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Correlation of genetic mutations with inflammatory markers and micronutrient status in familial Mediterranean fever

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ABSTRACT

Aims: Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disorder characterized by recurrent febrile episodes and serosal inflammation. This study aimed to investigate the association between CRP levels, MEFV genotypes, and serum levels of vitamin D, vitamin B12, and iron in adult FMF patients.

Methods: This retrospective cross-sectional study included 392 adult FMF patients. Laboratory parameters evaluated in the analysis included erythrocyte sedimentation rate, C-reactive protein (CRP), fibrinogen, hemoglobin, white blood cell count, serum iron, total iron-binding capacity, ferritin, vitamin B12, vitamin D, and genetic findings.

Results: CRP and other acute phase reactants were significantly higher in the homozygous ($p<0.001$) and compound heterozygous ($p=0.002$) FMF groups, and significantly lower in carriers ($p<0.001$). Univariate logistic regression showed compatibility between CRP and other markers (fibrinogen and WBC), with significant associations in the two FMF genotype groups. Carriers had a significantly lower risk of elevated CRP ($p<0.001$). In multivariate analysis, male sex and homozygous mutation remained significant predictors of high CRP levels. No significant differences were found in vitamin D and ferritin levels, while vitamin B12 levels were significantly lower in carriers ($p=0.04$).

Conclusion: This study demonstrates that CRP levels were elevated at the time of diagnosis, particularly in FMF patients with a homozygous genotype. Given the high prevalence of FMF in the region, clinicians—especially primary care providers—should consider FMF in the differential diagnosis and pursue genetic testing when appropriate.

Keywords: Familial Mediterranean fever, C-reactive protein, genotype, inflammation, MEFV, vitamin D, vitamin B12

INTRODUCTION

Familial Mediterranean fever (FMF) is an inherited autoinflammatory disorder characterized by recurrent episodes of systemic inflammation, primarily affecting populations from the Mediterranean region. The condition is associated with mutations in the Mediterranean fever (MEFV) gene and is marked by periodic febrile attacks and serosal inflammation, posing significant diagnostic and management challenges. This autosomal recessive disorder was first described in 1945 and characteristic mutations were subsequently identified in 1992.[1] It is caused by mutations in the MEFV gene located on the short arm of chromosome 16 (16p13.3). The MEFV gene consists of 10 exons, with over 370 variants identified to date.[2,3] A considerable fraction of the pathogenic or likely pathogenic variants is situated within exon 10, which encodes the B30.2/SPRY domain that is instrumental in the activation of caspase-1. Within populations residing in regions endemic to FMF, the most frequently observed MEFV mutation is M694V (c.2080A>G). Additionally, other exon 10 variants that are often identified include M694I (c.2082G>A), V726A (c.2177T>C), and M680I (c.2040G>C and c.2040G>A), which collectively constitute approximately 75% of all FMF cases. MEFV mutations result in the synthesis of a mutant form of the pyrin protein; this mutant pyrin activates caspase-1 and induces excessive secretion of interleukin-1 beta (IL-1β). IL-1β plays a critical role in promoting the production of acute-phase reactants, including C-reactive protein (CRP), serum amyloid A (SAA), and pentraxin-3 (PTX-3).[2] Elevated concentrations of IL-1, in conjunction with heightened levels of cytokines such as IL-6 and tumor necrosis factor-alpha (TNF-α), contribute to an increase in leukocyte counts and acute phase reactants, thereby exacerbating systemic inflammation.[3-5]

FMF exhibits a notably higher prevalence within certain ethnic populations, particularly among groups such as Turks, Arabs, non-Ashkenazi Jews, and Armenians, thereby indicating a potential genetic predisposition that may be influenced by the shared ancestry and environmental factors associated with these specific communities. The condition typically presents during childhood with recurrent febrile episodes

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accompanied by systemic inflammatory manifestations, particularly aseptic serositis, arthritis, and pseuderysipelas. The episodes in question typically have a duration that ranges from one day to three days, and during the periods in which individuals are free from any clinical manifestations, various inflammatory markers, including leukocyte counts and acute-phase reactants, tend to return to levels that are considered normal. Nevertheless, it is important to note that in a subset of individuals, these inflammatory markers can remain persistently elevated, even when there are no overt clinical symptoms present, a condition that is referred to in the academic literature as subclinical inflammation. In instances where this phenomenon occurs, acute-phase reactants can serve as valuable indicators for monitoring the presence of ongoing inflammation, even during intervals when the individual appears to be symptom-free. Therefore, it is essential for researchers and clinicians to recognize and understand the implications of these elevated markers in order to effectively assess and manage inflammation in affected patients throughout their clinical course.[2,4]

Vitamin D serves a dual purpose as both a pro-hormone and a crucial micronutrient that plays a significant role in human health and biological processes. Traditionally, its primary function is recognized in the context of maintaining calcium balance within the body; however, recent scientific investigations have begun to uncover a broader spectrum of activity, revealing that a variety of immune cell types not only express the vitamin D receptor but also possess the requisite enzymes for its metabolism, thereby suggesting that hormonal vitamin D may be intricately involved in the modulation and regulation of immune and inflammatory responses. The extent of this immunomodulatory function is further corroborated by a growing body of observational research that consistently illustrates a negative correlation between serum levels of 25-hydroxyvitamin D [25(OH)D] and concentrations of CRP, thereby highlighting the potential significance of adequate vitamin D levels in mitigating inflammatory processes within the body.[6-10] These cumulative findings underscore the necessity for a deeper understanding of vitamin D's multifaceted roles beyond its classical functions, emphasizing its potential impact on immune system dynamics and the broader implications for health and disease management. Serum 25(OH)D is widely regarded as the most reliable biomarker of vitamin D status, whereas CRP is among the most used indicators of systemic inflammation in clinical settings. Although the causality of the 25(OH)D–CRP association remains debated due to inconsistent findings from randomized controlled trials, recent bidirectional Mendelian randomization (MR) analyses suggest that the effect is likely unidirectional, with 25(OH)D exerting a suppressive influence on CRP levels rather than the reverse.[11]

Iron homeostasis is primarily regulated by the hepcidin–ferroportin axis; however, this regulatory system is susceptible to disruption by immune activation. Perturbations in iron metabolism may, in turn, impair immune competence and influence the functionality of various immune cell types, including macrophages, T lymphocytes, and B lymphocytes. During infection or inflammatory conditions, the tightly regulated balance of iron can be disturbed. Pro-inflammatory cytokines such as interleukin-6 (IL-6), along with microbial components like lipopolysaccharides (LPS), can stimulate hepcidin synthesis, resulting in iron sequestration within cells and a subsequent decline in serum iron levels. Dysregulation of iron availability affects both innate and adaptive immune responses by altering cytokine production in macrophages, enhancing antimicrobial mechanisms, and modulating lymphocyte effector functions.[12,13]

Vitamin B12, also known as cobalamin, is a water-soluble essential micronutrient. One of its primary physiological roles, particularly in conjunction with folic acid, is to facilitate DNA synthesis, a process critical for cellular proliferation and division, including the development of T lymphocytes. As such, adequate levels of vitamin B12 are crucial for sustaining normal lymphocyte counts and maintaining immune balance, such as preserving a healthy CD4/CD8 ratio.[14]

Inflammation is a complex biological process predominantly regulated by the immune system. In recent years, optimizing immune function has garnered significant global attention, not only for the prevention of chronic diseases but also for mitigating the severity of acute illnesses. The roles of essential micronutrients such as vitamin D, vitamin B12, and iron in supporting immune competence and modulating inflammatory responses are well established, as their effects on immune health have been extensively studied.[9] Evaluation of the last five years of the literature shows that only two studies of pediatric FMF patients under the age of 18 have examined the relationship between vitamin D, B12 and folic acid levels and the frequency of acute attacks, and the relationship between vitamin D and subclinical inflammation and oxidative stress.[2,15] The aim of this study was to evaluate whether there is an association between CRP, an indicator of clinical or subclinical inflammation, and different genotype groups and between CRP and iron, vitamin B12 and vitamin D levels in adult FMF patients. The secondary objective was to assess whether these vitamin and mineral deficiencies differ between genotypes.

METHODS

This comprehensive research endeavor was granted the necessary ethical approval by the esteemed Non-interventional Clinical Researches Ethics Committee of Ankara Etlik City Hospital (Date: 09.08.2023, Decision No: AEŞH-EK1-2023-428). The methodology employed throughout the duration of this study was meticulously designed and executed in strict accordance with the ethical principles delineated in the Declaration of Helsinki, originally established in 1975 and subsequently revised in 2013.

This retrospective, cross-sectional and descriptive study included 392 patients over the age of 18 who underwent genetic analysis and counseling for the molecular diagnosis of FMF between 01/01/2019 and 01/01/2023 in the medical genetics department of the tertiary hospital. Age, sex, results of acute phase reactants such as sediments, CRP, fibrinogen, hemoglobin, white blood cells, serum iron, iron binding capacity, ferritin, vitamin B12 level, vitamin D level and genetic analysis results of these patients were evaluated. Individuals

diagnosed with metabolic bone disorders, malnutrition, chronic renal or hepatic insufficiency, or any other chronic condition coexisting with FMF were excluded from the study.

The comprehensive genotype characteristics pertaining to the various cases that were incorporated into the study are meticulously compiled and presented in **Table 1**, which serves as a pivotal reference point for understanding the genetic underpinnings of the conditions being investigated. In a fundamental categorization, the patient population was systematically partitioned into two distinct groups, specifically comprising those individuals who have received a formal diagnosis of FMF and those individuals who are classified as carriers of the condition, thereby allowing for a clearer examination of the genetic implications associated with each group. All patients diagnosed with FMF were also classified as homozygous and compound heterozygous.

Table 1. Descriptors of demographic data and laboratory values

Variables		
Age	Median (min-max)	36.00 (18.00-73.00)
Gender, n (%)	Male/female	159 (40.6)/233 (59.4)
Sedimentation (mm/h)	Median (min-max)	14.00 (2.00-94.00)
CRP (mg/L)	Median (min-max)	7.56 (0.21-235.00)
Serum iron (µg/dl)	Median (min-max)	86.40 (11.00-998.00)
Iron binding capacity (µg/dl)	Median (min-max)	308.50 (173.00-503.00)
Ferritin (µg/L)	Median (min-max)	28.50 (2.30-704.00)
Fibrinogen (mg/dl)	Median (min-max)	343.00 (0.66-976.00)
Hemoglobin (g/dl)	Median (min-max)	13.60 (8.30-17.80)
WBC (10^3/ml)	Median (min-max)	7.51 (3.32-24.10)
Vitamin B12 (ng/L)	Median (min-max)	225.00 (36.00-819.00)
Vitamin D (µg/L)	Median (min-max)	13.03 (3.00-70.00)
M694V homozygous	Yes	44 (11.2)
M694V heterozygous	Yes	131 (33.4)
M680I homozygous	Yes	10 (2.6)
M680I heterozygous	Yes	24 (6.1)
V726A homozygous	Yes	2 (0.5)
V726A heterozygous	Yes	27 (6.9)
M694I heterozygous	Yes	2 (0.5)
R761H homozygous	Yes	1 (0.3)
R761H heterozygous	Yes	5 (13)
E148Q homozygous	Yes	1 (0.3)
E148Q heterozygous	Yes	6 (1.5)
Exon 10	Yes	377 (96.2)
Exon 2	Yes	8 (2.0)

Min: Minimum, Max: Maximum, CRP: C-reactive protein, WBC: White blood cell

The statistical analyses and comparisons were performed in four groups: homozygous diagnosed patients, compound heterozygous diagnosed patients, all diagnosed patients with a combination of these two groups, and the carrier group. Socio-demographic characteristics, vitamin and mineral levels and genotypes were compared in patients who were divided into three groups according to CRP values (normal between 0-5 mg/L, slightly elevated between 5-15 mg/L, elevated 16 mg/L and above). In addition, ferritin, vitamin B12 and vitamin D levels were compared in all groups.

In order to accurately assess the serum levels of CRP, blood specimens were collected within the time frame of 8 a.m. to 10 a.m., ensuring that the participants had undergone a minimum of 8 hours of fasting overnight prior to the collection; these samples were then placed directly into specialized tubes that contained heparin as an anticoagulant and subsequently subjected to centrifugation to separate the serum. The concentrations of vitamin D and vitamin B12 within the serum were determined utilizing the chemiluminescence immunoassay technique (Beckman Coulter Access II system, California, USA). Furthermore, a comprehensive complete blood count (CBC) was conducted utilizing an automated hematology analyzer,–(Beckman Coulter LH780 California, USA). The evaluation of serum CRP levels was performed using the Cobas c702 autoanalyzer.

Vitamin D deficiency was defined as serum 25(OH)D levels below 20 ng/ml, whereas levels ranging from 20 to 30 ng/ml were classified as vitamin D insufficiency.[16] Vitamin B12 deficiency was defined by serum concentrations lower than 150 pg/ml.[17] Normal iron status was characterized by a serum ferritin concentration above 50 ng/ml, serum iron levels exceeding 60 µg/dl, and a total iron-binding capacity (TIBC) within the range of 250–450 µg/dl.[18]

The genotype data of the patients who had previously undergone MEFV genetic analysis were scanned and noted electronically. The diagnoses and phenotypic data of these patients at the time of diagnosis were also retrospectively analyzed in the hospital electronic system.

Statistical Analysis

The statistical evaluations and data analyses were meticulously conducted utilizing the sophisticated capabilities of SPSS software, specifically version 11.5, which is renowned for its robust data management and statistical analysis functionalities. In this detailed examination, quantitative variables were articulated in terms of their mean accompanied by the standard deviation or alternatively represented as the median alongside the minimum and maximum values, thereby providing a comprehensive overview of the data distribution, while qualitative variables were systematically presented in the form of counts and percentages to facilitate a clearer understanding of categorical data. This methodological approach ensures that the results are not only statistically sound but also easily interpretable, thereby enhancing the overall rigor and reliability of the findings. The Mann–Whitney U test was applied to compare two groups of

a qualitative variable with respect to a quantitative outcome, given that the assumption of normality was not satisfied. For comparisons involving qualitative variables with more than two categories, the One-Way ANOVA test was used when normal distribution assumptions were met; otherwise, the Kruskal–Wallis H test was employed. The relationship between the two qualitative variables was analyzed by Fisher-exact test. Risk factors affecting the dependent qualitative variable were analyzed by Univariate and Multivariate Logistic Regression analysis. Statistical significance level was taken as 0.05.

RESULTS

Some demographic data such as age and gender, acute phase reactants, vitamin and mineral levels and genotype characteristics of 392 genetically analyzed patients are shown in **Table 1**.

Comparisons of variables between CRP categories were analyzed and significant differences were found between the three CRP groups in terms of age, gender, WBC, ferritin, fibrinogen and genotype variables ($p<0.05$). Patients in homozygous, compound heterozygous and both diagnosed groups had statistically significantly higher CRP values. Approximately half of the patients undiagnosed- carriers had normal CRP levels (**Table 2**).

The risk factors affecting the CRP value were analyzed by Univariate Logistic Regression analysis. Gender, WBC, fibrinogen, homozygous, compound heterozygous, all diagnosed, and carrier groups were found to be significant risk factors ($p<0.05$). Male patients were 2.350 times more likely to have high CRP values than female patients. As the WBC value increased by one unit, the risk of high CRP value increased 1.304 times. As the fibrinogen value increased by one unit, the risk of high CRP value increased 1.007 times. The risk of elevated CRP was 2.926 times higher in homozygotes, 1.850 times higher in compound heterozygotes, and 2.722 times higher in all diagnosed patients. The risk of high CRP was 0.396 times lower in carriers (**Table 3**).

The variables of gender, WBC, fibrinogen, homozygous, compound heterozygous, all diagnosed and heterozygous-carrier, which were significant because of Univariate Logistic Regression analysis, were included in the Multivariate Logistic Regression analysis and gender, WBC, fibrinogen, homozygous variables were found to be significant risk factors together. In the presence of other variables, the risk of high CRP values was 2.019 times higher in male patients than in female patients. In the presence of other variables, as the WBC value increased by one unit, the risk of high CRP value increased 1.253 times. In the presence of other variables, as the fibrinogen value increased by one unit, the risk of high CRP value increased 1.007 times. In the presence of other variables, the risk of high CRP value was 2.515 times higher in homozygotes (**Table 4**).

Vitamin D levels and ferritin levels were compared in all groups and no significant results were found (**Table 5, 6**).

Vitamin B12 levels were compared in all groups. It is shown in **Table 7** that vitamin B12 levels were significantly lower in the carrier group ($p=0.04$).

Table 2. Comparisons of variables for CRP

Variables		Normal	Mild	Moderate-high	p value
Age	Median (min-max)	35.00 (19.00-66.00)	42.00 (18.00-73.00)	34.00 (21.00-70.00)	0.038[b]
Gender, n (%)	Female	110 (48.5)	61 (26.9)	56 (24.6)	<0.001[c]
	Male	44 (28.6)	38 (24.7)	72 (46.7)	
Hemoglobin	Median (min-max)	13.75 (9.40-17.80)	13.40 (9.70-17.30)	13.60 (8.30-17.30)	0.269[b]
WBC	Median (min-max)	6.90 (3.32-14.90)	7.60 (3.80-17.50)	8.75 (3.76-24.10)	<0.001[b]
Serum iron	Median (min-max)	111.00 (11.00-998.00)	56.70 (11.00-678.00)	122.00 (12.00-921.00)	0.471[b]
Iron binding capacity	Median (min-max)	307.50 (240.40-503.00)	309.75 (230.00-483.00)	310.50 (173.00-481.00)	0.783[a]
Ferritin	Median (min-max)	15.40 (2.30-227.00)	40.00 (4.00-294.00)	41.40 (2.80-704.00)	0.016[b]
Fibrinogen	Median (min-max)	305.00 (2.32-625.00)	362.50 (2.84-538.00)	435.50 (4.32-976.00)	<0.001[b]
Vitamin B12	Median (min-max)	225.00 (95.00-555.00)	231.00 (36.00-646.00)	224.00 (81.00-819.00)	0.858[b]
Vitamin D	Median (min-max)	14.10 (3.00-48.84)	11.38 (5.09-70.00)	13.50 (3.00-39.00)	0.365[b]
Homozygous diagnosed	Negative	142 (43.8)	88 (27.2)	94 (29.0)	<0.001[c]
	Positive	12 (21.1)	11 (19.3)	34 (59.6)	
CH diagnosed	Negative	128 (43.7)	80 (27.3)	85 (29.0)	0.002[c]
	Positive	26 (29.5)	19 (21.6)	43 (48.9)	
Heterozygous (carriers)	Negative	44 (27.8)	32 (20.3)	82 (51.9)	<0.001[c]
	Positive	110 (49.3)	67 (30.0)	46 (20.7)	
All diagnosed	Negative	116 (49.2)	69 (29.2)	51 (21.6)	<0.001[c]
	Positive	38 (26.2)	30 (20.7)	77 (53.1)	

CRP: C-reactive protein, WBC: White blood cell, CH: Compound heterozygous, Min: Minimum, Max: Maximum, a: One Way ANOVA test, b: Kruskal Wallis H test, c: Chi-square test

Table 3. Univariate regression analysis results for risk factors affecting CRP value

Variables (references)		β	SD	p value	OR	95% CI for OR	
						Lower Limit	Upper limit
Age		0.011	0.008	0.197	1.011	0.994	1.028
Gender (female)	Male	0.855	0.222	<0.001	2.350	1.520	3.634
Hemoglobin		-0.109	0.060	0.070	0.897	0.797	1.009
WBC		0.266	0.050	<0.001	1.304	1.183	1.438
Serum iron		-0.002	0.001	0.111	0.998	0.996	1.000
Iron binding capacity		-0.003	0.004	0.518	0.997	0.990	1.005
Ferritin		0.008	0.005	0.090	1.008	0.999	1.017
Fibrinogen		0.007	0.001	<0.001	1.007	1.004	1.009
Vitamin B12		0.001	0.001	0.434	1.001	0.999	1.003
Vitamin D		-0.015	0.016	0.354	0.985	0.955	1.017
Homozygous diagnosed (negative)	Positive	1.074	0.344	0.002	2.926	1.492	5.738
CH diagnosed (negative)	Positive	0.615	0.262	0.019	1.850	1.108	3.089
All diagnosed (negative)	Positive	1.001	0.229	<0.001	2.722	1.736	4.267
Heterozygous (carriers) (negative)	Positive	-0.925	0.222	<0.001	0.396	0.256	0.613

CRP: C-reactive protein, WBC: White blood cell, SD: Standard deviation, OR: Odds ratio, CI: Confidence interval CH: Compound heterozygous

Table 4. Multivariate regression analysis results for risk factors affecting CRP value

Variables (references)		β	SD	p value	OR	95% CI for OR	
						Lower Limit	Upper limit
Constant		-4.076	0.687	<0.001	-	-	-
Gender (female)	Male	0.703	0.317	0.027	2.019	1.084	3.761
WBC		0.225	0.064	<0.001	1.253	1.106	1.419
Fibrinogen		0.007	0.001	<0.001	1.007	1.004	1.009
Homozygous diagnosed (negative)	Positive	0.922	0.457	0.043	2.515	1.028	6.153

CRP: C-reactive protein, WBC: White blood cell, SD:Standard deviation, OR:Odds ratio, CI: Confidence interval

Table 5. Comparison of vitamin D levels in different genetic combinations

Variables		Vitamin D		p value
		Mean±SD	Median (min-max)	
Homozygous diagnosed	Negative	15.18±9.20	13.00 (3.00-70.00)	0.485[a]
	Positive	16.02±8.57	14.20 (4.81-39.00)	
CH diagnosed	Negative	15.82±9.61	13.39 (3.00-70.00)	0.221[a]
	Positive	13.37±6.52	12.57 (3.00-33.46)	
All diagnosed	Negative	15.78±9.88	13.37 (3.00-70.00)	0.610[a]
	Positive	14.49±7.52	12.90 (3.00-39.00)	
Heterozygous (carriers)	Negative	14.90±7.60	13.10 (3.00-39.00)	0.925[a]
	Positive	15.60±10.06	13.03 (3.00-70.00)	

CH: Compound heterozygous, SD: Standard deviation, Min: Minimum, Max: Maximum, a: Mann-Whitney U test

Table 6. Comparison of ferritin levels in different genetic combinations

Variables		Ferritin		p value
		Mean±SD	Median (min-max)	
Homozygous diagnosed	Negative	70.71±129.51	28.00 (2.30-704.00)	0.184[a]
	Positive	114.58±152.39	37.20 (2.80-570.00)	
CH diagnosed	Negative	84.71±138.74	29.70 (2.30-704.00)	0.571[a]
	Positive	71.45±129.01	25.30 (4.00-500.00)	
All diagnosed	Negative	70.46±131.16	29.35 (2.30-704.00)	0.465[a]
	Positive	96.61±142.81	26.65 (2.80-570.00)	
Heterozygous (carriers)	Negative	109.03±163.06	31.10 (2.80-704.00)	0.106[a]
	Positive	49.47±85.11	27.45 (2.30-443.00)	

CH: Compound heterozygous, SD: Standard deviation, Min: Minimum, Max: Maximum, a: Mann-Whitney U test

Table 7. Comparison of vitamin B12 levels in different genetic combinations

Variables		Vitamin B12		p value
		Mean±SD	Median (min-max)	
Homozygous diagnosed	Negative	255.55±128.75	219.00 (36.00-819.00)	0.072[a]
	Positive	275.82±109.06	254.00 (92.00-555.00)	
CH diagnosed	Negative	256.27±119.14	225.00 (36.00-819.00)	0.949[a]
	Positive	268.39±147.94	225.00 (77.00-808.00)	
All diagnosed	Negative	251.04±121.47	217.00 (36.00-819.00)	0.186[a]
	Positive	271.61±131.98	239.50 (77.00-808.00)	
Heterozygous (carriers)	Negative	278.23±138.36	242.00 (77.00-819.00)	0.040[a]
	Positive	244.53±113.82	212.00 (36.00-654.00)	

CH: Compound heterozygous, SD: Standard deviation, Min: Minimum, Max: Maximum, a: Mann-Whitney U test

DISCUSSION

The primary objective of this study, which included 392 patients, was to evaluate the potential association between CRP levels measured at the time of genetic testing and the various MEFV genotypes identified. The levels of CRP along with fibrinogen, ferritin and WBC were observed to be statistically significantly elevated in patients diagnosed with FMF, while conversely, these biomarker levels were found to be reduced in individuals classified as carriers of the genetic mutation associated with this condition.

Univariate Logistic Regression analysis revealed that CRP and other acute phase reactants (fibrinogen and WBC) were compatible, while significant results were obtained in the two genotype groups (homozygous and compound heterozygous) diagnosed with FMF. In addition, the carrier group had a statistically significant lower risk of having high CRP values. Multivariate logistic regression analysis, incorporating the variables found to be significant in the univariate analysis, revealed that male sex and the presence of a homozygous mutation were independently associated with statistically significant outcomes. There are a limited number of recent studies in the literature showing a strong association between genotype and disease severity and clinical manifestations in FMF.[3-5,19-21] The results of our study, in which we found a strong association between FMF genotype and CRP levels at the time of genetic diagnosis, are consistent with the findings of studies in the literature.

The contemporary diagnostic criteria established by the EUROFEVER/PRINTO (Pediatric Rheumatology International Trials Organization) place significant emphasis on the clinical diagnosis of FMF in patients who exhibit the presence of solely one pathogenic variant alongside compatible clinical manifestations indicative of the disease.[22,23] It is noteworthy that a substantial majority, exceeding 98% of individuals who possess a single mutation, even those characterized by a variant that is considered to be highly pathogenic, tend to remain clinically asymptomatic and healthy, although there exists a subset of these individuals who may experience heightened levels of subclinical inflammation that are not readily apparent. Furthermore, it is important to highlight that a mere fraction, specifically less than 2%, of mutation carriers go on to develop a disease phenotype that resembles FMF, with this group exhibiting a relative risk that ranges from 6.3 to 8.1 when compared to individuals who are deemed to be healthy controls in the broader population.[5] In a parallel context, our own research findings demonstrated that the levels of CRP, were substantially lower within the cohort of carriers. This observation underscores the complexity of the relationship between genetic predisposition and clinical manifestation, as well as the necessity for further exploration into the underlying mechanisms that contribute to the observed variances in inflammatory responses among mutation carriers. Ultimately, these findings provide valuable insights into the nuanced interplay between genetic factors and clinical outcomes in the context of FMF and related inflammatory conditions.

In the current investigation, which was meticulously designed to assess various biochemical markers, it was observed that the serum concentrations of 25(OH)D, commonly referred to as 25(OH)D, did not exhibit any statistically significant variations or discrepancies when analyzed across the different genotype classifications, thereby suggesting a potential uniformity in vitamin D status regardless of genetic predisposition. A previous study conducted in our country investigated the relationship between markers of inflammation and oxidative stress and vitamin D levels during acute attacks in pediatric FMF patients, reporting a negative correlation between these parameters.[2] In another study conducted in a pediatric FMF patient group in our country, vitamin D levels were compared with the frequency of attacks in children and no significant relationship was found.[15] However, considering that vitamin D levels in our country are generally inadequate at a very high rate of 60-80%, it may be explained that it was found to be low in both genotype groups in FMF patients and carriers.[24] It was reported that 86.5% of 178 FMF patients treated in pediatric nephrology clinic were diagnosed with vitamin D deficiency.[25] Conversely, one study reported that pediatric FMF patients exhibited lower vitamin D levels during remission periods compared to acute attack phases; however, no significant association was observed between serum vitamin D concentrations and disease activity.[26] In addition, it was found that children with FMF had significantly lower vitamin D levels than healthy control groups,[26,27] and that this decrease in vitamin D levels was positively correlated with increasing age.[27] Consistent with these findings, Erten et al.[28] also reported markedly lower serum vitamin D

concentrations in FMF patients when compared to age- and sex-matched healthy controls. In another study from the literature, decreased vitamin D levels were detected in children with FMF receiving colchicine treatment and it was noted that the cumulative colchicine dose negatively affected vitamin D levels.[29]

The observation that ferritin levels were significantly elevated in the group with CRP >16 mg/dl suggests that this increase may be attributed to its role as an acute phase reactant. This interpretation is supported by the absence of significant differences among the three CRP groups with respect to hemoglobin levels, serum iron, and TIBC. Furthermore, no statistically significant difference in ferritin levels was observed between patients diagnosed with FMF and heterozygous carriers.

No significant difference was found when comparing vitamin B12 levels in the three groups stratified by CRP levels. Vitamin B12 levels were significantly lower only in the carrier group when compared in all groups. In the study in which vitamin B12 levels were compared in a total of three pediatric groups including two FMF patient groups with frequent and infrequent attacks and a healthy control group, it was found that B12 levels were significantly lower in the group with frequent attacks.[15] Although our results and the findings of this study appear to be contradictory, the reason for this difference may be that our patient group was adult patients, and it was not known whether these patients were using colchicine at the time of genetic analysis. Because in this study conducted with pediatric FMF patients, it was reported that all patients with frequent attacks received regular colchicine treatment, and it was emphasized that colchicine may lead to intestinal malabsorption and cause low vitamin B12 levels. Chronic colchicine consumption impairs the reversible absorption of vitamin B12 absorbed from the terminal ileum. Colchicine has been reported to exert a detrimental effect on the villous architecture of ileal mucosal cells, leading to a reduction in villus number and impairing receptor-mediated absorption of vitamin B12 in the ileum. Colchicine also limits the absorption of vitamin B12 by reducing the receptor level of the B12-intrinsic factor (IF) structure in mucosal cells, an effect that is dose-dependent and reversible. Colchicine also impairs the function of receptors that limit the number of villi by damaging the villi structure in the ileal mucosal cell, thereby limiting the absorption of vitamin B12 in the ileum.[30,31] Another study investigating vitamin B12 levels in adult FMF patients on regular colchicine treatment found more cases of vitamin B12 deficiency in the patient group than in healthy controls.[32] Although there are not many current articles in the literature about colchicine impairing the absorption vitamin B12 from the terminal ileum, this issue is emphasized in various sources.[33-34]

Limitations

This investigation is characterized by a number of inherent limitations that must be acknowledged and considered when interpreting the results. To begin with, the research was executed within the confines of a singular medical center, which inherently restricts the potential for the findings to be universally applicable across diverse populations and clinical settings. Furthermore, the methodological framework employed in this study, specifically its retrospective and cross-sectional design, imposes significant constraints on the capacity to draw definitive causal inferences regarding the relationships between the variables under examination. Due to the retrospective design of the study, it was not possible to obtain information on the frequency of attacks, clinical symptoms, complications such as amyloidosis and the number of years of disease duration. Despite these limitations, we believe that this study is valuable in emphasizing the importance of genotype analysis in adult FMF patients not diagnosed in childhood. Because it is important for adult patients to receive genetic diagnosis due to the possibility that it may cause different complications in advanced ages or that the clinical symptoms it causes may be confused with other diseases.

CONCLUSION

In this study, it was found that adults diagnosed with FMF by genetic analysis for the first time, especially those with homozygous genotype, had higher CRP levels at the time of diagnosis. There were no significant differences in ferritin, and vitamin D levels in FMF patients with different genotypes and carriers. No correlation was found between acute or subacute inflammation states, expressed by normal or elevated CRP values, and the levels of these vitamins and mineral. Considering that FMF is more common in our country, it is important for all clinicians, especially family physicians, to keep this hereditary disease in mind in terms of differential diagnosis in all age groups and to request genetic analysis when necessary.

ETHICAL DECLARATIONS

Ethics Committee Approval

This comprehensive research endeavor was granted the necessary ethical approval by the esteemed Non-interventional Clinical Researches Ethics Committee of Ankara Etlik City Hospital (Date: 09.08.2023, Decision No: AEŞH-EK1-2023-428).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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