ORIGINAL RESEARCH

Resul Yilmaz, MD¹
Erhan Karaaslan, MD¹
Samet Ozer, MD¹
Ergun Sonmezgoz, MD¹
Nafia O. Kazanci, MD¹
Nursen Cakan, MD¹
Ali Akbas, MD²

¹ Department of Pediatrics, Gaziosmanpasa University School of Medicine, Tokat, Turkey.
² Department of Biochemistry, Gaziosmanpasa University School of Medicine, Tokat, Turkey.

Hypovitaminosis D in Children with Familial Mediterranean Fever

Abstract

Purpose: Vitamin D deficiency or insufficiency plays a role in the initiation and perpetuation of certain autoimmune diseases. The purpose of this study was to measure the vitamin D status of children with Familial Mediterranean Fever (FMF) and compare it to their healthy peers.

Methods: A total of 50 FMF patients and 49 healthy children were enrolled in this prospective study. Vitamin D levels were measured via HPLC. Demographic data, FMF symptom severity scores and the levels of other disease activity markers were retrieved from our hospital database.

Results: The mean age and gender balance of patients and controls were similar, being 8.4 ± 3.8 years and 19 male patients, and 9.1 ± 3.0 years and 25 male controls, respectively. The mean 25(-OH) vitamin D3 levels were 15.94 ± 9.66 µg/L in FMF patients and 41.22 ± 21.31 µg/L in controls. Vitamin D levels were normal in 12% of FMF patients, insufficient in 62% and deficient in 26%. No vitamin D deficiency was evident in any control subject; 30% had insufficient and 70% had normal vitamin D levels. Plasma vitamin D3 levels were similar in all patients despite varying FMF symptom severity scores.

Conclusions: Vitamin D deficiency is frequent in children with FMF but is not associated with disease severity score.

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Correspondence to:
Dr. Resul Yilmaz
Gaziosmanpasa University School of Medicine,
Department of Pediatrics,
60030 Tokat, Turkey
Email: drresul@hotmail.com

Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disease characterized by recurrent self-limiting attacks of fever and serosal inflammation [1]. FMF predominantly affects Turks, Arabs, Jews and Armenians [2,3]. The relevant gene, *Mediterranean fever* (*MEFV*), is located on chromosome 16 and encodes a protein, pyrin/marennosin, that plays a role in the suppression of inflammatory pathways [4-6]. Upon mutation of the *MEFV* gene, production of interleukin (IL)-1 β and tumor necrosis factor (TNF)- α increase, stimulating the innate immune response [6-8]. Vitamin D is one member of a group of steroids (secosteroids). Secosteroids are not considered true vitamins because adequate exposure to sunlight (specifically, ultraviolet-B light) eliminates the need for dietary vitamin D supplementation (which typically supplies only 20% of the required daily amount of the compound) [9].

Vitamin D is involved in phosphate-calcium homeostasis and bone formation and resorption [10]. It plays pivotal roles in the regulation of cell proliferation and differentiation and in cytokine production [11,12]. Indeed, vitamin D deficiency/insufficiency is associated with poor development, a higher prevalence of various malignancies (colorectal, uterine, prostate and skin), development of chronic diseases (osteoporosis and inflammatory bowel disease) and development of autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus [SLE], multiple sclerosis and type I diabetes mellitus) [13-17].

Of relevant prior studies, only two studied serum vitamin D levels in adult FMF patients [18,19]. Thus, we explored whether FMF children exhibit hypovitaminosis D compared with healthy peers.

Materials and Methods

A total of 132 pediatric FMF patients were treated in our outpatient clinic. Twenty patients were too old for inclusion in the study (> 19 years of age). Of the remaining 112 FMF patients, 50 agreed to participate in the study. Serum vitamin D levels were measured via HPLC in all 50 patients admitted for follow-up examinations during the period from July to September 2012. Fifty healthy age- and gender-matched children, who visited our outpatient clinic for routine checkups, were recruited as control subjects but one later withdrew from the study.

The study was approved by the Gaziosmanpasa University Clinical Research Ethics Committee. All procedures were conducted as dictated by the Declaration of Helsinki after written informed consent was obtained from patients and/or guardians.

Blood samples were drawn for routine testing and 25(-OH) vitamin D levels were measured in residual samples using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) with the aid of a 25(-OH) Vitamin D3 kit (RECIPE Chemicals+Instruments GmbH, Munich, Germany).

Subjects were screened for the presence of all 12 known FMF mutations. Genomic DNA was extracted from 5 mL amounts of whole blood obtained via standard procedures. FMF mutations were detected using a reverse-hybridization test strip-based assay (FMF StripAssay, ViennaLab Labordiagnostika, Vienna, Austria) allowing detection of the 12 most common *MEFV* mutations: p.E148Q (c.442G>C) in exon 2; p.P369S (c.1105C>T) in exon 3; p.F479L (c.1437C>G) in exon 5; and p.M680I (c.2040G>C), p.M680I (c.2040G>A), I692del (c.2076>2078del), p.M694V (c.2080A>G), p.M694I (c.2082G>A), p.K695R (c.2084A>G), p.V726A (c.2177T>C), p.A744S (c.2230G>T); and, p.R761H (c.2282G>A) in exon 10.

Diagnoses were guided by the Tel-Hashomer Criteria.(20) All patients were in remission and all were taking colchicine. No patient or control was taking any other drug (anticoagulant, anticonvulsant or growth hormone), vitamin, or medicinal plant material or supplement that could affect vitamin D levels. All blood samples were drawn over the same period (July September 2012). Plasma 25(-OH) vitamin D3 levels less than 30 and 10 μ g/L were considered to indicate vitamin D insufficiency and deficiency, respectively [21,22].

FMF disease severity scores were calculated with reference to age at disease onset, number of attacks per month, amyloidosis status, arthritis status, colchicine dose and the presence or absence of erysipelas-like erythema. Scores of 3–5 were considered to reflect mild disease, 6–8 moderate disease, and ≥ 9 severe disease [23]. Demographic data, information on disease duration and current colchicine dose were retrieved from our hospital database or collected during interviews of patients.

Statistical analysis was performed using SPSS version 19 (IBM SPSS, Somers, NY, USA). Continuous variables are presented as means $\pm$ standard deviations and categorical variables are presented as counts with percentages. Differences in categorical variables were compared using the chi-squared test. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of variables was normal. One-way analysis of variance (ANOVA) and Kruskal-Wallis analysis of variance were used to compare continuous data that were normally and non-normally distributed, respectively. The unpaired *t*-test was used to compare the means of normally distributed variables. Multivariate logistic regression was used to explore the effects of

TABLE 1. Clinical features of FMF patients at the time of diagnosis

		Mean $\pm$ SD or n (%)
Disease duration (years)		8.2 $\pm$ 5.7
Duration of diagnosis (months)		36 $\pm$ 49
Family history of FMF		12 (24)
Fever		37 (74)
Abdominal pain		44 (88)
Arthritis		24 (48)
Mutation	Carrying M694V allele	27 (54)
	Homozygous for any mutation on MEFV gene	19 (38)

FMF: Familial Mediterranean Fever

categorical variables on vitamin D3 status. A p-value <0.05 was considered to reflect significance.

Results

The mean age and gender balance of the patient and control groups were similar (8.4 $\pm$ 3.8 years, 19 boys and 9.1 $\pm$ 3.0 years, 25 boys, respectively; both p values > 0.05). Demographic and clinical findings are shown in Table 1. All subjects were Caucasian. Serum calcium, phosphate, and vitamin D levels are shown in Table 2. The mean 25(-OH) vitamin D3 levels were 15.94 $\pm$ 9.66 μ g/L in FMF patients and 41.22 $\pm$ 21.31 μ g/L in controls.

Vitamin D deficiency and insufficiency were evident in 26% and 62% of FMF patients, respectively (Table 3) and the proportions of subjects with vitamin D deficiency and insufficiency was higher in FMF patients than controls (p<0.05). Lower vitamin D levels were evident in female FMF patients (p<0.05) whereas no between-gender difference was evident in controls (p>0.05). Plasma vitamin D3 levels were similar in

FIGURE 1. Vitamin D levels in Familial Mediterranean Fever patients with different symptom severity scores. No among-subgroup differences in vitamin D3 status were evident (p>0.05).

patients, despite different FMF symptom severity scores (p>0.05; Figure 1).

No correlations were evident among vitamin D3 status and allelic status (homozygous or heterozygous), presence of the M694V mutation (on one or both chromosomes) and disease severity score (Table 4). Table 5 lists the significant outcomes of multivariable logistic regression analysis of vitamin D3 status. This analysis included the variables listed in Table 4: allelic status, presence of the M694V mutation and disease severity score. The latter three variables did not influence vitamin D3 status.

TABLE 2. Plasma Vitamin D levels and serum calcium, phosphate levels of FMF patients and control group.

	FMF Patients (n=50)	Control Group (n=49)	p
Age (years)	8.4 $\pm$ 3.8	9.1 $\pm$ 3.0	0,668
Gender (Boys)(%)	19 (38)	25 (50)	0,271
Vitamin D (μ g/L)	15.94 $\pm$ 9.66	41.22 $\pm$ 21.31	<0,001
Calcium (mg/dL)	9.81 $\pm$ 0.42	10.28 $\pm$ 0.91	0,002
Phosphate (mg/dL)	4.65 $\pm$ 0.78	5.39 $\pm$ 0.85	<0,001

FMF: Familial Mediterranean Fever

Data are shown as Mean $\pm$ SD or n (%)

TABLE 4. Distribution among levels of Vitamin D3 Status according to allelic status, M694V and Disease Severity Score

		Vitamin D3 Status			p
		Deficiency	Insufficiency	Normal	
		N(%)	N(%)	N(%)	
Allelic status	homozygous	4(30.8)	13(41.9)	2(33.3)	0.760
	heterozygous	9(69.2)	18(58.1)	4(66.7)	
M694V	M694V positive	4(30.8)	19(61.3)	4(66.7)	0.144
	M694V negative	9(69.2)	12(38.7)	2(33.3)	
Disease Severity Score	mild	1(7.7)	8(25.8)	0(0)	0.338
	moderate	10(76.9)	16(51.6)	4(66.7)	
	severe	2(15.4)	7(22.6)	2(33.3)	

Discussion

This is the first study to show that plasma vitamin D3 levels are lower in FMF children than in age- and gender-matched healthy controls.

T helper cells play a crucial role in FMF pathogenesis and many other autoimmune and autoinflammatory diseases [13-17]. T helper 1 cells produce IFN- γ and TNF- β . Aypar *et al.* [24] showed that the IFN- γ levels produced by Th1 cells were significantly higher than those of healthy controls during FMF attacks, indicating that Th1 polarization triggered inflammation. Koklu *et al.* [25] reported high IFN- γ levels in FMF patients during both attack and remission periods.

Recently, the vitamin D receptor (VDR) has been shown to be expressed by cells of the immune system, upon which vitamin D exerts immunomodulatory effects [11,12,26,27]. Vitamin D inhibits the differentiation and maturation of both myeloid cells¹¹ and pathogenic proinflammatory T cells including Th1 and Th17 cells [9,26,28]. It also inhibits the production of IL-6 and INF- γ [29,30]. Thus, low vitamin D levels might activate the inflammatory networks of FMF patients, triggering attacks.

FMF is an episodic disease featuring inflammatory attacks, but subclinical inflammation is also present during attack-free periods and many affect clinical outcomes via a pathophysiological process that remains poorly understood [1,31]. As vitamin D has immunomodulatory effects on Th cells and affects cytokine production, it is possible that the low vitamin D levels of FMF patients may explain the presence of subclinical inflammation.

Vitamin D deficiency is evident in many patients treated by rheumatologists; therefore, FMF patients were tested to see if they were similarly deficient. Three studies from Turkey re-

ported low vitamin D levels in adult and child FMF patients [18,19,32]. Our results are in agreement with these data.

Although some studies have described relationships between low vitamin D levels and the severity of various rheumatological and autoimmune diseases, others have reported no association between vitamin D levels and the extent of disease activity in adult SLE patients [33-37] and Anik *et al.* [32] found no significant correlation between vitamin D levels and disease severity scores in FMF children from Turkey. In the present study, no correlation was found between FMF symptom severity score and vitamin D level; however, all FMF patients, whether they had mild, moderate or severe symptom scores, had low vitamin D levels. Such contradictory findings may be explained by the fact that our patient numbers were small and VDR genetic polymorphism was evident. VDR gene polymorphism may be responsible for various disease activities [22,37]. We will study genetic polymorphisms further in the context of FMF.

A recent study performed in western Turkey found that vitamin D levels in FMF children were inversely correlated with the cumulative colchicine dose [32] It was concluded that

TABLE 5. Effect of explanatory variables (allelic status, M694V, Disease Severity Score) on Vitamin D3 Status in multivariate logistic regression model (Likelihood Ratio Test)

Effect	χ^2	P
Allelic status	1.114	0.573
M694V	4.545	0.103
Disease Severity Score	5.036	0.284
<i>Model</i>		
Final	10.366	0.240

colchicine inhibited the functions of intracellular microtubules, which are essential components of the normal genomic response to vitamin D. Defects in the microtubular network attributable to colchicine use may reduce vitamin D levels because of increased production of 1,25(-OH)₂ vitamin D₃ and 24,25(-OH)₂ vitamin D₃ [32,38].

Another reason why vitamin D₃ levels were low in FMF patients receiving colchicine may be that the drug triggers malabsorption by, and other adverse events in, the gastrointestinal system. Colchicine may decrease serum vitamin B₁₂ levels by affecting the function of the intestinal mucosa [39,40]. Although malabsorption of Vitamin B₁₂ has been proposed, no study has yet investigated the effects of colchicine on vitamin D absorption.

Several factors including season, age, exposure to sunlight, use of certain medications, traditions, dress habits and geographical location influence vitamin D metabolism [18,19,41]. Vitamin D levels are higher in summer and autumn because of increased exposure to sunlight [42,43]. Although the present study was conducted between July and September 2012, vitamin D levels were low in patients but normal in controls; thus, seasonality cannot explain this difference.

Our study had certain limitations. First, vitamin D levels were determined in only about one-third of 132 FMF patients admitted to our clinic. Second, no information on dietary calcium or vitamin D intake, dress habits, exposure to the sun, or exercise habits was collected, and so the effects of such factors on plasma vitamin D levels could not be evaluated.

In conclusion, vitamin D deficiency is frequent in children with FMF. It remains unclear whether low-level vitamin D₃ status triggers FMF attacks or whether a lower vitamin D₃ level is an unwanted side-effect of colchicine treatment. It would be useful to explore the possible relationship between vitamin D deficiency and the numbers of FMF attacks and disease severity scores in large FMF cohorts, and to evaluate the possible beneficial effects of vitamin D supplementation.

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