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Original Article

Serum Calprotectin in Children with Familial Mediterranean Fever

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Abstract:

Background: Familial Mediterranean fever (FMF) is the most prevalent inherited autoinflammatory disease worldwide. Ongoing subclinical inflammation, induces amyloidosis, even during the attack-free periods despite colchicine therapy. Serum calprotectin (CLP) belongs to the S100 protein family and was proposed as an indicator of inflammation in several diseases.

Aim of the work: To evaluate serum calprotectin (CLP) levels among children with FMF during the attack-free periods.

Patients and Methods: This cross-sectional case-control study included 35 children diagnosed with FMF in the attack-free period who were following at the Pediatric Rheumatology Outpatient Clinic, Children's Hospital, Faculty of Medicine Cairo University, and 35 children as a control group. This study was conducted during November 2020 to April 2021. Serum CLP was measured by ELISA.

Results: The mean ± SD age of our studied FMF cohort was 9.23 ± 2.6 years; 15 (42.9%) were males, and 20 (57.1%) were females compared to the mean ± SD age of the control group 9.0 ± 2.6, 21 (60%) were males, and 14 (40%) were females(p=0.665 and p=0.151 respectively). The range of serum calprotectin levels among our FMF patients during the attack-free period was (15.4-1306) ng/ml, while the range in the control group, its range was (0.2 - 77) ng/ml with mean± SD was 24.62±16.66 ng/ml, (p-value = 0.001). Of those with FMF 25 (71.4%) had normal level of CLP (mean± SD= 31.6± 9.2 and range=15.4 – 48.6 ng/ml) and 10 (28.6%) had elevated CLP (mean± SD= 296.79±442.33 and range= 55 – 1306 ng/ml). There were no significant correlations between serum CLP levels and FMF clinical presentations, disease severity scores, different laboratory data, or types of MEFV gene mutation p- values = 0.697, 0.696 and 0.146 respectively.

Conclusion: Serum CLP levels were elevated in a subset of children with FMF during the attack-free period. Serum CLP did not correlate with any studied parameter. The specificity and sensitivity of diagnostic accuracy and outcome prognostic ability of CLP in FMF remain to be studied.

Level of Evidence of Study: IV (*1*)**.**

Keywords: Serum calprotectin; Colchicine; FMF; Familial Mediterranean fever; attack-free period

Abbreviations: CLP: calprotectin; FMF: familial Mediterranean fever; IL-1ß: Interleukin 1ß; ISSF: International Severity Scoring System for FMF; SAA: serum amyloid-A; SD: standard deviation

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Introduction

Familial Mediterranean fever (FMF) is a monogenic autoinflammatory disease brought by mutations in Mediterranean fever gene situated on chromosome 16p13, which encodes for pyrin (*2*). Pyrin is a constituent of a large molecular assembly called the inflammasome (*3*). Pro-inflammatory cytokines, primarily Interleukin-1ß (IL-1ß), are enzymatically processed when the pyrin inflammasome is activated (*4*). Recurrent episodes of fever, serositis, peritonitis, pleuritis, arthritis, or an erysipelas-like skin rash characterize FMF. Amyloidosis is the most devastating consequence (*5*). Although individuals of Mediterranean heritage, such as Ashkenazi Jews, Turks, Kurds, Armenians, and Arabs, are especially vulnerable to it, it can affect people from a variety of ethnic backgrounds (*6*). The main targets in managing FMF are to avert clinical episodes in patients and to reduce chronic subclinical inflammation. Thus, minimizing the chance of amyloidosis (*7*). Calprotectin is a protein that interacts with calcium and zinc and is classified as

a member of the S100 family. It is found in high concentrations in neutrophil granulocytes and lower amounts in activated monocytes and macrophages (*8*). Referred to as a damage-associated molecular pattern (DAMP) protein, calprotectin has the potential to serve as a reliable biomarker in rheumatic diseases, offering an alternative to acute-phase proteins (*9*). Familial Mediterranean fever (FMF) was reported to be associated with increased levels of fecal calprotectin (CLP), suggesting its potential utility in diagnosing and monitoring FMF patients (*10*). Increased serum CLP levels was also observed in adults with FMF during episodes (*11*) and between attacks (*12*). Our study aimed to assess serum calprotectin (CLP) levels among children with FMF during the attack-free periods compared to healthy age and sex-matched control group.

Subjects and Methods

This study was a case-control cross-sectional study that enrolled 35 children diagnosed with FMF, who fulfilled the pediatric criteria (*13*). They were following up at the Pediatric Rheumatology Outpatient Clinic, Children's Hospital, Faculty of Medicine Cairo University, and 35 sex and age-matched apparently healthy children as a control group. The study was performed over a timeframe of six months, from November 2020 to April 2021. The study was approved by the Ethical Committee of the Faculty of Medicine, Cairo University; Egypt (MS 21-2021). All research participants' caregivers, or their children if they were older than 12 years old, were informed about the study's goals, procedures, and possible advantages. All participants were enrolled after receiving their caregivers' informed consent.

Participants

All recruited children with FMF were under the age of 14 years. They had no concomitant rheumatological, infectious, or other chronic disease. They were in the attack-free period (at least two weeks clinically free from the end of the last attack).

Methods

All patients were subjected to comprehensive history taking, including demographic data, age of onset of FMF, age at diagnosis, disease duration, manifestations of FMF during illness course, number of attacks in last 6 months, dose of colchicine in mg, and compliance to colchicine. Response to colchicine was assessed according to the FMF 50 score (*14*). Assessment of FMF severity by the International Severity Scoring System for FMF (ISSF) (*15*) was done. FMF disease was categorized as mild with scores of 0 to 2, moderate with scores of 3 to 5, and severe with scores of 6 to 10. Complete general and systemic examinations were done at the time of study. Results of MEFV gene mutations were collected from patients' files. Laboratory investigations were done during the study, including complete blood picture, ESR, CRP, BUN, creatinine, serum amyloid A (SAA), urine analysis and serum calprotectin. Measurement of serum calprotectin was done by using the ELISA technique, and it was studied by competitive enzyme immunoassay method, Catalogue number E4010 Hu (Bioassay technology laboratory; England/China), according to the manufacturer (*16*). In this study good response, according to the FMF50 score, was coined to improvement by ≥ 50% in five of six criteria (attack frequency, attack duration, global patient assessment, global physician assessment, frequency of attacks with arthritis, and levels of acute-phase reactants) without worsening of the sixth criterion (*14*). The value of serum calprotectin in this work was estimated to be less than 50.18 ng/dL which represented 2 SD of mean of calprotectin level among the control group cohort estimated to represent the 16^{th} – 84^{th} percentiles.

Statistical Analysis

The data was coded, tabulated and analyzed by the statistical software program for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). The data was outlined by calculating the mean, standard deviation (SD), median, minimum, and maximum for quantitative data and by determining the frequency (count) and relative frequency (%) for categorical data. Quantitative variables were compared using non-parametric statistical tests, namely the Kruskal-Wallis test and the Mann-Whitney test. A Chi-square (X2) test was conducted to compare categorical data. The exact test was employed in cases where the anticipated frequency is below 5. Spearman correlation coefficient was utilized to establish correlations between quantitative variables. Statistical significance was determined for P values less than 0.05.

Results

This study was conducted with 70 children; 35 of them were diagnosed with FMF according to the new pediatric diagnostic criteria of FMF (12), and all FMF patients were during the attack-free period. Thirty-five apparently healthy age and sex-matched children were enrolled as a control group. The mean age ± standard deviation (SD) of FMF patients at the time of the study was 9.23 ± 2.6 years, while the mean age ± SD of the control group was 9 ± 2.56 years (p=0.665). Among our FMF patients, 15 (42.9%) were males, and 20 (57.1%) were females while the control group comprised 21 (60%) were males, and 14 (40%) were females (p= 0.151). Demographic and clinical characteristics of both FMF patients and control groups are shown in Table 1.

Table 1. The demographic and laboratory findings of the study groups

		FMF group n=35		**Control group** n=35	**P value**
Age (years)	Range Mean ±SD	3-13 9.23 ± 2.6		3-13 9.23 ± 2.6	0.665
Sex (%)	Males Females	15 (42.9%) 20 (57.1%)		21 (60%) 14 (20%)	0.151
TLC (10^9/L)	Range Mean ± SD	3.4 - 11 6.51± 1.6		4.7 – 12.2 7.51 ± 1.99	0.051
Hb(g/dl)	Range Mean ±SD	10.4 – 13.9 12.31 ± 0.83		10.8 – 14 12.02 ± 0.75	0.424
Platelets(10^9/L)	Range Mean ±SD	176 - 490 280.06 ± 59.92		172 – 480 318.8 ± 93.96	0.007
Creatinine (mg/dl)	Range Mean ±SD	0.20 – 0.90 0.61± 0.18		0.45 - 0.80 0.63 ± 0.10	0.858
ESR (mm/h)	Range Mean ±SD	4 -35 14.06 ± 8.26		4-30 11.06 ± 7.48	0.063
CRP (%)	Positive Negative	9 (25.7%) 26 (74.3%)		4 (11.4%) 31 (88.6%)	0.124
Serum amyloid A (mg/l)	Range Mean ±SD	1-191 9.89 ± 31.82		1.03 – 4.99 2.97 ± 1.19	0.209
		Normal Calprotectin Number= 35	Increased Calprotectin Number=10		
Calprotectin(ng/ml)	Range Mean ±SD	15.4-48.6 31.6± 9.2	55-1306 296± 442.3	0.20 – 77 24.62 ± 16.66	0.001

CRP: C reactive protein; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; P value: probability value; SD: standard deviation; TLC: total leukocyte count

Family history of FMF was present in 21 (60%) of those with FMF. The mean age ± SD of onset of FMF symptoms was 4.09 ± 2.59 years, while the mean age ± SD at diagnosis was 5.71 ± 2.33 years. The mean ± SD number of FMF attacks in the last six months was 2.89 ± 2.10. According to the ISSF score, 26(74.3%) of our patients had mild disease severity, and 10 (25.7%) had intermediate disease severity scores. All FMF patients were on colchicine treatment, and the mean ± SD colchicine dose among the studied group was 1.057 ± 0.511 mg/day. Of them 26 (74.3%) were compliant with colchicine. According to the FMF 50 score, 25(71.4%) of our patients were good responders to colchicine. (Table 2). All of patients with homozygous gene mutation had poor response to colchicine, while 90% of patients with heterozygous mutation had good response (p= 0.001). (Table 3). The mean ± SD disease duration among FMF patients was 5.14 ± 2.86 years.

There was a significant difference between FMF patients and the control group regarding platelet count and CLP levels, with p-values = 0.007 and 0.001 respectively. However, there was no statistically significant difference between cases and control groups regarding total leukocytic count or hemoglobin. There was no statistically significant relationship between CRP, ESR, and CLP (p- value = 0.527 and 0.620 respectively). Only 10 (28.6%) FMF cases had elevated serum CLP levels (above 50.18ng/dL), and there was a statistically significant difference between FMF cases with high serum CLP levels and those with normal serum CLP levels as regards age at diagnosis and number of attacks during the last 6 months with p values= 0.005 and 0.038 respectively. There was no correlation between serum CLP and weight or body mass index (BMI) among FMF cases with p = 0.587 and p=0.985 respectively.

Table 2. The clinical characteristics of FMF patients included in our study

	Total cases Number=35	Normal Calprotectin Number =25	High Calprotectin Number=10	P value
Age (years)				
Range	3-13	4-13	3-12	0.113
Mean± SD	9.2 ± 2.6	9.76 ± 2.2	7.9 ± 3.14	
Age at disease onset (years)				
Range	1-9	1-9	1-7	0.287
Mean± SD	4.09 ± 2.59	4.44± 2.75	3.2 ± 1.99	
Age at diagnosis (years)				
Range	1-9	2-9	1-7	0.005
Mean± SD	5.71 ± 2.33	6.4 ±2.1	4 ± 2.05	
Disease duration (years)				
Range	1-11	1-11	2-9	0.733
Mean± SD	5.14 ± 2.89	5.32 ± 3.05	4.7± 2.41	
FMF symptoms (%)				
Abdominal pain	33 (94.3%)	25(100%)	9(90%)	0.286
Fever	29(82.9%)	18(72%)	9(90%)	0.390
Arthralgia	22(62.9%)	14(56%)	8(80%)	0.259
Chest pain	5(14.3%)	4(16%)	1(10%)	1
MEFV gene mutation(%)				
Homozygous	5(14.3%)	3(12%)	2(20%)_	0.642
Heterozygous	22(62.9%)	17(68%)	5(50%)	
Compound heterozygous	8(22.9%)	5(20%)	3(30%)	
FMF genotype(%)				
M694I	12(34.3%)	8(32%)	4(40%)	0.706
E148Q	10(28.6%)	7(28%)	3(30%)	1
M680I	5(14.3%)	4(16%)	2(20%)	1
V726A	4(11.4%)	2(8%)	2(20%)	0.561
M694V	3(8.6%)	3(12%)	0(0%)	0.542
R761H	3(8.6%)	1(4%)	1(10%)	0.496
I692del	2(5.7%)	1(4%)	0(0%)	1
A744S	1(2.9%)	1(4%)	0(0%)	1
M620I	1(2.9%)	1(4%)	0(0%)	1
M094I	1(2.9%)	0(0%)	1(10%)	1
P369S	1(2.9%)	1(4%)	0(0%)	1
Number of attacks in last 6 months				
Range	0-7	0-6	0-7	0.038
Mean± SD	2.89 ± 2.1	2.36 ± 1.75	4.2 ± 2.39	
Colchicine dose(mg/day)				
Range	0.5-2	1-2	1-2	0.225
Mean± SD	1.06 ± 0.511	1.45± 0.47	1.35 ± 0.47	
Colchicine usage duration(years)				
Range	0.5-7	0.5-6	2-7	0.377
Mean± SD	3.13 ± 1.61	2.9± 1.47	3.7± 1.89	
Compliance to colchicine (%)				
Complaint	26(74.3%)	19(76%)	7(70%)	0.694
Not-complaint	9(25.7%)	6(24%)	3(30%)	
FMF 50 score (%)				
Good response	25(71.4%)	18(72%)	6(60%)	0.689
Poor response	10(28.6%)	7(28%)	4(40%)	
ISSF score(%)				
Mild	26(74.3%)	20(80%)	6(60%)	0.393
Intermediate	9(25.7%)	5(20%)	4(40%)	
Serum amyloid A (mg/ml)				
Range	1-191	1.2-9.9	1-191	0.627
Mean± SD	9.89 ± 31.82	3.83 ± 1.74	25.08 ± 58.82	

FMF: Familial Mediterranean Fever, IL-1β: Interleukin 1β, ISSF: International Severity Scoring system for FMF; MEFV: Mediterranean Fever

SAA was elevated among 6 (17.1%) of our FMF cases, and there were statistically significant differences between FMF patients with high SAA levels and those with normal levels as regards disease duration, number of attacks during the last 6 months, ISSF score, being higher in FMF patients with high SAA (p = 0.027, p= 0.003 and p= 0.002 respectively).

There was no correlation between SAA and serum CLP levels (p = 0.949).

Table 3. Mediterranean fever MEFV gene mutations and response to colchicine

		FMF gene mutation			P value
		Homozygous	Heterozygous	Compound	
Response to colchicine according to FMF 50 score	Poor	5 (100%)	2 (9.1%)	3 (37.5%)	<0.001
	Good	0 (0.00%)	20 (90.9%)	5 (62.5%)	

Discussion

FMF is the earliest and most prevalent among all documented inherited periodic fever disorders. The occurrence of mutations in the MEFV gene, in which pyrin is encoded, has been found to influence the regulation of inflammation mediated by IL-1b. This inflammatory process is known to contribute significantly to the development of FMF (*17*). Acute phase reactants, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum amyloid-A (SAA), and fibrinogen, are widely utilized indicators of inflammation in medical practicing. These markers typically rise during acute FMF attacks and revert to normal levels during periods without attacks in most FMF patients (*18*). Nevertheless, subclinical inflammation can endure throughout the period without any attacks. This prolonged subclinical inflammation can lead to severe consequences that pose a threat to life, particularly secondary systemic amyloidosis (*19*).

Calprotectin is an upstream ligand of various inflammatory proteins secreted by TLR4 and RAGE (receptor for advanced glycation end products); tissue damage may increase the synthesis of calprotectin, resulting in a detrimental hyperinflammation loop (*20*).

Increased levels of serum calprotectin have been reported in autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel diseases, and a correlation was found between its levels and disease activity (*21–23*). In pediatrics, studies investigated CLP as a potential biomarker for JIA disease activity and revealed that these patients exhibited higher serum CLP levels than the healthy control group (*24*, *25*).

In this work, only a subset of FMF children had elevated serum CLP during the attack-free periods, while the majority had within normal level of serum CLP. FMF patients with high serum CLP had higher number of attacks during last 6 months than FMF patients with normal serum CLP levels, it seems that the attack free periods are not devoid of inflammation. Yet, there was no significant correlation between serum CLP and disease severity, and this is in agreement with previous work(*25*). Also, we did not find a correlation between serum CLP levels and different types of FMF gene mutations, which is a consistent with others (*26*). The value of CLP is not clear, yet we did not study CLP as a predictor of future attacks.

Previous studies detected significantly higher serum concentrations of calprotectin among obese children and it was suggested that obesity may generate chronic inflammation in children (*27*), but our study did not observe any correlation between serum CLP levels and weight or BMI. So, the higher serum CLP levels among our studied cases may be related more to persistent subclinical inflammation.

The significant difference in platelet count between FMF patients and the control group indicates persistent subclinical inflammation in our FMF patients during the attack-free period. According to the FMF 50 score, 71.4% of our FMF patients were good responders to colchicine. Good response to colchicine was significantly common in FMF patients with the heterozygous mutation, while most poor responders had homozygous MEFV gene mutation. Yet, these findings are not unanimous, and the discrepancy could be related to varying disease severity in various studies (*28*, *29*).

The statistically significant differences between FMF patients with high SAA levels and those with normal levels as regards disease duration, number of attacks during the last 6 months, ISSF score, makes SAA a reliable marker in children with FMF, in assessing disease activity, severity score and risk of amyloidosis.

There were certain limitations to our research such as the small sample size and the study was not prospective to evaluate the predictive value of CLP in dictating duration of the attack-free period, or relapses. Hence, further studies enrolling a larger sample size and including FMF patients during the attack and the attack-free periods are recommended to determine the role of serum CLP in the pathophysiology of FMF, which will improve the follow-up and treatment protocols among children with FMF.

Conclusion

CLP levels were higher in a subset of children with FMF during the attack-free period than in the control group. It might prove to be a marker for detecting subclinical inflammation in children with FMF. We need more comprehensive understanding of the role of serum CLP in diagnosing of subclinical inflammation in FMF in order to assess our results more thoroughly.

Author Contributions:

All authors have contributed to the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with the reported study. Authors declare veracity of information.

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