4.8	80.8 ± 6.8
Fasting Blood Sugar (mg/dL)	123.2 ± 36.9[a]	117.2 ± 47.3[a]	82.1 ± 7.7[b]	82.5 ± 8.2[b]
Post-Prandial Blood Sugar (mg/dL)	193.6 ± 66.0[a]	179.9 ± 83.1[a]	102.5 ± 7.1[b]	104.9 ± 7.0[b]

Each value is the mean ± standard deviation. Values with non-identical superscripts within rows are statistically significant ($p<0.05$).

Analytical Methods

The serum levels of vitamin K1 and vitamin K2 in the form of MK-7 were assessed by high-performance liquid chromatography (HPLC) coupled with a fluorescence detector. The vitamin K1 and MK-7 content in food were evaluated with the same HPLC analytical method. A reverse phase HPLC method [5-7] involving post column derivatization and fluorescent detection was used with some modifications. The method was validated as per the ICH guidelines [8]. Our laboratory participates with UK *Vitamin K External Quality Assurance Scheme* (KEQAS) for quality control.

Analytical standards K1, MK-7, the internal standard K2-6 and zinc dust (<10 µM) for post column reduction of vitamin K were purchased from Sigma Chemical Co. All the other chemicals used were Analytical Reagent grade and all solvents for HPLC were HPLC grade.

An HPLC (Shimadzu) system was used which was equipped with a degasser

(DGU_20A5R), pump (LC-20AD), auto-sampler (SIL-20AC HT), column oven (CTO-10 AS), and fluorescence detector (RF 20A). A reverse phase C18 column (Kinetex C18, 10 cm x 4.6 mm, 2.6 µ, 100°A) was employed with the column oven adjusted to 25°C. A post column (30 x 4 mm) assembly was inserted between the analytical column, which was packed with zinc dust for the post column reduction of vitamin K, and the detector. The mobile phase contained the following: 2 ml of zinc solution (0.136% zinc chloride, 0.04% sodium acetate, 30 µL acetic acid in 75% methanol) in 2 liters of methanol and filtered through 0.45 µm filter paper. Injected samples or standards were eluted at 1.2 ml/min isocratic flow rate and a 12-minute run time. Eluted peaks were monitored with the fluorescent detector set at excitation and emission wavelengths 248 nm and 430 nm, respectively.

Six-point calibration curves of vitamin K were established by spiking plasma samples with 0.16 ng/ml to 20 ng/ml concentrations of the vitamins. All samples were extracted as follows. To each plasma sample, 500 µl internal standard (vitamin K2-6, 20 ng/ml in methanol) was added followed by 3.5 ml of ethanol and 0.5 ml of sodium chloride (0.9 % in water). Each mixture was vortexed for 2 minutes and 10 ml hexane was added and the tubes were again vortexed. The hexane layers were separated by centrifugation, collected in separate tubes and evaporated under nitrogen. The residues were dissolved in 100 µl methanol and 50 µl of this solution was injected on the column. For all food samples, about 1000 mg of each test sample was placed in a 100 mL volumetric flask to which was added 10 mL tetrahydrofuran,1 mL of the internal standard and 70 ml of ethanol. The contents were sonicated for 1 hour and diluted to volume with ethanol. The samples were filtered, and aliquots of the filtrate were injected onto the column.

Statistical Methods

Data in this study were analyzed by using the unpaired *t*-test method. This method was used to determine statistical significance between two sets of data (p<0.05) using a computer software program. Statistical analyses were made between male and female subjects within and between patients with diabetes mellitus and healthy subjects.

RESULTS

The levels of menaquinone-7 (MK-7) and phylloquinone (K-1) in healthy and Type 2 diabetes mellitus populations are presented in Table 2. The differences in values of vitamins K1 and MK-7 in diabetic male subjects as well as male healthy subjects was statistically significant (P<0.05) while the differences between the two forms of vitamin K in these two groups of female subjects were not statistically significant. The vitamin K1 serum levels in the studied healthy and diabetic populations were within the normal range based on the physiological values of vitamin K-1in serum in an adult population, and ranged from 0.116 to 1.056 ng/ml [9]. However, the serum levels of vitamin K2 in the form of MK-7 in both healthy and diabetic sample of this population were found in the low range.

Table 2. Levels of Menaquinone-7 MK-7) and Phylloquinone (K-1) in healthy and Type 2 diabetes mellitus populations

Study Groups	Type 2 diabetes mellitus patients		Healthy volunteers	
No. of subjects (N)	M 100	F 109	M 25	F 25
Menaquinone-7 MK-7) (ng/ml)	0.41 ± 0.37	0.42 ± 0.49	0.31 ± 0.23	0.39 ± 0.20
Phylloquinone (K-1) (ng/ml)	0.55 ± 0.50	0.53 ± 0.46	0.70 ± 0.65	0.48 ± 0.39

Values expressed in Mean ± SD; SD- Standard Deviation, M-Males, F- Females

Table 3 provides data regarding the analysis of vitamins MK-7 and K-1 content (µg/100 g or µg/100 ml) in staple foods of India. As can be seen, vitamin MK-7 is absent or below the limits of detection from all these foods while the K-1 content varies widely, and will therefore result in wide variations in serum levels based on dietary intake.

Table 3. Staple foods in India evaluated for menaquinone-7 (MK-7) and phylloquinone (K-1) content (µg/100 g or µg/100 ml)

No.	Food Item	Description	Menaquinone-7	Phylloquinone
1	Dhokla	Fermented batter derived from rice and split chickpeas	ND	2.76 ± 0.17
2	Naan	Leavened with yeast or with bread starter, flatbread	ND	ND
3	Jalebi	Sweet snack made by deep-fried wheat flour	ND	ND
4	Idli	Savory cake made of fermented lentils and rice.	ND	1.67 ± 2.13
5	Handvo	A cake based on gram flour with vegetables and peanuts	ND	37.14 ± 3.02
6	Yogurt	A dairy product made by coagulating milk with any culinary acid e.g. lemon juice	ND	ND
7	Cheese	Processed Cheddar Cheese	ND	4.71 ± 0.68
8	Buttermilk	A fermented dairy drink after churning butter out of cultured cream	ND	0.51 ± 0.05
9	Butter	Made of pure milk Fat	ND	4.60 ± 0.82
10	Milk	Cow's milk	ND	0.97 ± 0.19
11	Charoli	Almond-flavored seeds of a bush, *Buchananialanzan.*	ND	2.85 ± 0.40

Four samples from each food were analysed and the mean values with standard deviations presented. ND-- Not Detected

It should be noted that the analytical laboratory involved in these studies participates in the UK *Vitamin K External Quality Assurance Scheme* (KEQAS) for quality control. To ensure accuracy of analytical procedures for vitamin K, on six occasions two serum and one standard vitamin K sample were received from KEQAS by the laboratory for the determination of vitamin K by the HPLC method. On all six occasions the method employed gave ‘Satisfactory’ results with a ‘Green’ certification being awarded to the laboratory. The target for results was ± 20% deviation from the all-laboratory trimmed mean representing the target concentrations for the unknown samples that were provided by KEQAS.

DISCUSSION

Studies have indicated that serum levels of vitamin K vary throughout the world and are largely dependent upon dietary intake [10]. For example, the serum levels of MK-7 in Japan have been reported to vary by region, varying from 5.26 ± 6.13 ng/ml in women in Eastern Japan (e.g., Kanto region, Tokyo) to 1.22 ± 1.85 ng/ml in women in Western Japan (e.g., Kasai region, Hiroshima) [10]. MK-7 levels have been reported as 0.37 ± 0.20 ng/ml in British women [10].

The recommendations for the daily intake of vitamin K have been inconsistent, in part due to the unquantified contribution of intestinal bacteria, with the levels of intake that may be adequate for blood clotting being insufficient for other functions of the vitamin [11]. A daily intake of 1 µg per kg body weight may be adequate for blood clotting [11]. The intake of vitamin K is estimated to be from 60 µg to 200 µg per day in a Western diet, of which phylloquinone (K-1) constitutes about 90%, versus 10% for menaquinones (vitamin K-2) [12].

Findings for MK-7 serum levels indicate wide variations in dietary MK-7 content due to different dietary staples with varying contents of the vitamin [10]. For example, the fermented beans known as natto are consumed regularly at breakfast in Eastern Japan and provide approximately 1,000 µg of MK-7 per 100 g of natto. However, consuming natto is an infrequent dietary practice in the Western part of Japan [10]. This epidemiological literature citation provides a possible explanation of higher MK-7 serum levels in women from Eastern than Western Japan, and may explain the lower cardiovascular and skeletal morbidity in the former group [10].

While the typical nutritional schedules in India may provide nutritional vitamin K sufficient to support the life-sustaining blood coagulation cascade, its nutritional content may be insufficient to fulfill the multi-tasking role of vitamin K in preventing disease and sustaining health in a growing number of vitamin K-dependent health conditions. One of the recently discovered health conditions linked with vitamin K insufficiency has been infection with the coronavirus-2, resulting in the viral disease known as COVID-19 [9]. The scientists behind this epidemiological finding propose that insufficient serum levels of vitamin K could result in a build-up of the inflammatory response associated with COVID-19, contributing to multiorgan failure in these patients. This hypothesis is supported by another vitamin K epidemiological study where patients with COVID-19 exhibited reduced vitamin K status and a poor prognosis [10]. Further studies are needed to explore and affirm the possible role of virama K in susceptibility and recovery from COVID-19 as well as other viral diseases as influenza.

CONCLUSIONS

The results demonstrated that the serum levels of vitamin K2 in the form of MK-7 in both healthy and diabetic sample of this population were in the low range. As a consequence, vitamin K insufficiency may be a common occurrence. Based on results of the current study indicating low serum levels of MK-7 as well as low levels in the typical diet, the authors propose that the general population could benefit from the consumption of vitamin K2 in the form of MK-7 supplements, with emphasis on patients with diabetes, elevated blood pressure, a harbinger of cardiovascular disease, and compromised immune systems. Although this study was conducted in India, the results can be extrapolated world-wide.

ACKNOWLEDGEMENTS

The study was funded by Synergia Life Sciences Pvt, Ltd., Mumbai, India.

Conflicts of Interest

DM, UM and SJ are associated with Synergia Life Sciences. RV, ADBV, JS and VB serve on an advisory board of Synergia Life Sciences Pvt. Ltd. SJS has no potential conflicts to disclose.

Data sharing

Data described in the article, code book, and analytic code will be made available upon request.

ORCID IDs

Rama Vaidya: http://orcid.org/0000-0002-5656-4601

Ashok Vaidya: http://orcid.org/0000-0002-5459-9165

Jayesh Sheth: http://orcid.org/0000-0001-5936-5192

Shashank Jadhav: http://orcid.org/0000-0001-7723-4110

Umakant Mahale: http://orcid.org/0000-0002-9124-8129

Dilip Mehta: http://orcid.org/ 0000-0003-4076-1480

Vladimir Badmaev: http://orcid.org/0000-0003-3737-2847

Sidney J. Stohs: http://orcid.org/0000-0001-5575-5759

REFERENCES

1. Karpiński M, Popko J, Maresz K, Badmaev V, Stohs SJ. Roles of Vitamins D and K, Nutrition, and Lifestyle in Low-Energy Bone Fractures in Children and Young Adults. J Am Coll Nutr. 2017; 36(5):399-412. PMID: 28686548. DOI: 10.1080/07315724.2017.1307791

2. McCann JC, Ames BN. Vitamin K, an example of triage theory: is micronutrition inadequacy linked to diseases of aging? Am. J Clin Nutr. 2009; 90(4): 889-907.PMID: 19692494. DOI: 10.3945/ajcn.2009.27930

3. El Asmar MS, Naoum JJ, Adbid EJ. Vitamin K dependent proteins and the role of vitamin K2 in the modulation of vascular calcification: a review. Oman Med J. 2014; 29: 172-177. PMID: 24936265. DOI: 10.5001/omj.2014.44

4. Walther B, Karl JP, Booth SL, Boyaval P. Menaquinones, bacteria, and the food supply: the relevance of dairy and fermented food products to vitamin K requirements. Adv Nutr. 2013; 4(4): 463-73. PMID: 23858094. DOI: 10.3945/an.113.003855

5. Paroni, R, Faioni EM, Razzari C, Fontana G, Cattaneo M. Determination of vitamin K1 in plasma by solid phase extraction and HPLC with fluorescence detection. J Chromatog B.2009; 877: 351–354. PMID: 19129011. DOI: 10.1016/j.jchromb.2008.12.044

6. Wanga LY, Bates CJ, Yan L, Harrington DJ, Shearer MJ, Prentice A. Determination of phylloquinone (vitamin K1) in plasma and serum by HPLC with fluorescence detection. Clin Chim Acta. 2004; 347: 199–207. PMID: 15313159.DOI: 10.1016/j.cccn.2004.04.030

7. Davidson KW, Sadowski JA. Determination of vitamin K compounds in plasma or

serum by high-performance liquid chromatography using post-column chemical reduction and fluorometric detection. In: *Methods in Enzymology.* 1997; 282:408-409. Publisher: Academic Press.

8. International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use. Bioanalytical Method Validation M10. Endorsed on 26 February 2019. https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation

9. Sadowski JA, Hood SJ, Dallal GE, Garry PJ. Phylloquinone in plasma from elderly and young adults: factors influencing its concentration. Am. J Clin Nutr. 1989; 50:100-8. PMID: 2750682. DOI: 10.1093/ajcn/50.1.100

10. Kaneki M, Hodges SJ, Hosoi T, Fujiwara S, Lyons A, Crean SJ, Ishida N, Nakagawa M, Takechi M, Sano Y, Mizuno Y, Hoshino S, Miyao M, Inoue S, Horiki K, Shiraki M, Ouchi Y, H Orimo H. Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K2: possible implications for hip-fracture risk. Nutrition. 2001; 17(4): 315-21. PMID: 11369171. DOI: 10.1016/s0899-9007(00)00554-2

11. Fusaro M, Gallieni M, Rizzo MA, Stucchi A, Delanaye P, Cavalier E. Moysés RMA, Jorgetti V, Iervasi , Giannini S, Fabris F, Aghi A, Sella S, Galli F, Viola V, Plebani M. Vitamin K plasma levels determination in human health. Clin Chem Lab Med. 2017; 55(6): 789–799. PMID: 27732556. DOI: 10.1515/cclm-2016-0783

12. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. J Nutr. 1998; 128: 785–788. PMID: 9566982. DOI: 10.1093/jn/128.5.785