Fecal Calprotectin in assessing Familial Mediterranean Fever Patients

Aya Mohammed Mahros[1] Eslam El Shenawy[2] Mohammed Hussien Ahmed[1]

[1]*Department of Hepatology, Gastroenterology and Infectious Disease, Faculty of Medicine, Kafrelsheikh University, Kafr Elshikh, Egypt.*

[2]*Department of Radiology, Faculty of Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.*

Corresponding Author
Aya Mohammed Mahros

Mobile:
+201004039954

E mail:
yoye_85@hotmail.com

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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, in monocytes and reactive macrophages. It 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 predictor of disease activity.

Patients/Material and Methods: This is a Prospective cross-sectional study. Between May 2020 and May 2022, 158 patients diagnosed with FMF in our outpatient clinic in 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 blood picture, ESR, CRP, Amyloid A, Faecal 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. Largest number of patients was diagnosed at 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 time of diagnosis ($P < 0.001616$). The level Calprotectin at time of diagnosis of 100 pg/mL had 96.40% sensitivity and 96.60% Specificity. Homozygous mutation had positive correlation with Colchicine dose needed to give response. Calprotectin level have negative correlation with 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 autoinflammatory disease presented by repeated episodes of fever and serositis **[1, 2]**. The etiology of FMF is not properly recognized but, 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 clinical manifestation of disease. Homozygus mutation are more liable to severe symptoms and complication **[3, 4]**.

Four Types of gastrointestinal affection may be found in FMF patients. The first one is associated with the attack, while the second not related to the attack . The third GIT affection is related to treatment side effects 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 are correlated with severity of intestinal inflammation **[6,7]**. Many conditions are associated with increase the level of fecal calprotectin such as IBD and

(NSAID) use.[8, 9] Many recent studies suggest that FC may be 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 a expensive, difficult in children and invasive procedure **[13]**. So there is a need for a simple non invasive methods for evaluation of mucosal inflammation .

This study aimed to evaluate fecal calprotectin levels in FMF patients as 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 subjected to history taking, 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 temperature 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. Largest number of patients was diagnosed at age of twenties. Only 5 patients were diagnosed at 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 history of abdominal surgery. We reported that 130 patients had heterozygous mutation and 28 patients had homozygous mutations confirmed by PCR **(Figure 1)**.

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

Our study reported that FC levels were raised at 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 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 correlation between quantitative variables. The area under the ROC curve were 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 $P < 0.05$.

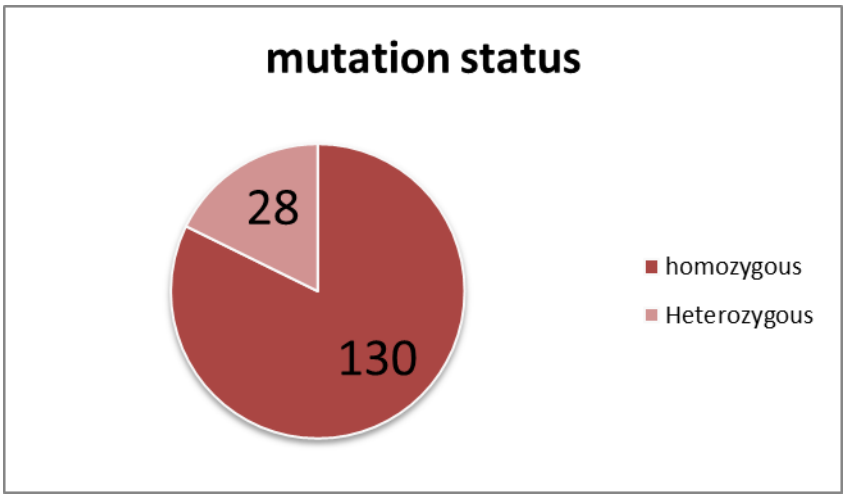

Fig. (1): Show number of homozygous and Heterozygous subtype.

Table (1): Showed lab finding 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 Colachicine needed to give response.

	Heterozygous	Homozygous	P value
	130	28	
Fecal calprotectin at 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[a]	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

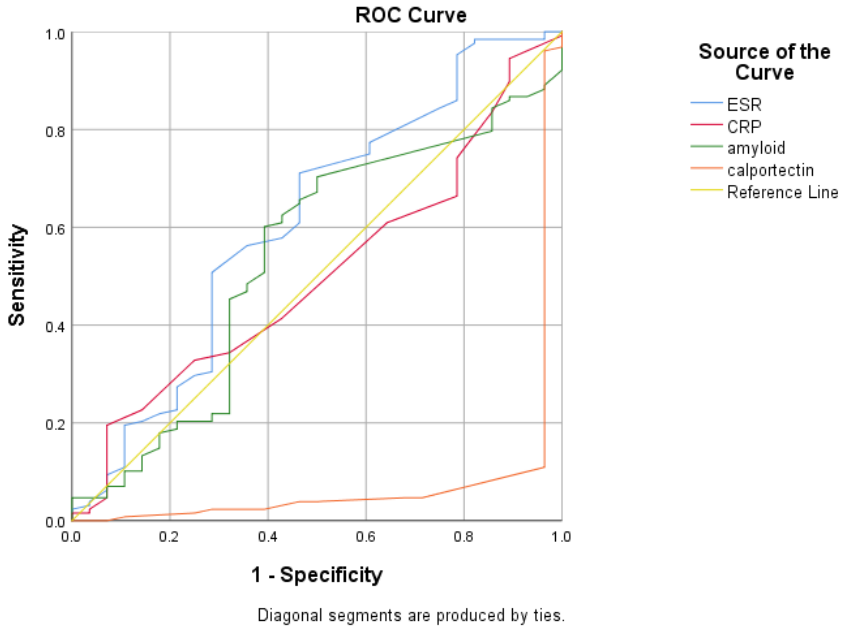

Fig. (2): ROC curve analysis.

DISCUSSION

Familial Mediterranean fever (FMF) is autoinflammatory disease presented by repeated episodes of fever and serositis**.** Several markers as CRP, ESR, Amyloid, 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 lymphocyte and neutrophiles in intestinal mucosa **[15]**. However, there are other etiologies to raise its level **[16-18]**. To exclude other cause of raised FC, we excluded patients with any chronic disease, those known to have IBD or other intestinal pathology on colonoscopy, 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 homozygous mutation have more sever disease than those with heterozygote mutation, our study revealed increase platelet count in homozygotes than heterozygote, 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 were significantly higher in homozygous than heterozygous group. This is in line with a study by Gucenmezet al. found that Fecal calprotectin level in FMF patients was significantly higher than in healthy patients **[10]**. So we suggest that FC can be used for diagnosis and follow up of FMF patients. High level of FMF may be due to

chronic inflammation in FMF patients. Another study by Fatma et al concluded that FC is noninvasive simple method for assessing inflammation in childern 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 biomarkers to assess the severity of inflammation in FMF patients
- FMF is autoimmune disease with chronic inflammation in different organ
- Future study with large number is highly recommended

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