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The influence of vitamin D administration on the clinical presentation, body mass index, and osteoprotegerin (OPG) level in a sample of Egyptian children with familial Mediterranean fever

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Abstract

Background: Familial Mediterranean fever (FMF) is autosomal recessive chronic disease represents by recurring attacks of polyserositis, fever, and joint pain. Vitamin D deficiency in FMF children has been recently mentioned in literature and linked to delayed physical growth. Osteoporosis in FMF patients can be linked to low levels of vitamin D, too. Osteoprotegerin (OPG) might be used as an indicator for osteoporosis. Therefore, this work aimed to investigate the impact of vitamin D administration on clinical status, BMI, and bone mineral density represented by alterations in the OPG serum levels in a group of Egyptian children with FMF. This was a prospective longitudinal study carried out on 33 children, aged 4–16 years, with FMF cases. Patients were on colchicine 0.5–2 mg/day and received vitamin D_3 oral drops 2800 IU/ml; each drop contains 100 IU in a dose of 600 IU/day for 6 months. The effect of vitamin D administration was evaluated clinically, anthropometrically and by assessment of serum vitamin D and osteoprotegerin at baseline and 6 months later.

Results: Serum vitamin D levels were below the normal range before intervention and showed significant improvement ($p < 0.001$) 6 months after intervention. Significant increase in both BMI *Z* scores ($p < 0.05$) and OPG serum levels and improvement in the clinical status as illustrated by significant decrease in the number of cases with fever, arthritis, and abdominal pain and significant decrease in the frequency and duration of the attacks ($p < 0.001$).

Conclusion: Our results intensely indicate that vitamin D supplementation improved the clinical condition, BMI, and bone mineral density in children with FMF.

Keywords: Body mass index (BMI), Bone mineral density (BMD), Familial Mediterranean fever (FMF), Osteoprotegerin (OPG), Vitamin D

Background

Familial Mediterranean fever (FMF) is an autosomal recessive disease, which is represented by recurring attacks of polyserositis, fever, and joint pain, persisting for 24–72 h. FMF is common in subjects of Mediterranean origin [1]. MEFV is the mutated gene in FMF patients, which encrypts pyrin, responsible for

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inflammation by altering activation of caspase-1, apoptosis, stimulation of the NF-κB pathway, and formation of interleukin (IL)-1β by innate immune system. Renal amyloidosis is the major complication of FMF [2–4].

Clinically, nearly, 10% of FMF patients have resistance to colchicine which is detected by complaining of monthly attacks or persistent elevation of acute-phase reactants in spite of the compliance to maximum tolerated colchicine dose [5]. MEV genotypes is the main determinant factor of phenotypic variation and FMF severity [6].

FMF management aims to inhibit the attacks and decrease subclinical chronic inflammation and its consequences, especially amyloidosis. Colchicine, the principle effective drug in FMF treatment, prevents the attacks in 60–70% of cases. Nevertheless, 20–30% of cases have partial response to the maximum allowed colchicine doses [7].

Vitamin D plays a very important role in calcium metabolism and mineral deposition in bones; it has immunomodulatory influence on innate and acquired immunity. It was found that vitamin D abnormalities in metabolism cause secretion of proinflammatory cytokines preventing the manufacture of T regulatory cells [8].

Vitamin D deficiency in FMF has been recently mentioned in the literature [9, 10]. Several researches detected vitamin D correlation with the clinical status of FMF patients. Deficiency of vitamin D was found to exacerbate the symptoms of FMF and increase colchicine resistance [11].

Furthermore, some studies linked decreased levels of vitamin D to malabsorption as a consequence of treatment with colchicine. Padeh et al. stated that 14% of FMF cases acquired diarrhea while treated with colchicine [12].

It has been established that bone mineral density decreases in patients with different inflammatory diseases as a result of chronic inflammation, and attack-free patients with FMF have significantly low bone mineral density than normal subjects, indicating early-onset osteoporosis predisposition [13]. The pathophysiology of bone loss is attributed mainly to inflammatory activity in patients with FMF [14]. In addition, alterations in metabolism of vitamin D may lead to increased resorption of bone. Osteoporosis in FMF patients may be linked to low levels of vitamin D [15].

Osteoprotegerin (OPG) is secreted by osteoblasts, mesenchymal stem cells, fibroblasts, endothelial cells, and human adipose tissue [16]. It is one of the members of the tumor necrosis factor (TNF) receptor superfamily and has a pleiotropic impact on bone metabolism as well as an endocrine role. OPG inhibits production and differentiation of immature osteoclast precursors to mature osteoclasts by attaching to the RANK receptors present on immature osteoclasts, leading to the inhibition of bone resorption [17].

In a study done by Chen Y-H et al. (2012), OPG was suggested as a predictor of bone mineral density and alternate to DEXA to detect the possibility for osteoporosis. They concluded that OPG can be used as an indicator for osteopenia or osteoporosis and bone turnover evaluation [18]. Therefore, this work aimed to assess the impact of vitamin D administration on the clinical status & the bone mineral density represented by alterations in the OPG serum levels in a group of Egyptian children with FMF.

Methods

This prospective longitudinal study was carried out at the Clinical Genetics Clinic at Medical Research Center of Excellence at the National Research Centre of Egypt (NRC) where 33 FMF children, aged 4–16 years were recruited from the patients treated and followed up in this clinic. The diagnosis of FMF cases was based on history, clinical examination, and laboratory investigations according to the Tel-Hashomer criteria [19] and confirmed by molecular diagnosis of MEFV gene mutations.

Clinical examination

All patients were subjected to full history taking and thorough clinical examination. Patients were evaluated according to frequency, duration, and the presence or absence of attacks and the presence of symptoms, mainly fever, arthritis, and abdominal pain. Patients were on colchicine dose (0.5–2 mg/day), and none of the patients were receiving any drugs other than colchicine that could influence vitamin D level, had bone diseases, or using drugs that could affect bone metabolism. Patients received vitamin D orally in a dose of 600 IU/day. Regarding the preparation used, it was Cholecalciferol (Vit. D_3) oral drops 2800 IU/ml (each drop contains 100 IU) [20–22] for 6 months in addition to their regular colchicine doses.

The effect of vitamin D administration was evaluated through the following procedures performed at baseline and repeated 6 months later:

- Clinically, according to symptoms including abdominal and chest pains, fever, arthritis, myalgia, erysipelas-like erythema (duration, frequency, and severity of attacks) in the FMF patients [23, 24].
- Assessment of body mass index (BMI)
- Laboratory investigations:

a. Assessment of serum vitamin D
b. Assessment of serum osteoprotegerin (OPG)

Anthropometric measurements included weight (measured using a calibrated digital scale to the nearest 0.01 kg) and height (measured to the nearest 0.1 cm using Harpenden Stadiometer); body mass index (BMI) was calculated as weight (kg)/height (m^2). *Z* scores of BMI were calculated. All these measurements were following the methods of the international biological program [25].

Laboratory investigations

MEFV genotyping

Genomic DNA isolation, PCR amplification, and sequencing Peripheral blood was collected from each participant, and the genomic DNA samples were extracted from blood lymphocytes using DNA Isolation Kit for Mammalian Blood (Roche Diagnostics, Mannheim, Germany). For each patient, both MEFV exons 2 and 10, which are considered as mutation hot spots, were individually amplified by PCR using 2 pairs of corresponding primers:

- Exon 2: F: 5′- GCCTGAAGACTCCAGACCACC CCG-3′, R: 5′- AGGCCCTCCGAGGCCTTCTCT CTG-3′
- Exon 10: F: 5′- GAGGTGGAGGTTGGAGACAA-3′, R: 5′- TGACCACCCACTGGACAGAT-3′.

PCR was performed in a 25-ml reaction volume containing 60 ng of genomic DNA, 5 U of Taq160 (Invitrogen), 20 pmol of each primer, 50 mM MgCl2, 10 mM d NTP, and 10× PCR buffer (Invitrogen) in the Veriti 96-well Thermal Cycler 9902 (Applied Biosystems, Foster City, CA, USA). The PCR conditions was as follows: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s and 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. Bidirectional direct sequencing of purified PCR products was performed using the Big Dye Terminator V1.1 Cycle Sequencing Kit (ABI prism, Foster City, CA, USA) and an Applied Biosystems 3500DX Genetic Analyzer. The resulting chromatogram was analyzed using the Sequencing Analysis SeqA V5.4 (Applied Biosystems) program. The sequencing results were compared with the MEFV reference coding sequence available at NCBI with Gene Bank [26].

Biochemical investigations

Venous blood samples of patients were taken, centrifuged, separated, and stored at − 80 °C until they were analyzed. Assessment of Serum 25 hydroxyvitamin D (25 (OH) D) was assessed by vitamin D direct ELISA Kit (EIA-4696) (DRG ® International, Inc. USA) [27] and serum osteoprotegerin was estimated by using Human Osteoprotegerin (OPG) ELISA Kit.

Statistical analysis

The collected data was statistically analyzed using SPSS statistics (Statistical Package for Social Sciences) version 18. Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean ± SD (standard deviation), while it was done for qualitative data as number and percentage. One sample *t* test was used to compare results of patients before and after vitamin D administration, and ANOVA test was used to compare more than two variables. The Mc-Nemar test was also used, while correlations were done using Pearson correlation. $p < 0.05$ was considered as significant.

Results

Eighteen of our cases were males (54.5%), while 15 (45.5%) were females. The mean age of our cases was (7.5 ± 2.4) years. The demographic characteristics and molecular diagnosis of the studied cases are presented in Table 1. The homozygous mutations of genotypes M694I and M694I + M680I were the most common (63.6% & 27.3%, consequently).

Our results delineated that vitamin D serum levels were beneath the normal references before interference. After the intervention, these levels showed a significant increase ($p < 0.001$) and a significant increase in both BMI *Z* scores ($p < 0.05$) and OPG serum levels, while significant reduction in the duration of the attacks ($p < 0.001$) after vitamin D administration was detected (Table 2).

There was significant improvement in the clinical characteristics of our cases as illustrated by significant decrease in the number of cases with fever, arthritis, and abdominal pain. The frequency of the attacks was significantly decreased also ($p < 0.001$) (Table 3).

Our study detected also significant positive correlation between the serum level of vitamin D and OPG before the administration of vitamin D. Additionally, a significant negative correlation between the duration of the attacks and the percent of alteration in the OPG serum level was found (Table 4). There was no significant difference in ZBMI, OPG, and vitamin D serum levels before or after treatment regarding sex or different MEFV mutations (Table 5). Our results showed that cases with *arthritis* had significantly lower vitamin D serum level, while the cases that manifested with fever significantly had lower OPG and vitamin D serum levels before vitamin D administration, as shown in Table 6.

Table 1 Demographic and molecular characteristics of the studied cases

Variables		Mean ± SD	Range
Age (years)		7.5 ± 2.4	4.0–13.0
Bone age (years)		8.0 ± 2.3	4.0–13.5
Age of onset (year)		4.8 ± 2.3	2.0–10.0
Colchicine daily dose (mg)		1.3 ± 0.6	0.5–2.0
Disease duration (years)		2.7 ± 2.3	0.5–8.8
		N	**Percentage (%)**
Sex	**Male**	18	54.5
	Female	15	45.5
Consanguinity		8	24.2
Family history		13	39.4
Molecular diagnosis	**M694I**	21	63.6
	M694I + M680I	9	27.3
	V726A	1	3.0
	M762V + M694V	1	3.0
	E148Q+	1	3.0

Total *N* = 33

Table 2 Effect of vitamin D administration on ZBMI, attack duration, OPG, and vitamin D

Variables	Before	After	Change (after-before)	*p*
Z BMI	− 0.2 ± 1.3	0.2 ± 1.2	0.3 ± 0.8	**0.045***
Attack duration (h)	44.6 ± 26.9	30.5 ± 18.2	− 14.0 ± 15.1	**< 0.001***
OPG (pg/ml)	328.3 ± 147.1	677.7 ± 187.3	339.4 ± 205.5	**< 0.001***
Vitamin D (ng/ml)	22.3 ± 9.3	41.1 ± 10.4	18.8 ± 7.2	**< 0.001***

Total = 33. Data expressed as mean ± SD. Negative values indicate reduction. ^Paired t test. *Significant

Discussion

Vitamin D effects on bone health and calcium homeostasis are well recognized. Recently, great attention has been given to its extra-skeletal effects. Epidemiological, basic, and clinical researches showed rising evidence that vitamin D status is accompanied with actions impacting function of muscles, body adipose tissue, immunity, and risk of cardiovascular disease [28].

Previous studies assessed the deficiency of the serum level of vitamin D in FMF and noted its relation to disease severity. Vitamin D deficiency was associated with higher disease severity and pain in patients with FMF. Ozer et al., reported that serum vitamin D was significantly reduced in colchicine-resistant FMF cases than in non-colchicine–resistant FMF patients. This may be a factor that plays a role in the etio-pathogenesis of colchicine resistance [29].

In an aforementioned study done by Zaki et al., serum levels of 25-hydroxyvitamin D were reduced in patients with FMF compared with healthy subjects. In addition, Zaki et al., recommended that vitamin D serum levels must be assessed frequently, then supplementation should be prescribed to patients with FMF [30]. This was also reported in previous studies done by Anik et al., Kisacik et al., & Garip et al. which demonstrated lower vitamin D levels in FMF than normal subjects [11, 31, 32].

Table 3 Effect of vitamin D administration on fever, arthritis, pain, and attack frequency

Condition		No. of cases before	No. of cases after	Improvement	*p*
Fever		31 (93.9%)	17 (51.5%)	14 (42.4%)	**< 0.001***
Arthritis		27 (81.8%)	14 (42.4%)	13 (39.4%)	**< 0.001***
Abdominal pain		26 (78.8%)	14 (42.4%)	11 (33.3%)	**< 0.001***
Attack frequency	**Mild**	6 (18.2%)	18 (54.5%)	19 (57.6%)	**< 0.001***
	Moderate	16 (48.5%)	13 (39.4%)		
	Severe	11 (33.3%)	2 (6.1%)		

Total = 33. Attack frequency: Mild (1/month), Moderate (2/month), Severe (≥ 3/month). ^McNemar test. *Significant

Table 4 Correlations among the studied cases before and after vitamin D administration

		Before			After			Change		
Variable		**ZBMI**	**OPG**	**Vit. D**	**ZBMI**	**OPG**	**Vit. D**	**ZBMI**	**OPG**	**Vit. D**
Colchicine daily dose	***r***	0.609	− 0.218	− 0.013	0.473	0.018	− 0.094	− 0.191	0.164	− 0.099
	p	**0.004***	0.295	0.952	**0.035***	0.932	0.655	0.420	0.433	0.636
Colchicine TTT duration	***r***	0.002	0.092	− 0.546	− 0.078	− 0.291	0.033	− 0.125	− 0.230	− 0.506
	p	0.991	0.616	**0.001***	0.712	0.106	0.860	0.553	0.205	**0.003***
Duration of attack	***r***	0.230	− 0.265	− 0.188	0.395	0.216	0.112	− 0.165	− 0.445	− 0.175
	p	0.268	0.136	0.296	0.151	0.228	0.536	0.432	**0.009***	0.330
OPG	***r***	0.036		0.559	0.262		0.150	0.043		0.306
	p	0.866		**0.001***	0.205		0.404	0.838		0.083
Vit. D serum level	***r***	− 0.227	0.559		− 0.074	0.150		− 0.170	0.306	
	p	0.274	**0.001***		0.724	0.404		0.417	0.083	

Total = 33. *ZBMI*, body mass index Z scores, *TTT* treatment, *OPG* osteoprotogerin, *r* correlation coefficient, $p < 0.05$ is a significant correlation. Pearson correlation.
*Significant

Moreover, Lotfy et al., reported that vitamin D levels were lower in Egyptian FMF children. In their study, no statistically significant associations were identified between level of vitamin D and various clinical manifestations, laboratory findings, and genotypes. They speculated that vitamin D deficiency in FMF patients may be related to inflammation; thus, administration of vitamin D large doses appears to be suitable for FMF children [33].

Based on these former studies, the current study aimed at investigating the effects of vitamin D administration in children with FMF.

The best available indicator of vitamin D condition is the serum level of 25(OH) D [34]. There is no universal consensus on the serum level of 25(OH) D needed for sufficient status. Classifications of vitamin D status within clinical practice guidelines in pediatric are debatable. 25(OH) D concentrations > 50.0 nmol/l are cutoff values considered to be "sufficient" by The American Academy of Pediatrics (AAP) [35], while 25(OH) D serum concentration between 75–125 nmol/l (30–50 ng/ml) is considered to be "sufficient" for adolescents by the Society for Adolescent Health and Medicine (SAHM) [36].

Furthermore, the reference ranges for vitamin D metabolites depend on the method of their detection, and the lower value of 25.OH-D vitamin levels considered adequate for health is controversial due to seasonal variations of vitamin D levels, localization, type of skin, nutritional status, exposure to sun, and lifestyle [37].

In a report by Hollis et al., the circulating levels of 25(OH) D3 < 32 ng/ml were considered as vitamin D deficiency [38].

Accordingly, in the current study, the mean 25(OH) D3 serum concentration at baseline was 22.3 ± 9.3 ng/ml before starting vitamin D administration which was considered deficiency and increased to 41.1 ± 10.4 ng/ml, accompanied by a significant improvement in the clinical presentation after vitamin D_3 oral administration for 6 months in a dose of 600 IU/day. This difference was statistically significant ($p < 0.001$). The results of our study coincide with the outcomes mentioned before in literature by Zaki et al. and Ozen et al. [30, 39].

In addition, the results of this study are in agreement with those of Kazem et al.'s, which reported low vitamin D serum level in FMF cases with significant improvement of the clinical status concerning the attack duration, frequency, and severity after vitamin D supplementation for 6 months [40].

Concomitantly, Zhang et al. agreed with the opinion that serum concentrations of vitamin D should be sustained at more than 30 ng/ml to attain adequate anti-inflammatory impacts [41]. This corroborates with our results where vitamin D serum level reached 41.1 ± 10.4 ng/ml after 6 months of vitamin D administration.

Although, some previous studies by Yilmaz et al., Anik et al., Lange et al., & Kisacik et al. also reported lower serum vitamin D concentrations in FMF patients [10, 11, 15, 31]. Others showed discrepant results, which may be related to the presence of VDR polymorphisms and colchicine use. To date, the association between colchicine use and intestinal malabsorption of low vitamin D concentrations has not been demonstrated, although colchicine has been linked to impaired absorption of different nutrients such as vitamin B12 and lactose [42].

On the contrary, Anik et al. and Karatay et al. showed a strong relationship between colchicine use and low serum vitamin D concentrations in patients with FMF and Behçet's disease, respectively [10, 43].

Moreover, Anik et al. declared that decreased vitamin D levels in FMF cases could be attributed mainly

Table 5 Comparison between ZBMI, OPG, and vitamin D serum levels according to different demographic and molecular characteristics

Condition		Lab	Male	Female	^*p*
Sex	**Before**	**ZBMI**	− 0.3 ± 1.1	0.0 ± 1.5	0.639
		OPG	281.3 ± 67.0	384.7 ± 194.3	0.066
		Vitamin D	21.1 ± 8.8	23.7 ± 10.0	0.429
	After	**ZBMI**	0.0 ± 1.1	0.4 ± 1.3	0.363
		OPG	638.8 ± 154.5	724.2 ± 216.8	0.197
		Vitamin D	38.8 ± 9.3	43.9 ± 11.4	0.170
	Change	**ZBMI**	0.2 ± 0.5	0.4 ± 1.0	0.548
		OPG	357.5 ± 162.1	317.6 ± 252.4	0.587
		Vitamin D	17.7 ± 7.3	20.2 ± 7.0	0.344
Molecular diagnosis			**M694I**	**M694I + M680I**	
	Before	**ZBMI**	− 0.1 ± 1.4	− 0.4 ± 1.0	0.561
		OPG	327.1 ± 145.6	308.5 ± 117.2	0.738
		Vitamin D	22.3 ± 7.8	20.0 ± 10.8	0.516
	After	**ZBMI**	0.2 ± 1.3	0.2 ± 0.9	0.967
		OPG	688.0 ± 188.7	672.5 ± 170.0	0.834
		Vitamin D	42.5 ± 9.3	35.6 ± 9.0	0.072
	Change	**ZBMI**	0.3 ± 0.7	0.6 ± 0.8	0.353
		OPG	345.2 ± 225.4	364.0 ± 172.8	0.825
		Vitamin D	20.2 ± 6.9	15.6 ± 8.1	0.127
Consanguinity			**Present**	**Absent**	
	Before	**ZBMI**	− 0.4 ± 0.6	− 0.1 ± 1.4	0.354
		OPG	354.1 ± 204.5	320.0 ± 127.9	0.668
		Vitamin D	19.2 ± 6.8	23.3 ± 9.9	0.293
	After	**ZBMI**	− 0.3 ± 1.1	0.3 ± 1.2	0.294
		OPG	660.3 ± 162.7	683.2 ± 197.3	0.768
		Vitamin D	39.3 ± 10.0	41.7 ± 10.7	0.571
	Change	**ZBMI**	0.2±1.0	0.4 ± 0.7	0.559
		OPG	306.2 ± 135.2	350.0 ± 224.7	0.607
		Vitamin D	20.1 ± 8.5	18.5 ± 6.8	0.591
Family history			**Present**	**Absent**	
	Before	**ZBMI**	− 0.4 ± 1.4	− 0.1 ± 1.2	0.569
		OPG	299.2 ± 126.6	347.2 ± 159.3	0.369
		Vitamin D	23.8 ± 11.3	21.3 ± 7.9	0.449
	After	**ZBMI**	0.2 ± 1.2	0.2 ± 1.2	0.977
		OPG	574.5 ± 153.5	744.7 ± 179.5	**0.008***
		Vitamin D	39.3 ± 11.9	42.3 ± 9.5	0.421
	Change	**ZBMI**	0.6 ± 0.9	0.2 ± 0.7	0.316
		OPG	275.3 ± 114.8	381.1 ± 241.2	0.102
		Vitamin D	15.4 ± 5.9	21.1 ± 7.2	**0.025***

^Independent *t* test. *Significant

to the colchicine dose and duration of treatment which reduces vitamin D absorption from the gastrointestinal tract or changes the metabolism of vitamin D. This is consistent with our results which displayed significant negative correlation between the duration of colchicine treatment and vitamin D serum level [10].

In another study done by Turhan et al., they attributed the lower vitamin D concentration in FMF patients to the colchicine use. No significant difference was determined in vitamin D concentrations according to the attack frequency. Accordingly, they speculated that vitamin D is not an important factor in triggering the attacks in FMF

Table 6 Comparison of ZBMI, OPG, and vitamin D serum levels according to different symptoms of FMF

Condition		Lab	Present	Absent	*p*
Fever	**Before**	**ZBMI**	− 0.2 ± 1.2	0.3 ± 2.2	0.570
		OPG	303.5 ± 112.3	712.4 ± 5.2	**< 0.001***
		Vitamin D	21.5 ± 9.0	35.0 ± 3.0	**0.046***
	After	**ZBMI**	0.3 ± 1.3	0.0 ± 1.1	0.525
		OPG	707.5 ± 178.6	646.0 ± 196.9	0.354
		Vitamin D	39.9 ± 9.7	42.4 ± 11.3	0.497
Arthritis	**Before**	**ZBMI**	− 0.1 ± 1.1	− 0.4 ± 1.7	0.574
		OPG	292.0 ± 90.2	491.4 ± 239.0	0.097
		Vitamin D	20.0 ± 7.2	32.5 ± 11.6	**0.002***
	After	**ZBMI**	0.3 ± 1.0	0.1 ± 1.3	0.637
		OPG	683.0 ± 199.5	673.7 ± 183.4	0.891
		Vitamin D	38.8 ± 10.4	42.9 ± 10.3	0.268
Abdominal Pain	**Before**	**ZBMI**	− 0.1 ± 1.3	− 0.3 ± 1.0	0.791
		OPG	346.9 ± 160.9	259.2 ± 22.8	0.062
		Vitamin D	23.6 ± 9.8	17.3 ± 5.3	0.110
	After	**ZBMI**	0.1 ± 1.3	0.2 ± 1.1	0.870
		OPG	735.8 ± 182.3	634.8 ± 183.9	0.127
		Vitamin D	41.8 ± 11.3	40.6 ± 10.0	0.749

Independent *t* test. ANOVA test. *Significant

[44]. This disagrees with our results which detected a significant decrease in the attack frequency after vitamin D administration. Kisacik et al. concluded that vitamin D deficiency in the FMF patients may trigger the attacks which goes parallel with our findings [31]. Onur et al., Kisacik et al., and Erten et al. reported low serum 25-hydroxy vitamin D concentrations in FMF patients, and female patients with FMF were most strongly affected [11, 15, 31]. However, most of these previous studies were conducted in adults. These results are in contrast to our study as we did not detect any significant difference in vitamin D serum level between males and females with FMF either before or after vit D treatment ($p > 0.05$).

In addition, Onur et al. concluded that there was no significant difference between vitamin D plasma concentrations in patients with and patients without articular symptoms. This is not concomitant with our results, which presented those cases with arthritis before vitamin D administration had significantly lower vitamin D serum level than those without ($p < 0.05$) [11].

The five most frequent mutations of the MEFV gene are E148Q, M680I, M694V, M694I, and V726A [45–47]. In the current study, the homozygous genotype mutations of M694I and M694I + M680I were the most frequent (63.6% & 27.3%, consequently). This coincides with the results of Kazem et al., who reported M694I, M694V, M680I, E148Q, and V726A mutations in a sample of Egyptian FMF patients [40].

Regarding the relation between vitamin D serum levels and different MEFV gene mutations, the present study demonstrated no significant difference in vitamin D serum levels among our cases in relation to MEFV gene mutations ($p > 0.05$). This is in agreement with the study of Onur et al., in which vitamin D plasma concentrations were similar in FMF cases with different MEFV mutations [11].

Many studies as those done by La Montagna et al., Carbone et al., & Harrison et al., revealed that chronic inflammation lowers BMD [48–50]. Recently, it has been shown that subclinical inflammation might persist in patients with FMF, even in the attack-free periods [51]. The continuous subclinical inflammation in FMF patients may lead to osteoporosis. Past researches revealed low BMD levels in patients with FMF whether children or adults which cannot be prevented by regular administration of colchicine [52–54].

Osteoprotegerin (OPG), is identified as "osteoclast inhibiting factor", prevents excessive resorption of bone by inhibiting the final steps of osteoclastogenesis. It prevents osteoclast differentiation, inhibits vascular calcification, and controls apoptosis [55].

In prior researches, the serum level of OPG was established to increase in chronic inflammatory illnesses, like rheumatoid arthritis, juvenile idiopathic arthritis [56], and inflammatory bowel disease [57], and in females who had osteoporosis [58]. High OPG concentrations can be a reaction as a defensive mechanism

against bone loss or against the inflammatory cytokines [54].

Administration of vitamin D inhibited damage of bone in some diseases as osteoporosis [59]. Moreover, vitamin D was supposed to enhance formation of bone and/or prevent bone destruction in diseases connected to bone [60].

The current study detected significant increase in serum OPG level after vitamin D administration. While Yuksel et al. detected significant higher OPG serum levels and lower BMD in patients with FMF. FMF and OPG present were independent factors for osteopenia and/or osteoporosis. They explained that the presence of periodic and/or subclinical inflammation could reduce BMD, that in turn might elevate OPG serum concentrations as a compensatory mechanism. These increases in OPG levels may prevent excessive osteoporosis [54].

In addition, Feng et al. concluded that 1,25 $(OH)_2$ D3 may increase OPG/RANKL ratio and facilitate anti-inflammatory response in an inflammatory environment of synoviocyte, leading to inhibition of osteoclastogenesis induced by inflammation in rheumatoid arthritis [61]. This is in agreement with our findings as there was significant increase in the OPG serum levels after vitamin D administration.

In a study carried out by Stern et al., it was found that OPG levels and bone mineral density were greater in mice receiving vitamin D. This goes in parallel with our results as we detected increase in OPG level after vit D administration in FMF patients [62].

On the other hand, to determine the relationship between genotype and OPG, vitamin D serum levels, and BMI and the clinical response to vitamin D administration, we analyzed the difference among different MEFV mutations of our patients and we did not detect any statistically significant differences among these mutations regarding BMI, OPG, and vitamin D serum levels before or after vitamin D treatment in our cases. This agrees with the results of Yuksel et al., in which the differences between these mutations were not statistically significant, in relation to bone mineral density and serum levels of osteoprotegerin [54]. Nevertheless, five common mutations were only investigated in our study. There are more than 160 well established mutations in FMF patients [3]. Consequently, further studies with greater number of cases are needed.

Moreover, in our study, we were unable to find any correlation between colchicine dose and vitamin D or OPG serum levels. This is similar to Yuksel et al., who did not find any relation between parameters of osteoporosis and colchicine treatment and stated that consistent administration of colchicine had no effect in the prevention of reduction in BMD [54]. However, our study group was limited in number. Two prior studies done by Duzova et al. & Suyani et al. evaluated children and adult FMF cases for BMD, which was significantly reduced compared with the healthy individuals. Nevertheless, the exact influence of colchicine on bone mineral density cannot be determined as all patients had been taking colchicine [52, 53].

The definitive effect of vitamin D versus colchicine on BMD, BMI, and control of disease severity in patients with FMF requires prospective case–control study comparing a group given vitamin D without colchicine versus a group receiving colchicine only; however, it is unethical to deprive individuals diagnosed as FMF of colchicine.

Consequently, we thought that administration of vitamin D especially, with its known immunological effects as an adjuvant therapy to colchicine may help those patients to increase BMD, improve their growth, decrease disease severity, and lead a better normal life. Interestingly, this was proved by our study as it demonstrated a significant increase in BMI and OPG serum levels, while significant reduction in duration of the attacks was detected ($p < 0.001$) after administration of vitamin D. Moreover, we detected significant improvement in the clinical characteristics of our patients as illustrated by a significant decrease in the number of patients having fever, arthritis, and abdominal pain ($p < 0.001$). There was also a significant decrease in the attack frequency ($p < 0.001$).

Surprisingly, in our study, no correlation between OPG serum level and BMI could be detected. In contrast to our findings, in a study done by Lambrinoudaki et al. [63], the serum levels of OPG were inversely associated with BMI [64]. In agreement with those results, lately, certain studies by Vik et al. & Dimitri et al. [65, 66] and another study done on children by Xiang et al. [67] described negative correlation between BMI and OPG, whereas coinciding with our results, many studies as those done by Wasilewska et al., Gannage-Yared et al., & Anand et al. reported no impact of BMI on OPG serum level [68–70].

Furthermore, OPG serum level has been positively correlated to age in adults as found by Dimitri et al. & Anand et al. [66, 70]; however, opposing findings have been obtained in children and adolescents by Wasilewska et al. & Gannage-Yared et al. [68, 69]. OPG was negatively associated with age in the study of Lambrinoudaki et al. also [64]. Contradicting to these studies, we could not detect any correlation between OPG serum level and the age of our patients.

Vitamin D is necessary to preserve bone health. Numerous molecules mainly receptor activator of NF-kB ligand (RANKL and a RANKL antagonist (osteoprotegerin) are made by osteoblasts, and activated $CD4^+$ T lymphocytes and are significant controllers of bone remodeling [71]. The relative ratio of RANKL to OPG

in the osteoclast precursor microenvironment can determine mature osteoclast formation. Vitamin D has been found to decrease OPG, and the combination of increased RANKL expression and decreased expression of OPG as vitamin D enhances maturation and stimulation of osteoclasts and increases bone resorption [72].

Considering this close relationship of vitamin D with osteoprotegerin, we assessed the relation between serum levels of vitamin D and OPG; there was a significant positive correlation between their serum levels ($p = 0.01$).

However, Kitazawa et al. stated that despite the fact that vitamin D firstly releases OPG, long-standing administration of vitamin D resulted in a recovery of OPG expression. This proposed that the catabolic impacts of vitamin D may be temporary. Actually, vitamin D has numerous anabolic impacts on osteoblasts, comprising stimulation of osteopontin and alkaline phosphatase. Thus, vitamin D seems to encourage bone resorption, which is essential for bone remodeling and construction of new bone. In addition, prolonged administration of vitamin D may facilitate osteoblast proliferation and differentiation [72].

$1,25(OH)_2$ D3 was found to prevent osteoclastogenesis stimulated by inflammation in rheumatoid arthritis in a study conducted by Yavuzer et al. in China. That study explained this by rising of the OPG/RANKL ratio by inducing OPG more than RANKL. This is supportive to our results [72].

Our study seems to support the opinion that although vitamin D induces RANKL expression that accelerates osteoclast differentiation and osteoclast activity, it may prevent osteoclastogenesis through inducing the compensatory OPG production.

The study limitation is the small sample size.

Conclusion

Vitamin D supplementation improved the clinical presentation, BMI, and bone mineral density as indicated by increase in serum OPG level in children with FMF. We endorse that our results should be established by greater studies.

Abbreviations

BMD: Bone mineral density; BMI: Body mass index; FMF: Familial Mediterranean fever; Ht: Height; MEFV: Familial Mediterranean fever gene; OPG: Osteoprotegerin; TNF: Tumor necrosis factor; VDR: Vitamin D receptor; Wt: Weight.

Authors' contributions

HR: conceptualization and design of the study, anthropometric measures of patients, collecting and entering data, literature search, writing the original draft, preparation, and editing of the final manuscript. *MM*: clinical examination of patients, collecting data, investigations, visualization, and resources. *WA*: literature search, visualization, and resources. *AA*: clinical examination of patients, collecting data, investigations, visualization, and resources. *ER*: methodology, investigations and resources. *HH*: statistical analysis of data and interpretation and writing the results. *HT*: conceptualization, clinical examination of patients, collecting data, methodology, resources, supervision, and writing, reviewing and editing the manuscript. The authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Medical Research Ethics Committee of National Research Center (no: 19/018) according to the "World Medical Association Declaration of Helsinki" in 1995 (as revised in Seoul 2008). Written informed consent was obtained from parents of the children enrolled in the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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