Original Article

The Evaluation of Vitamin K Status in Children with Febrile Seizure

Kürşat Bora Carman[1], Yasemin Karal[2], Gül Gülen Mert[3], Arzu Ekici[4], Peren Perk[5], Coşkun Yarar[1], Çağatay Nuhoğlu[6], Gürkan Bozan[7], Ömer Kılıç[7], Meltem Dinleyici[7], Sabiha Şahin[7], Emrah Atay[8], Uğur Tokdemir[7], Baver Demir[5], Ener Çağrı Dinleyici[7]

[1]Department of Pediatric Neurology, Eskişehir Osmangazi University School of Medicine, Eskişehir, Turkey
[2]Department of Pediatrics, Trakya University School of Medicine, Edirne, Turkey
[3]Department of Pediatric Neurology, Çukurova University School of Medicine, Adana, Turkey
[4]Clinic of Pediatric Neurology, Yüksek İhtisas Hospital, Bursa, Turkey
[5]Gaziantep Children's Hospital, Gaziantep, Turkey
[6]Clinic of Pediatrics, Haydarpasa Numune Training and Research Hospital, İstanbul, Turkey
[7]Department of Pediatrics, Eskişehir Osmangazi University School of Medicine, Eskişehir, Turkey
[8]Department of Public Health, Eskişehir Osmangazi University School of Medicine, Eskisehir, Turkey

Address for Correspondence: Kürşat Bora Carman, Department of Pediatrics, Department of Pediatric Neurology, Eskişehir Osmangazi University School of Medicine, Eskişehir, Turkey
kbcarman@gmail.com
+90 222 239 79 29-27 64

Received: 16 May 2020
Accepted: 6 October 2020

DOI: 10.4274/balkanmedj.galenos.2020.2020.5.70

Cite this article as: Carman KB, Karal Y, Mert GG, Ekici A, Perk P, Yarar C, Nuhoğlu Ç, Bozan G, Kılıç Ö, Dinleyici M, Şahin S, Atay E, Tokdemir U, Demir B, Dinleyici EÇ. The Evaluation of Vitamin K Status in Children with Febrile Seizure. Balkan Med J

Background: Febrile seizure (FS) is the most common neurological disorder of childhood. The exact pathophysiology of FS is unknown. Recent studies showed that the role of vitamin K in non-hematological and inflammatory disorders. The present study aimed to investigate serum vitamin K levels in children with FS.
Aim: To evaluate the vitamin K levels in children with febrile seizure.
Study Design: Prospective case-control study
Methods: This multicenter study examined representative populations in eight different cities in Turkey between April 1, 2018 and April 1, 2019. Blood samples were taken from all children at presentation. Vitamin K 1, Vitamin K2, tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) levels searched by enzyme-linked immuno-sorbent assay.
Results: The study was conducted with 155 children: 84 children with FS and 71 children in febrile control group. Serum vitamin K1 and vitamin K2 were also higher in FS group than the controls. The results of statistical analysis showed that vitamin K1 and K2 levels were correlated with TNF-α, IL-1β and IL-6 levels. The median vitamin K1 and vitamin K2 levels of children experiencing their first FS were higher than children with recurrent FS. The type of FS has no effect on serum vitamin K1 and vitamin K2 levels.
Conclusion: This study demonstrates that the vitamin K levels in children with febrile seizure was higher than control group. These new findings may contribute to clarifying the etiopathogenesis of FS.
Keywords: Children, febrile seizure, Vitamin K

Introduction

The precise pathophysiology of febrile seizure (FS) is uncertain. The clinical and experimental studies showed genetics, inflammation and cytokines are involved. The most searched cytokines include interleukin 6 (IL-6), interleukin 1 beta (IL-1□) and tumor necrosis factor alpha (TNF- α)[1-4].
The two natural sources of vitamin K are phylloquinone (PK, vitamin K1), and menaquinone (K2). Vitamin K1, synthesized in plants is the main source of dietary vitamin K in the western world. Vitamin K2 are derived mainly from intestinal microbiota and fermented food [5]. Based on the variations in the side chain structure, vitamin K2 divides into sequence of chemically related compounds called menaquinones (MKs) [5-7]. It serves as a

cofactor in vitamin K-dependent proteins for the enzymatic modification of glutamic acid (Glu) residues into gamma-carboxyglutamic acid (Gla) through vitamin K-dependent gamma-glutamyl carboxylase [6-8].
Vitamin K has important biological actions including in the synthesis of several blood coagulation factors, and connection to bone metabolism and vascular changes. Recent studies showed that vitamin K levels was found to be related with inflammation [5, 9-12]. The process of carboxylase is present in nearly all mammalian tissues and two proteins dependent on vitamin K (VKDP) have been found in the brain (Gas6 and protein S). Gas6 has been demonstrated to encourage brain cell survival and protect them from TNF-α-induced injury [13-14]. Results from a research by Reddi et al. [9] showed that the status of vitamin K in humans is inversely correlated with the levels of inflammatory markers that circulate. The role of vitamin K has been rarely searched neurological diseases[15-16] and there are no previous study about the presence of seizure. In present study we aimed to investigate serum vitamin K levels in children with FS and compare to febrile controls.

Material and Method

Patient information

This prospective multicenter research, conducted between April 1, 2018 and April 1, 2019 in eight different cities. The study was approved by local ethical committee and supported by university research grant. Documented written consent had been received from the parents of all children.

Previous medical history and demographical characteristics of all children have been recorded. Usage of antibiotics was recorded within the last 15 days. Detailed physical exams including neurological examinations were performed.

The control group included age-matched children and diagnosed with febrile disease but who did not have seizures and had no known history of previous febrile seizures.

Laboratory analysis

Blood samples (3 cc) was collected in a serum separation tube (SST) within one hour from the seizure. The serum was immediately centrifuged and stored at - 80^0 C. Vitamin K 1, Vitamin K 2, tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) levels were analyzed using an enzyme-linked immuno-sorbent assay (ELISA) kit, which is available commercially. The experiments were carried out on the instructions of manufacturer (ELISA Assay Kits and BioTEK ELISA KIT, United States). To improve accuracy all samples were measured in duplicate.

Statistical analysis

The levels of Vitamin K1, K2, TNF-alpha, IL-1β and IL-6 are presented in the descriptive statistics tables as mean, median, standard deviation, minimum and maximum values. Due to non-parametric distribution of these parameters, Mann-Whitney U and Kruskall-Wallis analyses were conducted to compare the medians of these parameters between the patients with febrile seizures and controls. Analysis of correlation was conducted with Spearman correlation analysis and multivariate logistic regression analysis was performed. SPSS for Windows 15.0 (Chicago, IL, US) was used for analyses and is considered significant if $p < 0.05$

Results

Patient characteristics

The study was conducted with 155 children: 84 children with FS (51 boys and 33 girls) and 71 children (45 boys and 26 girls) in febrile control group. Gender distributions were similar between FS group and control group (p=0.431). The mean age of FS group was 25.59 ±14.58 (3-60) months and mean age of febrile control group was 36.36 ±20.33 (6-60) months (p=0.156). Laboratory features including white blood cell counts, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were similar.

Furthermore, 49 (58.3%) children experienced their first FS episode and the median age of recurrent group was higher than others. In study group, 71.4 % (60/84) of seizures were classified as simple FS. The positive family history for FS was present in 52.4% (44/84) of children and no correlation was determined between positive family history and the number of FS experienced. Demographic and clinical features are summarized in Table 1.

3.2 Laboratory analysis

Serum vitamin K1, vitamin K2, IL-1β, TNF-α, IL-6 levels in FS group were all higher than those in controls ($p<0.001$ for all) (Table 2). In children with first FS, median vitamin K1 and vitamin K2 levels higher than children with recurrent FS ($p= 0.025$ for vitamin K1 and $p= 0.034$ for vitamin K2). Serum TNF-α and IL-1βlevels in first seizure group were all higher than those recurrent seizure group ($p=0.012$ and $p=0.029$) while serum IL-6 levels were similar between two groups ($p=0.053$) (Table 3). Serum vitamin K1, vitamin K2, TNF-α, IL-1β, IL-6 levels in first seizure group and recurrent seizure group were all higher than those in controls (Table 3). The type of FS has no influence on levels of both vitamin K1 and vitamin K2 levels ($p > 0.05$). There was no significant correlation between both vitamin K1 and vitamin K2 levels and patient age, PT, aPTT results, antibiotic usage within previous 15 days and final diagnosis of patients.

In this study, vitamin K1 and vitamin K2 levels were positively correlated ($r=0.933$, $p<0.001$). In addition, detailed statistical analysis showed that both levels of vitamin K1 andK2 levels were independently associated with levels of TNF-α, IL-1β, and IL-6 levels [($r=0.973$, $p<0.001$) and ($r=0.926$, $p<0.001$); ($r=0.935$, $p<0.001$) and ($r=0.912$, $p<0.001$); ($r=0.925$, $p<0.001$) and ($r=0.899$, $p<0.001$)].

The multivariate logistic regression analysis was performed to determine the effect of some related factors (independent variables) on dependent variable. According to results: age, family history, febrile seizure, vitamin K1 and vitamin K2 variables found significant with the binary logistic regression analysis by controlling other variables. The increase in age and it K2 levels decrease the probability of FS. On the contrary positive family history and increase in vitamin K1 levels increase the probability of being patient. The results are given in Table 4.

DISCUSSION

In present study, the results showed higher levels of serum vitamin K1 and vitamin K2 levels in children with FS comparing the febrile children. Vitamin K is a cofactor of carboxylation of vitamin K dependent proteins (VKDP) in brain. Gas6 is expressed in cerebral, piriform cortex, hippocampus, thalamic and hypothalamic structure, midbrain in adult rats[17-19]. Different researches demonstrated that Gas6 has functions in cell growth, chemotaxis, survival, mitogenesis and myelination[13-14].

Prieto et al. [20] reported decline in the expression of Gas6 in a tissue specific manner with age and the reduction was most prominent in the frontal cortex and levels in 24-month-old rats being more than 84% lower than those aged 6months. However, in hippocampus striatum, these ratios were more than 50% [21]. This age dependent changes in vitamin K level might have role in pathogenesis of febrile seizure which occurs in a specific age period.

Vitamin K1 is of plant origin. It is rich in green tea, bean, olive and leafy green vegetables like broccoli, spinach. Whereas vitamin K2 has a bacterial origin and synthesized by *Bacteroides, Veillonella*, and *Enterobacter* which are the usual members of the intestinal microbiota[15-16]. Karl et al. [22] investigated the relationship between bacterially derived vitamin K levels and gut microbiota and reported that the relative abundance of *Bacteroides* and *Prevotella* were associated with menaquinone forms. The development of gut microbiota begins at birth and its stability achieved during adulthood. The colonization and expansion of gut bacteria dominated by *Actinobacteria* and *Proteobacteria* that shifts towards one dominated by *Bacteroidetes and Firmicutes* [16]. This process coincides in time with the time of febrile seizure occurrence. Febrile seizure occurs in children under the age of five, with peaks in the age of 12 to 18 months[1-4]. It might be speculated that intestinal dysbiosis might be involved in the pathogenesis of febrile seizure.

Inflammation and cytokines play a crucial role in the pathogenesis of febrile seizure[4]. The evidences indicate that vitamin K has anti-inflammatory activity and this effect is mediated by cytokines, and vitamin K status in humans has been reported to be inversely correlated with circulating inflammatory marker levels[9-10].

The findings of an animal study showed that vitamin K decreases rotenone induced TNF-α and IL-1β, IL-6 production[23,24]. Also, Grommes et al.[25] disclosed that reduced expression of proinflammatory cytokines in Gas6 treated cells. In contrast to these reports, the results of present research showed a positive correlation between vitamin K levels and proinflammatory markers (TNF-α, IL-1β, IL-6). The increased expression of vitamin K might be an answer to proinflammatory cytokines.

Our study has some limitations. The healthy, non-febrile children were not taken as a control group. This point might be an important challenge of present research. Synthetic short-chain vitamin K1 is widely used in food supplements, and some foods might contain bacteria derived menaquinone such as salami, cheese and milk[26,27]. A research done with healthy volunteers, also showed that the circulating K vitamins were plotted as time function. The peak values for both vitamin K were seen at about four hours after mealtime, followed by a rapid decline. Vitamin K returned to near baseline concentrations at eight hours after mealtime [26]. The blood samples were collected within one hour after seizure in this study. The feeding habits of the participants and the last time patients ate food were not questioned in present research. This point might be considered second limitation of present study. While, this is the pilot study, we could not perform power analysis at the beginning of the study. Regarding to post-hoc our preliminary results regarding to vitamin K1 and K2 levels, power of this study is 99% and 97%, consecutively.

In conclusion, this study demonstrates that the vitamin K levels in children with febrile seizure was higher than control group. According to our best, the present study is the first research about role of vitamin K in FS. These new findings may contribute to clarifying the pathogenesis of FS.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

Acknowledgement

Authors thanks to Samet ECE from Diagen Laboratories Ltd, Ankara, Turkey, for their kind support during laboratory analysis. We also thanks to our patients and their parents.

Ethical approval

This study was approved by university ethics committee. The study was performed according to the principles of Helsinki Declaration.

Funding
This study has been supported by a Eskisehir Osmangazi University Research Grant.

Author Contributions
KBC and ECD participated in protocol development. MC, YK, OK, AO, GB, CN, CS, OK, MD, SS, UT, AE, PP, BD and CY were collected the samples and performed clinical part of the study. EA analyzed the results statistically. KBC and ECD participated primary data analysis, interpretation and wrote the first version of the manuscript and also finalized the manuscript.

REFERENCES

1- Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. Epilepsia. 1993; 34: 592-6.
2- Waruiru C, Appleton R. Febrile seizures: an update. Arch Dis Child 2004; 89: 751-6
3- Kwon A, Kwak BO, Kim K, Ha J, Kim SJ, Bae SH et al. Cytokine levels in febrile seizure patients: A systematic review and meta- analysis. Seizure. 2018; 59: 5-10
4- Saghazadeh A, Gharedaghi M, Meysamie A, Bauer S, Rezaei N. Proinflammatory and anti-inflammatory cytokines in febrile seizures and epilepsy: systematic review and meta-analysis. Rev Neurosci. 2014; 25: 281-305.
5- Fusaro M, Gallieni M, Rizzo MA, Stucchi A, Delanaye P, Cavalier E, et al. Vitamin K plasma levels determination in human health. Clin Chem Lab Med. 2017;55:789-99.
6- Vermer C, De Boer-Van den Berg MA. Vitamin K -dependent carboxylase. Haematologia (Budap) 195; 18:71
7- Shearer MJ, Newman P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. J Lipid Res. 2014; 55: 345-62
8- Beulens JW, Booth SL, van den Heuvel EG, Stoecklin E, Baka A, Vermeer C. The role of menaquinones (vitamin K□) in human health. Br J Nutr. 2013; 110:1357-68
9- Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hoopper C, Harris M, et al. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine. 1995; 7: 287–90.
10- Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino SR, Dawson-Huges B et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol. 2008; 167: 313–20.
11- Booth SL. Roles for vitamin K beyond coagulation. Annu Rev Nutr 2009; 29: 89-110
12- Khojah HM, Ahmed S, Abdel-Rahman MS, Alkhalil KM, Hamza AB. Vitamin homologs as potential biomarkers for disease activity in patients with rheumatoid arthritis. J Bone Miner Metab. 2017; 35: 529-35
13- Ferland G. Vitamin K, an emerging nutrient in brain function. Biofactors.2012; 38: 151-7.
14- Ferland G. Vitamin K and brain function. Semin Thromb Hemost. 2013;39:849-55.
15- Aydin S. Can vitamin K synthesis altered by dysbiosis of microbiota be blamed in the etiopathogenesis of venous thrombosis? Biosci Microbiota Food Health. 2017; 36: 73–4.
16- Ihekweazu FD, Versalovic J. Development of the Pediatric Gut Microbiome: Impact on health and disease. Am J Med Sci. 2018; 356: 413–23.
17- Popescu DC, Huang H, Singhal NK, Shiver L, McDonough J, Clements RJ, et al. Vitamin K enhances the production of brain sulfatides during remyelination. PLoS One. 2018; 13(8): e0203057.
18- Lasemi R, Kundi M, Moghadam NB, Moshammer H, Hainfellner JA. Vitamin K2 in multiple sclerosis patients. Wien Klin Wochenschr. 2018; 130: 307–13.
19- Ferland G. Vitamin K and the nervous system: an overview of its actions. Adv Nutr.2012 ;3: 204-12
20- Prieto AL, Weber JL, Tracy S, Heeb MJ, Lai C. Gas6, a ligand for the receptor protein-tyrosine kinase Tyro-3, is widely expressed in the central nervous system. Brain Res. 1999 ; 816: 646-61.
21- Tsaioun KI, Denisova NA, Obin M, Joseph J. Novel groth factor Gas6, phosphatidylserine and their age-related changes in the rat brain. Neurosci. Res. Commun 2000; 26:113-22.
22- Karl JP, Meydani M, Barnett JB, Vanega SM, Barger K, Fu X, et al. Fecal concentrations of bacterially derived vitamin K forms are associated with gut microbiota composition but not plasma or fecal cytokine concentrations in healthy adults. Am J Clin Nutr. 2017; 106: 1052–61.
23- Yu YX, Li YP, Gao F, Hu QS, Zhang Y, Chan D et al. Vitamin K2 suppresses rotenone-induced microglial activation in vitro. Acta Pharmacol Sin. 2016; 37: 1178–89.
24- Pan MH, Maresz K, Lee PS, Wu JC, Ho CTi Popko J et al. Inhibition of TNF-α, IL-1α, and IL-1β by pretreatment of human monocyte-derived macrophages with menaquinone-7 and cell activation with TLR agonists In Vitro. J Med Food. 2016; 19: 663–9.

25- Grommes C, Lee CY, Wilkinson BL, Jiang Q, Koesnigsknecht-Talboo JL, Varnum B et al. Regulation of microglial phagocytosis and inflammatory gene expression by Gas6 acting on the Axl/Mer family of tyrosine kinases. J Neuroimmune Pharmacol. 2008; 3: 130–140.
26- Schurgers LJ, Teunissen KJ, Hamulyák K, Knapen MH, Vik H, Vermeer C. Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood. 2007; 109: 3279–83.
27- 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;4:463–73.

Table 1. Demographic and clinical findings of study group			
	Febrile seizures n (%)	Control group n (%)	p
Age (months) (mean ± SD; min-max)	25.59 ± 14.58 (3-60)	36.36 ± 20.33 (6-60)	0.156
Gender (boys/girls)	51/33	45/26	0.431
Source of fever			
Upper respiratory tract infections	60 (71.4)	37 (52.1)	
Lower respiratory tract infections	8 (9.5)	19 (26.8)	
Acute gastroenteritis	10 (11.9)	14 (19.7)	
Other infections	6 (7.2)	1 (1.4)	

Table 2. Serum vitamin K1, vitamin K2 levels in febrile seizure and control group			
Laboratory parameters	**Febrile seizures (n=84)**	**Control group (n=71)**	**P**
Vitamin K1 (ug/ml)			
Median (Min-Max) *Mean (±SD)*	11.1 (0.85-25.9) 11.67(7.4)	3.36 (0.19-19.9) 6.03 (5.55)	***< 0.001****
Vitamin K 2 (ng/ml)			
Median (Min-Max) *Mean (±SD)*	659 (34-1403) 675.6 (418.6)	244 (1.77- 1295) 419.6 (371.6)	***< 0.001****
TNF-α (ng/ml)			
Median (Min-Max) *Mean (±SD)*	512.95 (31.9-1322.2) 618.9 (401.9)	212.6 (39.3- 1065.0) 332.7 (284.6)	***< 0.001****
IL-1β (pg/L)			
Median (Min-Max) *Mean (±SD)*	4426.6 (280.8-8568.3) 4658 (2732)	1776.7 (221.5- 7817.4) 2706 (2198)	***< 0.001****

IL-6 (ng/ml)			
Median (Min-Max) *Mean ([SD)*	314.1 (13.6-80.8) 368.1 (242.9)	123.1 (21.4- 610.7) 214.9 (176.9)	***< 0.001****
**p values are calculated by Mann-Whitney U test because of distribution of the parameter as non-parametric.*			

Table 3. Serum vitamin K1, vitamin K2, TNF-α, IL-1β, IL-6 levels between first seizure and recurrent seizure groups					
Laboratory parameters	**First seizure (N=49)**	**Recurrent seizure (N=35)**	**p1**	**CONTROL (n=71)**	**p2** **p3**
Vitamin K1 (ug/ml)					
Median (Min-Max)	14.1 (0.85-25.9)	6.9 (1.5- 23.7)	**0.025**	3.36 (0.19-19.9)	***p2< 0.001*** ***p3=0.01***
Mean ([SD)	13.32 (7.8)	9.36(6.3)		6.03 (5.55)	
Vitamin K 2 (ng/ml)					
Median (Min-Max)	814.8 (34-1403)	446 (83-1283)	**0.034**	244 (1.77- 1295)	***p2< 0.001*** ***p3=0.032***
Mean ([SD)	758.02(422.8)	560.30(389.8)		419.6 (371.6)	
TNF-α (ng/ml)					
Median (Min-Max)	855.9 (31.9-1322.2)	364.5 (48.7-1178.6)	**0.012**	212.6 (39.3- 1065.0)	***p2< 0.001*** ***p3<0.018***
Mean ([SD)	719.5 (413.6)	478.1(343.1)		332.7 (284.6)	
IL-1β (pg/L)					
Median (Min-Max)	6183.8 (280-8568)	3611.8 (489.5-8378)	**0.029**	1776.7 (221.5- 7817.4)	***p2< 0.001*** ***p3<0.010***
Mean ([SD)	5209 (2778)	3886(82504)		2706 (2198)	
IL-6 (ng/ml)					
Median (Min-Max)	403.4 (13.6-880.8)	259.7 (42.2-799.1)	0.053	123.1 (21.4- 610.7)	***p2< 0.001*** ***p3<0.020***
Mean ([SD)	417.4 (258.8)	299 (202.6)		214.9 (176.9)	
**p values are calculated by Mann-Whitney U test because of distribution of the parameter as non-parametric.* *p1: first seizure vs. recurrent seizure; p2: first seizure vs. control; p3: recurrent seizure vs.control*					

Table 4. Multivariate logistic regression results of the study parameters.

Variables	B	Exp(B)*	95% C.I.for EXP(B)		Sig.
			Lower	Upper	
Age	**-,027**	**,974**	**,953**	**,995**	**,016**
Positive family of FS	**1,644**	**5,178**	**1,975**	**13,573**	**,001**
Positive family of epilepsy	,190	1,209	,280	5,227	,799
Vitamin K1 level	**,276**	**1,318**	**1,020**	**1,703**	**,034**
Vitamin K2 level	**-,005**	**,996**	**,991**	**1,000**	**,031**
TNF-α	,002	1,002	,997	1,007	,551
IL-1β	,000	1,000	1,000	1,001	,359
IL-6	-,002	,998	,989	1,006	,588
Constant	-,322	,725			0,522
***Odds Ratio**					