Fecal Calprotectin in Assessing Familial Mediterranean Fever Patients

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Background and study aims: Familial Mediterranean fever (FMF) is an autoinflammatory disease presented by inflammatory attacks. Calprotectin (FC) is a calcium-binding protein that is found mainly in neutrophils, monocytes, and reactive macrophages. It is considered a cytokine released from monocytes and neutrophils as a result of tissue trauma and inflammation. This study aimed to investigate fecal calprotectin levels in FMF patients as a predictor of disease activity.

Patients/Material and Methods: This is a Prospective cross-sectional study. Between May 2020 and May 2022, 158 patients were diagnosed with FMF in our outpatient clinic at Kafrelsheikh University Hospital after confirmation by PCR and obtaining Medical consent for participation in this study. Blood was collected from a peripheral vein to complete a blood picture, ESR, CRP, Amyloid A, and Fecal calprotectin. Blood tests were examined by ELISA; the study protocol was approved by the local ethics committee.

Results: We enrolled 158 patients among them 102 patients were females, and 56 were males. The largest number of patients were diagnosed at the age of twenties. Attacks last in 116 patients for less than 48 hours while lasting for more than that in 42 patients. 130 patients had heterozygous mutation(Group 1), 28 patients had homozygous mutations(Group 2) confirmed by PCR . There was a statistically significant difference between the two groups in Fecal calprotectin level at the time of diagnosis ($P < 0.001616$). The level of Calprotectin at the time of diagnosis of 100 pg/mL had 96.40% sensitivity and 96.60% Specificity. The homozygous mutation had a positive correlation with the Colchicine dose needed to give a response. Calprotectin levels have a negative correlation with the Colchicine dose needed.

Conclusion: Our finding suggests that fecal calprotectin can be used to assess the diagnosis and severity of FMF patients. However, future studies are recommended .

INTRODUCTION

Familial Mediterranean fever (FMF) is an autoinflammatory disease presented by repeated episodes of fever and serositis **[1, 2]**. The etiology of FMF is not properly recognized but, a mutation of the Mediterranean fever gene that is responsible for encoding a pyrin protein. As a result, abnormal pyrin leads to marked inflammation leading to the clinical manifestation of disease. Homozygous mutations are more liable to severe symptoms and complications **[3, 4]**.

Four Types of gastrointestinal affection may be found in FMF patients. The first one is associated with the attack, while the second is not related to the attack. The third GIT affection is related to treatment side effects such as diarrhea while the last one is malabsorption and inflammatory bowel disease **[5]**.

Fecal calprotectin is a simple tool for assessment of the intestinal inflammation and its level is correlated with the severity of intestinal inflammation **[6,7]**. Many conditions are associated with an increase in the level of fecal calprotectin such as IBD and

(NSAID) use.[8, 9] Many recent studies suggest that FC may be a more accurate biomarker of rheumatic diseases than ESR and CRP **[10]**. Previous studies in FMF patients found high fecal calprotectin. Because episodic abdominal pain affects 95% of FMF patients, most of them undergo complete or partial abdominal imaging before the diagnosis is made. Subclinical inflammation may be present in FMF patients. Unfortunately, there are no diagnostic markers **[11]**.

Many inflammatory diseases are prevalent in FMF patients as amyloidosis, Vasculitis, and inflammatory bowel disease **[12]**.

Colonoscopy is the gold standard to evaluate gut mucosal inflammation, However, it is an expensive, difficult in children, and invasive procedure **[13]**. So there is a need for a simple non-invasive method for the evaluation of mucosal inflammation .

This study aimed to evaluate fecal calprotectin levels in FMF patients as a predictor of disease activity.

PATIENTS AND METHODS

We included patients diagnosed with FMF according to the Yalçınkaya criteria 13 in our outpatient clinic at Kafrelsheikh University Hospital between May 2020 and May 2022.

Patients were subjected to history taking, and clinical and laboratory evaluation (CBC, CRP, ESR, albumin, and Fecal calprotectin). We excluded patients with other causes of raised FC such as those who took NSAID. The stool samples were stored at a temperature of less than 8°C until assay. And we avoided alternating freezing and thawing of the samples. FC ≤50 µg/g was defined as normal, and >50 µg/g as abnormal. Other major causes of raised FC as drugs, infection, inflammatory bowel disease, and amyloidosis were excluded from our study by stool analysis, and by doing endoscopy and colonoscopy followed by histopathology

RESULTS

We included 158 patients. Among them 102 patients were females, and 56 were males. We included 142 patients from Baltium, 12 from Kalian, and 4 from Bela. The largest number of patients were diagnosed at the age of twenties. Only 5 patients were diagnosed in the fifties. 72 patients had more than 6 attacks, 65 patients had 3 to 6 attacks and 21 patients had less than 3 attacks per year. Attacks last in 116 patients for less than 48 hours while lasting for more than that in 42 patients. Ninety-six patients had negative parent consanguinity. 30 patients had a history of abdominal surgery. We reported that 130 patients had heterozygous mutations and 28 patients had homozygous mutations confirmed by PCR **(Figure 1)**.

Laboratory evaluation of our patients revealed that WBC and platelet count showed significant differences between Homozygous and Heterozygous groups **(P value = 0.000)** while ESR, CRP, and Amyloid A levels showed insignificant differences between the two groups **Table (1)**.

Our study reported that FC levels were raised at the time of diagnosis above the normal range in both homozygous and heterozygous patients (n <50 µg/g) with significant difference between both groups (P = 0.00)**Table (2)**

ROC analysis of Calprotectin level at the time of diagnosis showed significant difference from ESR, CRP and Amyloid A levels with calprotectin value of 100 pg/mL had 96.40% sensitivity and 96.60% Specificity. **Table (4) and Figure 2**

Statistical analysis

Statistical assessments were performed using SPSS 22 software. We calculate the mean and median for the data with normal and abnormal distribution. For quantitative data, Mann-Whitney and T-tests were used. A chi-square test was used to compare the categorical data. Spearman test was used to find out the correlation between quantitative variables. The area under the ROC curve was calculated with a 95% confidence interval (CI). The sensitivity, specificity, positive predictive value, and negative predictive value for optimum cut-off values were in the 95% CI. The significance level was $P < 0.05$.

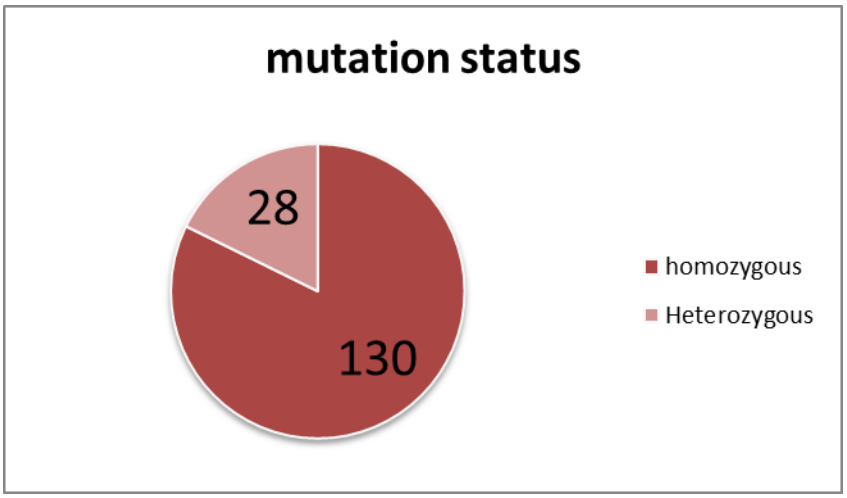

Fig. (1): Show the number of homozygous and Heterozygous subtypes.

Table (1): Showed lab findings in each group.

	Heterozygous	Homozygous	P value
	130 patients	28 patients	
Type of gene mutation			
E148 Q	73	15	
A744 S	15	6	
M680 I	22	5	
M694	31	6	
Level of Amyloid A	41.09 ± 47.13	40.06 ± 50.17	0.6263
Hb level (g/dl)	12 ± 1.71	11.76 ± 1.92	0.3923
WBC(g/dl)	250.52 ± 1268.22	1242.33 ± 2428.28	0.0000
Platelet count	10863.78 ± 49376.23	47499.96 ± 92505.72	0.0000
ESR	31.5 ± 13.88	26.29 ± 15.48	0.4188
CRP	10.02 ± 5.61	9.79 ± 5.44	0.8903

WBCs: white blood cells, RBCs: red blood cells, Hb: hemoglobin, MCV: mean corpuscular volume. ESR: erythrocyte sedimentation rate, CRP: c reactive protein.

Table (2): Showed calprotectin level and dose of Colchicine needed to give a response.

	Heterozygous	Homozygous	P value
	130	28	
Fecal calprotectin at the time of diagnosis (n <50 µg/g)			
mean ± SD	69.92 ± 30.92	158.21 ± 47.33	0.00
fecal calprotectin after 1 month of treatment			
mean ± SD	39.94 ± 14.09	36.04 ± 10.53	0.07995

Table (3): ROC analysis values.

Test Result Variable(s)	Area under curve	cut-off value	sensitivity	1 - Specificity	Std. Error	Asymptotic Sig. [b]	Asymptotic 95% Confidence Interval	
							Lower Bound	Upper Bound
ESR	0.611	19.5	84.40%	75.00%	0.063	0.066	0.488	0.735
CRP	0.503	7.5	66.41%	78.60%	0.057	0.961	0.391	0.615
Amyloid	0.539	9.3	70.31%	50%	0.062	0.524	0.417	0.660
Calprotectin	0.072	100	96.40%	96.60%	0.035	0.000	0.003	0.141

ESR: erythrocyte sedimentation rate, CRP: C reactive protein

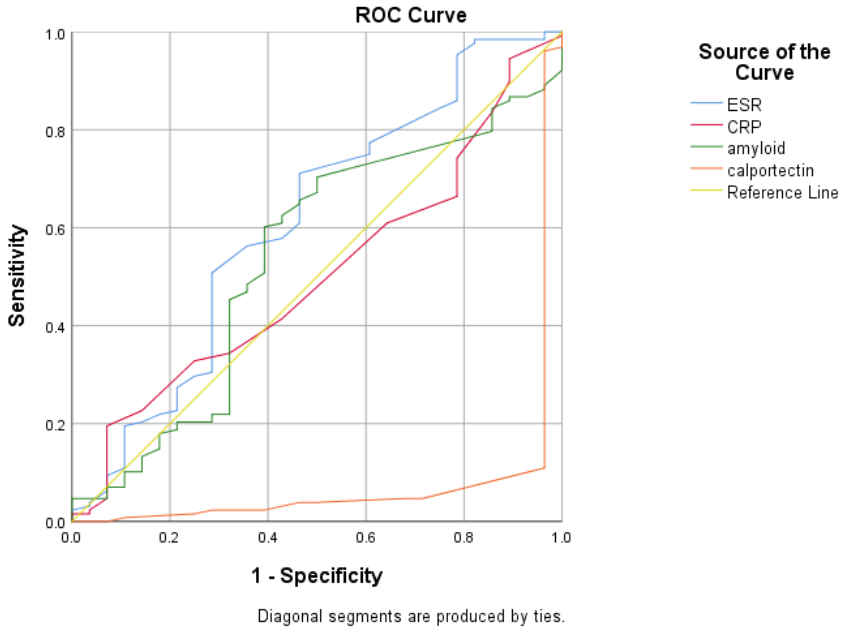

Fig. (2): ROC curve analysis.

DISCUSSION

Familial Mediterranean fever (FMF) is an autoinflammatory disease presented by repeated episodes of fever and serositis**.** Several markers such as CRP, ESR, Amyloid, and WBC used to detect systemic inflammation in FMF **[14]**. However these markers of low sensitivity and specificity for the diagnosis of subclinical intestinal inflammation.

Fc is a marker used to detect inflammation from activated lymphocytes and neutrophils in the intestinal mucosa **[15]**. However, there are other etiologies to raise its level **[16-18]**. To exclude other causes of raised FC, we excluded patients with any chronic disease, those known to have IBD or other intestinal pathology on colonoscopy, and those who were using NSAIDs and confirmed that all fecal samples were normal at the time of raised FC. Many studies addressed the role of FC in rheumatic diseases like rheumatoid arthritis, scleroderma, and Sjögren's syndrome **[19,20]**.

As patients with the homozygous mutation have more severe disease than those with heterozygote mutation, our study revealed an increased platelet count in homozygotes than heterozygotes, Similarly, a previous study revealed increased platelet activation in FMF patients **[21]**.

In the current study, the FC level in the FMF patient was higher than normal (>50 µg/g) in both FMF groups, and the level was significantly higher in the homozygous than the heterozygous group. This is in line with a study by Gucenmezet al. found that Fecal calprotectin levels in FMF patients were significantly higher than in healthy patients **[10]**. So we suggest that FC can be used for the diagnosis and follow-up

of FMF patients. High levels of FC may be due to chronic inflammation in FMF patients. Another study by Fatma et al concluded that FC is a noninvasive simple method for assessing inflammation in children with FMF patients **[22]**. The Study limitations were a relatively low number of patients and that it was cross-sectional.

CONCLUSION

Our finding suggests that fecal calprotectin can be used to assess the diagnosis and severity of FMF patients. However, future studies are recommended.

Abbreviations:

FC: Fecal calprotectin,

ESR: Erythrocyte sedimentation rate,

FMF: Familial Mediterranean fever

Conflict of interest: None

Funding: No

Ethical considerations:

The study was approved by the Ethics Committee of Kafrelsheikh University. Written informed consent was obtained from the patient.

HIGHLIGHTS

- FC Calprotectin may be a valuable biomarker to assess the severity of inflammation in FMF patients.
- FMF is an autoimmune disease with chronic inflammation in different organ
- Future study with large numbers is highly recommended

1. REFERENCES

1. Ahmed, M.H., Ibrahim, A.M., Ragab, S.M, Mahros, M.A. Musculoskeletal and neurological manifestations in a cohort of Egyptian Familial Mediterranean fever patients: genotype-phenotype correlation. *Egypt Rheumatol Rehabil*, 2022; 49**,** 6.
2. Gedalia A. Hereditary periodic fever syndromes. In: E Behrman, R Kleigman, eds. Nelson Textbook of Pediatrics. Philadelphia, PA: WB Saunders Company; 2008: 1030.
3. Samuels J, Aksentijevich I, Torosyan Y, Centola M, Deng Z, Sood R, Kastner DL. Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. Medicine (Baltimore). 1998 Jul; 77(4):268-97..
4. Kastner DL. Familial Mediterranean fever: the genetics of inflammation. *Hosp Prac*. 1998; 33: 131- 146.
5. Beser OF, Kasapcopur O, Cokugras FC, Kutlu T, Arsoy N, Erkan T. Association of inflammatory bowel disease with familial Mediterranean fever in Turkish children. *J Pediatr Gastroenterol Nutr*. 2013; 56: 498- 502.
6. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between fecal excretion of indium-111-labeled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 1999; 34: 50- 54.
7. Ahmed M, Emara M, Saeed E, Abouelfetouh M, Mahros A. 'How Valuable are Noninvasive Tests as Indicators of IBD Activity and Severity in the Primary Health Care ', *Afro-Egyptian Journal of Infectious and Endemic Diseases* 2021; 11(2), pp. 113-119.
8. Gisbert JP, Mcnicholl AG. Questions and answers on the role of fecal calprotectin as a biological marker in inflammatory bowel disease. *Dig Liver Dis*. 2009; 41: 56- 66.
9. Tibble JA, Sigthorsson G, Foster R, Scott D, Fagerhol MK, Roseth A, Bjarnason I. High prevalence of NSAID enteropathy as shown by a simple fecal test. *Gut*. 1999 Sep;45(3):362-6.
10. Gucenmez OA, Kume T, Makay B, Babayigit O, Arslan N, Unsal E. Role of fecal calprotectin in the assessment of intestinal inflammation in children with familial Mediterranean fever. Int J Rheum Dis. 2018;21(10):1844–1848.

11. Mor A, Gal R, Livneh A. Abdominal and digestive system associations of familial Mediterranean fever. *Am. J. Gastroenterol.* 2003; 98: 2594–604.

12. Fefferman DS, Farrell RJ. Endoscopy in inflammatory bowel disease: Indications, surveillance, and use in clinical practice. *Clin. Gastroenterol. Hepatol.* 2005; 3: 11– 24.

13. Yalçinkaya F, Ozen S, Ozçakar ZB, Aktay N, Cakar N, Düzova A, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. *Rheumatology (Oxford).* 2009 Apr;48(4):395-8.

14. Ahmed MH, El Henawy O, ElShennawy EM, Mahros AM. Clinical and genetic characterization of familial Mediterranean fever among a cohort of Egyptian patients. *Prz Gastroenterol.* 2022;17(3):240-244.

15. Canani RB, Terrin G, Rapacciuolo L, Miele E, Siani MC, Puzone C, et al. Faecal calprotectin is a reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. *Dig Liver Dis*. 2008 Jul;40(7):547-53..

16. Fallahi G, Motamed F, Yousefi A, Shafieyoun A, Najafi M, Khodadad A, et al. The effect of probiotics on fecal calprotectin in patients with cystic fibrosis. *Turk J Pediatr*. 2013 Sep-Oct;55(5):475-8.

17. Ashorn S, Honkanen T, Kolho KL, Ashorn M, Välineva T, Wei B, et al. Fecal calprotectin levels and serological responses to microbial agents among children and adolescents with inflammatory bowel disease. *Inflamm. Bowel Dis.*2009;15: 199–205.

18. Canani RB, de Horatio LT, Terrin G, Romano MT, Miele E, Staiano A, Rapacciuolo L, et al. Combined use of noninvasive tests is useful in the initial diagnostic approach to a child with suspected inflammatory bowel disease.*J. Pediatr.Gastroenterol. Nutr*.2006;42:9–15.

19. Inciarte-Mundo J, Victoria Hernández M, Ruiz-Esquide V, Raquel Cabrera-Villalba S, Ramirez J, Cuervo A, et al. Serum calprotectin versus acute-phase reactants in the discrimination of inflammatory disease activity in rheumatoid arthritis patients receiving tumor necrosis factor inhibitors. *Arthritis Care Res (Hoboken)* 2016;68(7):899–906.

20. Mads Abildtrup, Gabrielle H. Kingsley and David L. Scott, Calprotectin as a biomarker for rheumatoid arthritis: a systematic review. J Rheumatol. 2015;42(5):760–770.

21. Coban E, Adanir H. Platelet activation in patients with Familial Mediterranean Fever. *Platelets*. 2008 Sep;19(6):405-8.

22. Demirbaş F, Çaltepe G, Comba A, Abbasguliyev H, Uyar NY, Kalaycı AG. Fecal calprotectin in children with familial Mediterranean fever in the attack-free period. *Pediatr Int.* 2019 Nov;61(11):1140-1145.

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