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RESEARCH ARTICLE

Vitamin D status, serum lipid concentrations, and vitamin D receptor (VDR) gene polymorphisms in Familial Mediterranean fever

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ABSTRACT

Vitamin D (VitD) is critical for the regulation of inflammatory processes, and VitD deficiency has been linked to several chronic inflammatory disorders. We aimed to investigate the concentrations of serum 25(OH)D3, lipid parameters, and three known *VDR* polymorphisms (*BsmI*, *FokI*, and *TaqI*) in patients with Familial Mediterranean fever (FMF), an autosomal recessive autoinflammatory disease. The study included 123 FMF patients and 105 controls. A total of 38 patients were in acute attacks at the time of investigation. Serum 25(OH)D3 concentrations were determined using liquid chromatography–tandem mass spectrometry. *BsmI*, *FokI*, and *TaqI* polymorphisms were analyzed by a competitive allele specific polymerase chain reaction assay (KASPar). Serum lipid parameters were measured with enzymatic colorimetric methods. 25(OH)D3 concentrations were lower in FMF patients compared to controls ($p < 0.001$). No difference was observed in 25(OH)D3 concentration between patients with acute attack and those in attack-free period ($p = 0.193$). The distributions of *FokI* and *TaqI* genotypes were not significantly different between FMF patients and controls. There was a significant difference in the distribution of AA *BsmI* genotype between male FMF patients and male controls. Increased concentrations of triglycerides ($p = 0.012$) and decreased concentrations of high-density lipoprotein cholesterol [HDL-C] ($p = 0.006$) were found in FMF patients compared to controls. Although lower 25(OH)D3 concentrations were observed in FMF patients versus controls, no association was determined between FMF attack frequency and 25(OH)D3 concentrations. We showed that the AA genotype of *BsmI* polymorphism is associated with FMF in males but not in females. The effects of decreased HDL-C and increased triglyceride concentrations on cardiovascular events in FMF patients should be further investigated.

KEY WORDS: 25(OH)D3; FMF; Familial Mediterranean fever; serum lipids; VDR polymorphisms

INTRODUCTION

Familial Mediterranean fever (FMF), an autosomal recessive autoinflammatory disease, is characterized by recurrent fever, abdominal attacks, prodromes, and pericarditis [1]. Mutations in the Mediterranean fever (MEFV) gene encoding a protein called pyrin are found in many FMF cases. Mutations have been widely found in exons 1, 2, 3, 5, 9, and 10 of the *MEFV* gene. The five most frequent mutations are E148Q, M680I, M694V, M694I, and V726A [1-3]. FMF is common in Mediterranean and Middle Eastern populations but sporadic cases have been reported in many other populations [4,5]. Abnormal activation of innate immune system is important player in the pathogenesis of autoinflammatory diseases [6,7]. The proposed molecular mechanism in the pathogenesis of FMF is increased with inflammasome activation due to the restricted pyrin expression [8]. Cytokines activated by inflammasomes stimulate neutrophils and macrophages and induce inflammatory response [9].

The inflammatory process can be affected by different mechanisms such as the regulation of proinflammatory transcription factor and cytokine gene expressions. These effects lead to the inhibition of lymphocyte proliferation and the secretion of cytokines [10,11]. The actions of vitamin D (VitD) are mediated by the vitamin D receptor (*VDR*), which is a DNA binding transcription factor [12]. The human *VDR* gene is located on chromosome 12q12-14. The single nucleotide polymorphisms of the *VDR* gene, *BsmI*, *FokI* and *TaqI*, have associated with inflammation pathways [11]. Experimental and clinical studies have also demonstrated the role of inflammation in the development of cardiovascular events. Different factors such as serum lipid changes, endothelial dysfunction having found it to be associated with cardiovascular events [13]. The aim of this study was to investigate the 25-OH-Vit D3 and serum lipids concentrations and three known VDR polymorphism (*BsmI, FokI and TaqI*) in patients with FMF. We also evaluated

the association between attack frequency and 25-OH-Vit D3 concentration. Besides, the changes in the concentrations of serum lipids and their relationship with *VDR* polymorphisms were evaluated. The study had a larger sample size than previous studies [14-17]. To the best of our knowledge, no previous study has concurrently investigated *VDR* polymorphism, 25-OH-VitD3 and serum lipid concentrations in patients with FMF. This study provides an important opportunity to advance the understanding of the influence of VitD, *BsmI*, *FokI* and *TaqI polymorphisms* and serum lipids in FMF.

MATERIALS AND METHODS

Patients and controls

Study populations comprised 123 FMF patients [57 males and 66 females; aged 18–62 years (mean age: 37.01 ± 10.46 years)] and 105 healthy controls [52 males and 53 females; aged 19 - 57 years (mean age: 37.71 ± 8.06 years)]. In this study, we composed patients group randomly. No effort has been made to ensure that the number of women and men is equal. FMF diagnosis was made according to the Tel-Hashomer criteria [18]. A total of 38 patients were in acute attacks at the time of investigation. We also grouped patients according to attack frequency history within last three months. 70 patients had no attack (group 1), 30 patients had 1-2 attacks (group 2) and 23 patients had 3 or more attacks (group 3) within the last three months. The diagnosis of FMF attacks was confirmed by the presence of fever, clinical findings of serositis/arthritis, skin rash, and elevated C-reactive protein (CRP > 5mg/L) concentrations. 113 patients had been receiving only stable doses of colchicine (1.5 mg/day). In addition, two patients were taking Anakinra®and colchicine. 8 patients were not taking any drugs. Patients with impaired renal and thyroid function, diabetes mellitus, intestinal, musculoskeletal or skin diseases, liver disease, malignancy, or pregnancy were excluded from the study. For the healthy control group, the exclusion criteria included a clinical suspicion of any infections (body temperature out of the range of 36-38 °C, heart rate > 90 bpm, respiratory rate > 20/minute, and white blood count > 12,000/mm3 or < 4,000/mm3), the presence of liver disease, kidney disease, rheumatic disease, malignancy, pregnancy and smoking. Individuals taking VitD supplementation were not included in the study population. Genotype distributions of *MEFV* mutation, the values of creatinine, CRP, white blood cell count, and hemoglobin, were obtained from the Ankara Numune Education and Training Hospital laboratory information system. None of the patients or controls had any condition that could affect the lipid profile such as familial

dyslipidemia, obesity, metabolic syndrome and diabetes. Samples were sent by physicians from Ankara Numune Education and Research Hospital, Department of Rheumatology. The protocol was approved by the Ethics Committee of Ankara Numune Education and Training Hospital (E-15-422). Written informed consent was obtained from all participants.

Samples

Overnight fasting blood samples were collected from all participants into red top tubes and tubes containing ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson, UK). The red top tube was used for the analysis of 25-OH-Vit D3 and serum lipid analysis. The EDTA tube was used for molecular analysis of *BsmI (rs1544410), FokI (rs2228570) (tagging rs10735810), and TaqI (rs731236)* polymorphisms. The blood samples were obtained in the same season (03/16-08/16) from all of the patients and controls to avoid the seasonal variation of sun exposure on the 25-OH-Vit D3 status.

Determination of vitamin D and serum lipid concentrations

25-OH-Vit D3 concentrations were measured using liquid chromatography–tandem mass spectrometry (LC–MS/MS). LC–MS/MS was performed using the Shimadzu Prominence HPLC system (Kyoto, Japan) which is coupled to an AB Sciex API 3200 triple quadrupole mass spectrometer (Framingham, MA, USA).Total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations were determined by colorimetric enzymatic methods (Beckman Coulter, USA).

Genotyping

The genomic DNA was extracted using the RTA DNA Blood Kit (RTA, Istanbul, Turkey) according to the manufacturer's instructions. Single nucleotide polymorphisms (SNPs) were selected based on the functional relevance and minor allele frequency (> 0.05) using genotype data obtained from Caucasian individuals in the HapMap project (HapMap Data Rel 24/Phase II

Nov08, on NCBI B36 assembly, dbSNP b126). In this study, 3 SNPs were examined on the *VDR gene*: *BsmI* (rs1544410), *FokI*(rs2228570) (tagging rs10735810), and *TaqI* (rs731236). Genotyping was performed at the Dıskapı Yıldırım Beyazıt Traning and Research Hospital (Ankara, Turkey) using a previously validated allele-specific PCR-based KASPar SNP genotyping system (KBiosciences, Hoddesdon, UK). Thermocycling was performed according to the manufacturer's instructions. Detection was performed using a Rotor-Gene Q 6 plex Platform system with V2.0 software (Qiagen, Germany).

Statistical analysis

The conformity of the data to normal distribution was assessed with the Shapiro-Wilk test. The Mann-Whitney U-test was used to compare the differences of non-parametric variables. A $\chi 2$ analysis was used to compare the differences of categorical variables. The Kruskal-Wallis test was used to compare the 25-OH-Vit D3 concentrations between groups divided according to the attack frequencies. Genotype frequencies were compared between the patients and the control groups using the $\chi 2$ test. As an estimation of relative risk of the disease, odds ratios (OR) were calculated on the basis of 95% confidence intervals (CI). The independent-samples t-test was used to compare the HDL-C, LDL-C, total cholesterol, triglyceride and 25-OH-Vit D3 of the study groups between the wild-type and polymorphic genotype of rs1544410, rs731236 and rs222857polymorphisms in the patient and control groups. Analyses were performed using IBM SPSS software (release 20.0, IBM, SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the study population are shown in Table 1. *MEFV* mutations were detected in 96 patients. We did not detect any *MEFV* mutation in 27 FMF patients. The genotype distributions of *MEFV* mutations are shown in Table 2.

25-OH-Vit D3 concentrations in patients

In a report made by Hollis et al. [19] circulating levels of 25-OH-Vit D3 that are less than 32 ng/mL was considered as a vitamin deficiency. In our study, 25-OH-Vit D3 concentrations were above 32 ng/mL in two patients and 12 healthy controls. Median 25-OH-Vit D3 concentrations were 10.70 (7.70-14.40) and 17.40 (10.75-26.50) ng/mL in the patient and control groups, respectively ($p<0.001$). The median 25-OH-Vit D3 concentrations were 10.70 (7.74-14.33) ng/mL and 12.20 (7.70-25.61) ng/mL in patients with acute attack (n: 38) and attack-free period (n:85), respectively ($p = 0.193$). The mean 25-OH-Vit D3 concentrations were 10.20 (7.47-13.09), 10.45 (7.98-16.16) and 11.20 (7.95-15.17) ng/mL in groups 1, 2 and 3 respectively. No statistically significant difference was determined between the 3 groups in terms of 25-OH-Vit D3 concentrations (Figure 1).

Serum lipid concentrations in patients

A statistically significant difference was found in terms of HDL-C ($p = 0.006$) and triglyceride ($p = 0.012$) concentrations between the patient group and the control group. The results of the comparisons of the serum lipid parameters between the patient and control groups are shown in Table 3. We also compared serum lipid parameters between patients with acute attack and attack-free period. There was statistically significant difference were observed between groups in terms of HDL-C ($p < 0.001$) and triglyceride ($p = 0.043$) concentrations (Table 4.)

BsmI, FokI and TaqI polymorphisms of VDR genes in patients

The frequencies of genotypes and alleles for *BsmI* (rs1544410),*FokI* (rs2228570) and *TaqI* (rs731236) polymorphisms of *VDR* genes in FMF patients and the control group are shown in Table 5. There were no significant differences were observed in the distributions of genotypes for FokI (rs2228570) (chi-square=2.09;p=0.35) and TaqI (rs731236) (chi-square=0.091, p=0.95) between the patients and the control group. However, statistically significant differences were determined between the two groups in respect of BsmI (rs1544410) genotype distribution (chi-square=6.11, p=0.047).

When genotypes frequencies for the three SNPs were analyzed for gender separately in patient and control groups, no statistically significant difference is observed in the genotype distribution of *FokI* (rs2228570) and *TaqI* (rs731236) polymorphisms between groups in females (p=0.08; 0.27, respectively) or males (p=0.09; 0.15, respectively).There was no significant odds ratio for FMF in either the male or female groups (p>0.05). Statistically significant differences were found between the patient and control groups in genotype frequencies of *BsmI* (rs1544410) polymorphism in males (p=0.02), but not in females (p=0.58). Males carrying the AA genotype of the *BsmI* (rs1544410) polymorphism were determined to have a 2.62-fold significantly increased the riskof FMF (OR, 2.63; 95%CI, 1.12-6.01) compared to males carrying the GG or AG genotypes (Table 6).

When HDL-C, LDL-C, total cholesterol, triglyceride and VitD concentrations of the study groups were analyzed for the three SNPs of *VDR* gene in the patient and control groups, there were no statistically significant differences in any of these parameters between wild-type and polymorphic genotype of *BsmI* (rs1544410), *TaqI* (rs731236) and *FokI* (rs2228570) polymorphisms in both the patient and control groups.

DISCUSSION

Seven principal findings were emerged from the results of the current study: (i) 25-OH-Vit D3 concentrations were found to be lower in patients, (ii) there was no statistically significant difference between patients in an attack period and those in an attack-free period in terms of 25-OH-Vit D3 concentrations, (iii) no statistically significant difference was determined between patients grouped according to attack frequency in terms of serum 25-OH-Vit D3 concentrations, (iv) no significant association was observed between patients and controls in terms of the frequencies of genotype for *Fok1* (rs2228570) and *TaqI* (rs731236) polymorphisms , (v) males carrying the AA genotype of the *BsmI* rs1544410 polymorphism are at a 2.62-fold significantly increased risk of FMF (OR, 2.63; 95%CI 1.12-6.01) compared to males carrying the GG or AG genotypes of *BsmI* (rs1544410) polymorphisms, (vi) increased triglyceride and decreased HLD-C concentrations were found in patients,(vii) no association was found between *BsmI* (rs1544410), *FokI* (rs2228570), *TaqI* (rs731236) polymorphisms, serum lipid levels and 25-OH-Vit D3 concentrations in patients or the control group.

Lower serum VitD concentrations were detected in patients compared to healthy control group. This finding were consistent with previous studies [14-17]. The lower VitD concentration may arise from two possible reasons including VDR polymorphism and colchicine use. In the present study, no relationship was determined between VitD concentrations, *BsmI* (rs1544410), *FokI* (rs2228570) *and TaqI* (rs731236) polymorphisms. To date, no evidence has associated colchicine use with intestinal malabsorption of low VitD concentrations, although colchicine has been linked to impaired absorption of different endogen nutrients such as vitamin B12 and lactose [20,21]. In addition, in studies by Anık et al. [16] and Karatay et al. [22] a strong relationship was found between colchicine use and low serum VitD concentrations in patients with FMF and Behçet's disease, respectively. In the current study, 115 patients received stable doses of

colchicine (1.5 mg/day). The lower VitD concentration in patients might be related to colchicine use. A correlation was reported between a deficiency of VitD and FMF attacks [17], whereas no difference between patients with acute attack and attack-free periods were determined in the current study. In addition, no significant difference was determined in terms of VitD concentrations between patients grouped according to attack frequency. Accordingly, we speculated that VitD might not be considered as an important factor for triggering the FMF attack. Large proportion of the patients had no or 1-2 attacks within the last three months and the number of the patients who had 3 or more attacks within the last three months was low in this study. Therefore further studies with larger study population need to confirm this conclusion. The association between *VDR* polymorphism and rheumatological diseases such as rheumatoid arthritis, systemic lupus erythematous and Behcet's diseases has been investigated in different studies [23-26]. Conflicting results have been reported in these aforementioned studies. An association was reported between *FokI* (rs2228570) polymorphism and rheumatoid arthritis in a meta-analysis by Song et al. [23]. However, no association was found between *BsmI, TaqI* polymorphisms and rheumatoid arthritis in the same metanalysis. In a study byAteş et al. [24] the distributions of *BsmI*(rs1544410), *FokI*(rs2228570), *and TaqI*(rs731236) genotype frequencies were found to be similar in patients with rheumatoid arthritis and the control group. In 2015, Carvalho et al. [25] demonstrated a correlation between CT genotype of *Fok I* polymorphism, TT genotype of *TaqI* and a worse prognosis in patients with systemic lupus erythematosus. An association for the *FokI* polymorphism but not for *TaqI* and *BsmI* polymorphism in patients with Behçet's disease has also been reported [26]. To date, there has been only one study on the relationship between FMF and *VDR* polymorphism. In that study by Kızıldağ et al. [27], no association was found between the four common *VDR polymorphisms* (*FokI, TaqI, BsmI,* and *ApaI)* and FMF. The findings of the current study are in accordance with the results of Kızıldağ

et al. [27] in terms of *FokI(rs2228570), and TaqI (rs731236)*polymorphisms. Therefore, it is thought that the polymorphisms, *FokI*(rs2228570) *and TaqI*(rs731236), were not associated with susceptibility to FMF in the Turkish population. The results of the current study also showed a statistically significant difference in the genotype distribution of *BsmI* (rs1544410) polymorphism between the patient and control groups in males (p=0.02), but not in females (p=0.58). Males carrying the AA genotype of the *BsmI* (rs1544410) polymorphism were calculated to have a 2.63-fold significantly increased risk of FMF (OR, 2.63; 95%CI 1.12-6.01) compared to males carrying the *GG* or *AG* genotypes. This finding discordant with the study of Kızıldağ et al.[27]. Sex-related differences in the distribution the genotypes of *BsmI* polymorphism were also reported in several cases [28-30]. Bodoki et al. showed that significantly different *BsmI* polymorphism genotypes distribution between male and female idiopathic inflammatory myopathy patients [28]. Different genotype frequencies *BsmI* have been also observed between male and female patients with Graves' disease [29]. Finally, the AA genotype for *BsmI* polymorphism has been associated with higher body mass index, higher waist circumference, and lower adiponectin levels in randomly selected healthy individuals [30]. There is no study about the sex-linked incidence of FMF. In the study made by Dogan et al. no significant difference was observed between the male and female patients in terms of heterozygote and homozygote mutation carriage rate [3]. However, it was reported a correlation between amyloidosis and male gender [31,32]. Positive association was found between the b allele of the *BsmI* and the increased risk of developing breast cancer in the cases and controls from the Pakistani population [33]. The VDR *BsmI* BB genotype and B allele were found overrepresented among SLE patients. In the same study, the BB genotype of *BsmI* constituted a risk factor for the development of nephropathy among studied patients with SLE [34]. It was also found a correlation between AA genotype of *BsmI* and higher levels of antinuclear antibodies

(ANA) in patients with SLE. As a result, it was speculated that the AA genotype of *BsmI* (rs1544410) polymorphism might be related with the clinical findings of FMF in men. However; further studies are required with larger sample sizes to confirm this hypothesis.

There are conflicting data about serum lipids concentrations in FMF patients. In a study by Acay et al. [35] lower HDL-C and higher triglyceride concentrations were reported in patients with FMF. Candan et al. [36] found that the difference was observed only in terms of HDL-C concentrations compared to the healthy control group. It was shown that the FMF patients had lower concentrations of total cholesterol and HDL-C than in controls [37]. Additionally, higher triglyceride to HDL-C ratio was found in patients with chronic inflammatory diseases includes FMF [13]. In the present study, higher triglyceride and lower HDL-C concentrations were determined in patients compared to the control group. Besides, these differences were also observed between patients with an acute attack and those in an attack-free period. The present findings are consistent with the study by Acay et al. [35] and Keles et al. [13].In the current study, no association was found between *BsmI*(rs1544410), *FokI*(rs2228570), *and TaqI*(rs731236) polymorphisms and serum lipids. These inconsistent results may be related to differences in studies in respect of patient selection criteria, exposure to disease, sampling of patients at different stages of the disease, differences in *MEFV* mutations and study population number. We think that the changes in HDL-C and triglyceride concentrations may be related to the inflammatory process seen in FMF. It is well known that decreased concentrations of HDL-C and increased concentrations of triglyceride are associated with an increased risk of cardiovascular disease [38,39]. Therefore, we believed that increased triglyceride and decreased HDL-C concentrations may reflect increased atherosclerotic risk in patients with FMF. So, changes in HDL-C and triglyceride concentrations should be carefully monitored in patients with FMF to reduce the risk of future cardiovascular events.

CONCLUSION

The following conclusions can be drawn from the present study; (i) although 25-OH-Vit D3 concentrations were lower in patients with FMF than the healthy control group, no association was found between VitD concentrations and attack formation. (ii) There is no association between FMF and VDR *FokI* (rs2228570) and*TaqI* (rs731236) polymorphisms but the AA genotype of *BsmI*(rs1544410) polymorphism is associated with FMF in males but not in females. (iii) Because of the changes in serum concentrations of HDL-C and triglyceride further studies are needed to understand the effects of decreased HDL-C and increased triglyceride concentrations on cardiovascular events in FMF patients.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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FIGURES AND TABLES

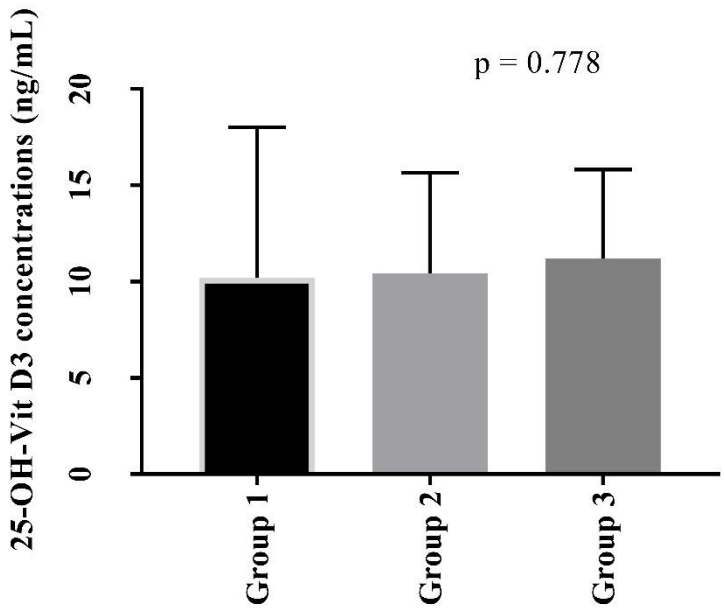

FIGURE 1. Comparisons of 25-OH-VitD3 concentrations in patients grouped according to attack frequency

TABLE 1. Baseline characteristics of study population

Characteristics	**Patients (n:123)**	**Controls (n:105)**	***P* value**
Age (years)	37.01 ± 10.46	37.71 ± 8.06	0.099
Male and female (n)	57/66	52/53	0.596
Creatinin (mg/dL)	0.78 (0.69-0.92)	0.79 (0.67-0.88)	0.192
CRP (mg/dL)	5.00 (1.00-12.00)	2.40 (1.17-4.33)	0.005
WBC (10^3mcL)	7.65 (6.37-9.30)	7.80 (6.70-8.80)	0.529
Hb (g/dL)	13.80 (12.50-15.25)	14.60 (13.60-15.70)	0.527
Disease duration (month)	91.76±85.80	none	
Disease onset (age)	28.76±10.84	none	
Family history (yes/no)	74/49	none	
Fever (yes/no)	59/64	none	
Abdominal pain (yes/no)	114/9	none	
Chest pain (yes/no)	35/88	none	
Erysipeloid (yes/no)	58/65	none	

CRP: C-reactive protein, WBC: White blood cell, Hb: Hemoglobin. Results are expressed as mean ± SD with 95% confidence intervals.

TABLE 2. Genotype and frequency of detected mutations in patients

Mutation type	***Genotype***	***Patients***
		n
Homozygote	M694V	18
	M680I	5
	A744S	1
Compound homozygote		
	M694V/R202Q	3
	E148Q/M694V	1
Heterozygote		
	M694V	11
	E148Q	9
	V726A	3
	M680I	2
	K695R	1
	G304R	1
	A744S	1
Compound heterozygote		
	M694V/V726A	13
	M680I/M694V	9
	M694V/E148Q	4
	V726A/R761H	1
	M694V/R202Q	2
	E148Q/M694I	2
	E148Q/M680I	2
	E148Q/L110P	1
	E148Q/R202Q	1
	M69V/R761H	1
	V726A/A744S	1
	V726A/R202Q	1
	A744S/M694V	1
	V726A/M680I	1
Total		96

TABLE 3. Comparison of median total cholesterol, triglyceride, LDL-C and HDL-C concentrations in patients and controls

Serum lipid parameters	**Patients (n:123)**	**Controls (n:105)**	***P***
Total cholesterol(mg/dL)	177.00(153.00-202.00)	184.00 (154.00-210.00)	0.570
Triglyceride (mg/dL)	99.00 (77.00-171.00)	87.00 (70.00-121.50)	0.012
HDL-C (mg/dL)	47.00 (38.00-54.00)	50.00 (43.00-57.00)	0.006
LDL-C (mg/dL)	106.00 (87.00-130.00)	107.00 (85.80-132.20)	0.807

LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol Results are expressed as median (25^{th} - 75^{th} percentiles) with 95% confidence interval

TABLE 4. Comparison of median total cholesterol, triglyceride, LDL-C and HDL-C concentrations in patients with acute attack and attack-free period

Serum lipid parameters	Acute attack (n:38)	Attack free period (n:85)	*P*
Total cholesterol(mg/dL)	160.00(136.00-192.00)	177.00 (155.00-205.00)	0.655
Triglyceride (mg/dL)	95.50 (80.75-151.25)	76.00 (103.00-173.00)	0.043
HDL-C (mg/dL)	35.00 (29.00-54.00)	49.00 (41.00-55.00)	< 0.001
LDL-C (mg/dL)	99.50 (82.00-116.25)	107.00 (87.00-128.00)	0.456

LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol Results are expressed as median (25^{th} - 75^{th} percentiles) with 95% confidence interval

TABLE 5. Genotype frequencies and OR values for SNPs of VDR rs1544410, rs222857 and rs731236 polymorphisms in both groups

Genotypes	Controls N (%)	Patients N (%)	P value	OR (95% CI)
***VDR* gene *BsmI* (rs1544410) (G> A) polymorphism**				
GG	45 (42.8)	54 (43.9)		Reference
GA	30 (28.6)	51 (41.5)	0.32	1.45 (0.75-2.77)
AA	30 (28.6)	18 (14.6)	0.12	0.53 (0.24-1.15)
GA+AA	60(57.2)	69 (56.1)	0.98	0.99 (0.56-1.76)
G	125 (58.1)	159 (64.6)		Reference
A	90(41.9)	87(35.4)	0.21	0.76 (0.5-1.15)
***VDR* gene *FokI* (rs2228570) (C > T) polymorphism**				
CC	77 (73.3)	100 (81.3)		Reference
CT	12 (12.4)	10 (8.1)	0.35	0.61 (0.24-1.57)
TT	15 (14.3)	13 (10.6)	0.29	0.61 (0.26-1.44)
CT+TT	27 (26.7)	23 (18.7)	0.15	1.64 (0.84-3.22)
C	166 (79.8)	210 (85.4)		Reference
T	42 (20.2)	36 (14.6)	0.09	0.63 (0.37-1.06)
***VDR* gene *TaqI*(rs731236) (T>*C*) polymorphism**				
TT	54 (51.4)	66 (53.7)		Reference
CT	28 (26.7)	32 (26.0)	0.86	0.93 (0.46-1.92)
CC	23 (21.9)	25 (20.3)	0.84	0.89 (0.41-1.94)
CT+CC	51 (48.6)	57 (46.3)	0.77	0.92 (0.50-1.67)
T	136 (64.8)	164 (66.7)		Reference
C	74 (35.2)	82 (33.3)	0.78	0.91 (0.52-1.58)

*P<0.05 confirmed as significant; A: adenine, G: guanine, T: thymine, C: cytosine, OR: odds ratio, CI: confidence interval

TABLE 6. Distribution of *genotypes*and OR values for SNPs of VDR rs1544410, rs222857 and rs731236 polymorphisms *according to gender* in both groups

	Genotypes	Controls N (%)	Patients N (%)	P value	OR (95% CI)
***VDR* gene rs1544410 (G> A) polymorphism**					
Female	GG	23 (43.4)	24 (36.4)		Reference
	AG	17 (32.1)	28 (42.4)	0.37	1,53 (0.62-3.79)
	AA	13 (24.5)	14 (21.2)	1.00	0.97 (0.34-2.77)
Male	GG	23 (44.2)	29 (50.9)		Reference
	AG	13 (25.0)	23 (40.3)	0.48	1.52 (0.59-3.9)
	AA	16 (30.8)	5 (8.8)	0.03*	2.62 (1.12-6.01)
***VDR* gene rs2228570 (C > T) polymorphism**					
Female	CC	38 (71.7)	58 (87.8)		Reference
	CT	8 (15.1)	4 (6.1)	0.10	0.29 (0.07-1.19)
	TT	7 (13.2)	4 (6.1)	0.18	0.34 (0.08-1.39)
Male	CC	40 (76.9)	43 (75.4)		Reference
	CT	4 (7.7)	6 (10.5)	0.73	1.36 (0.34-5.46)
	TT	8 (15.4)	8 (14.1)	1.00	0.95 (0.31-2.89)
VDR gene rs731236 (T>C) polymorphism					
Female	TT	30 (56.6)	31 (47.0)		Reference
	TC	12 (22.6)	26 (39.4)	0.21	2.07 (0.76-5.63)

	CC	11 (20.8)	9 (13.6)	1.00	0.82 (0.24-2.76)
Male	TT	24 (46.1)	34 (59.6)		Reference
	TC	16 (30.8)	8 (14.0)	0.34	0.09 (0.11-1.05)
	CC	12 (23.1)	15 (26.4)	1.00	0.88 (0.31-2.45)

*P<0.05 confirmed as significant; VDR: Vitamin D receptor, A: adenine, G: guanine, T: thymine, C: cytosine, OR: odds ratio, CI: confidence interval