ORIGINAL ARTICLE

Chemerin: an exploitable biomarker for familial Mediterranean fever in Egyptian patients

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ABSTRACT

Aim: The most prevalent autoinflammatory condition, Familial Mediterranean Fever (FMF), is characterized by persistent, subclinical inflammation. Chemerin plays a role in inflammation. The aim of the work is to assess if Chemerin can be used as a diagnostic index in Familial Mediterranean Fever patients.

Methods: A total of 66 Familial Mediterranean Fever patients and 60 controls matching age and sex were enrolled in the study. Serum Chemerin, vitamin D_3, oxidized LDL, lipid profile and Amyloid A were assessed by ELISA assay.

Results: Chemerin level which suggested chronic inflammation, was significantly elevated as compared to the controls. The Familial Mediterranean Fever patients had highly significant levels of total cholesterol, total triglycerides and LDL, while the HDL significantly inversely correlated in the Familial Mediterranean Fever patients compared to controls. Moreover, the vitamin D level was significantly lower in Familial Mediterranean Fever patients.

Conclusion: Chemerin that acts as a pro-inflammatory adipokine, could be a novel biomarker reflecting the chronic pro-inflammatory status in the Familial Mediterranean Fever patients and may facilitate the development of Chemerin as a new therapeutic modality in the treatment of Familial Mediterranean Fever. Furthermore, vitamin D supplementation is recommended in the Familial Mediterranean Fever patients.

Keywords: Familial Mediterranean Fever, Chemerin, Vitamin D_3, Lipid Profile

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INTRODUCTION

Familial Mediterranean Fever (FMF) is a chronic systemic autoinflammatory disorder, characterized by recurrent attacks of fever, headache, and inflammation of serous membranes (1). It is an inherited autosomal recessive trait associated with missense mutation in the *MEFV* gene located on the short arm of chromosome 16 (2). The *MEFV* gene encodes the protein pyrin-marenstrin which plays a role in the inflammatory pathway. The *MEFV* gene mutation leads to loss of Pyrin function resulting in uncontrolled inflammation (3).

Activated platelets in FMF patients react with neutrophils and endothelial cells producing proinflammatory compounds (4). The *MEFV* mutations are responsible for dysregulation of apoptosis and inflammation due to the diminished ability of the abnormal pyrin protein to modulate activity of the inflammasome and interleukin-1-beta. Accordingly, FMF has self-limited febrile attacks of polyserositis, followed by free periods. However, during the symptom-free intervals, FMF patients have subclinical inflammation. This persistent inflammation may cause endothelial dysfunction and atherosclerosis (5). Inflammation and vascular atherosclerosis are facilitated by this mechanism.

Chemerin, an inflammatory chemokine, is one of the adipokines that has been identified as playing a role in the link between obesity, inflammation, and atherosclerosis (6). Importantly, Chemerin plays a dual role in inflammation. In the early stages of inflammation, Chemerin acts as a pro-inflammatory molecule, attracting and activating immune cells to the site of inflammation. This helps to fight off infection and heal tissue damage. Whereas at the end of inflammation, Chemerin plays a significant role as an anti-inflammatory function (7). During the attack, the levels of serum Chemerin in patients were found to be significantly higher than those of healthy controls and the expression of Chemerin in serum is positively correlated with the erythrocyte sedimentation rate (ESR) and C-reactive protein levels (7). Moreover, Chemerin is mainly related to regulating adipogenesis and the progression of atherosclerosis (8). Therefore, Chemerin can be used as a predictor of inflammation and atherosclerosis in FMF patients. The aim of the study is to highlight the role of Chemerin in FMF inflammation.

SUBJECTS AND METHODS

Subjects

Our research comprised 66 patients diagnosed with FMF, according to the FMF criteria and 60 healthy children matching sex and age served as a control group. All patients were recruited from the Clinical Genetics Department, National Research Centre (NRC). This study received ethical approval from the Al-Azhar University Ethical Committee (No: -711) and adhered to the Declaration of Helsinki of the World Medical Association. After receiving a thorough explanation of the study, each participant and legal guardian in the study also signed a written informed consent agreement. All the datasets used and/or analysed during the current study are available from the corresponding author (Hala T El-Bassyouni) upon reasonable request.

Anthropometry

The International Biological Programme (IBP) was followed for the anthropometric measures. Tanita's SC-330 body composition analyser evaluated fat mass (9).

Biochemical examinations

Assessment of lipid profile

Total cholesterol (TC) and triglycerides (TG) in serum were assessed. Also, high-density lipoprotein cholesterol (HDL cholesterol) was assessed using an automatic biochemistry analyzer (Olympus America Inc., Centre Valley, Pennsylvania, USA), and the Friedewald algorithm was used to calculate low-density lipoprotein cholesterol (LDL cholesterol) (Olympus and Beckman Coulter).

Quantification of 25(OH) vitamin D_3

25(OH) vitamin D levels in serum were quantified using enzyme-linked immunosorbent assay (ELISA) (DiaSorin 25-OHD assay, still water, Minnesota, USA) (10).

Quantification of Chemerin

Chemerin was quantified in serum by enzyme-linked immunosorbent assay (ELISA) assay produced by Sinogeneclon Biotech, Co., Ltd. China, catalogue No.SG-10381 (7).

Quantification of oxidized LDL

Serum Oxidized LDL was determined in by enzyme-linked immunosorbent assay (ELISA) assay produced by Sinogeneclon Biotech, Co., Ltd. China, catalogue No.SG-11266 (11).

Quantification of Amyloid A

Amyloid A was determined by enzyme-linked immunosorbent assay (ELISA) assay produced by Sunlong Biotech Co., Ltd. China, catalog No.SL 1571Hu (12).

Molecular analysis

Peripheral blood sample of 3mL volume was collected from all participants in EDTA tubes and DNA was extracted from white blood cells using PREP-MP Genetics kit (MP Biomedicals, USA). The DNA was quantified by NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™) to be greater than or equal to 1.0ng per reaction.

Familial Mediterranean Fever Real-Time PCR Genotyping Kit (DNA Technology, Mosco, Russia) was used for mutation detection. PCR amplification was then conducted on a Light Cycler 480 Instrument (Software Version 1.2.9.11, Roche) according to the manufacturer's instructions. Exons 2, 3, 5 and 10 were amplified by a PCR program set as follows: 95°C denaturation for 30 seconds, 55°C hybridiation for 30 seconds and 72°C elongation for 30 seconds. The 13 gene variants analysed with the kit as follows: *E148Q* in exon 2, *P369S* and *R408Q* in exon 3, *F479L* in exon 5, and *M694V*, *M694I*, *M680IG/C*, *M680IG/A, I692del, V726A, A744S, K695R* and *R761H* in exon 10.

Statistical analysis

Statistics used to characterize the data included mean (+/- SD), range, and median, or frequencies (number of cases). The Kolmogorov-Smirnov test was used to determine whether numerical data supported the normal premise. The Mann Whitney U test for independent samples was used to compare non-normal data and the student test for independent samples was used to compare numerical variables between the research groups. We used the Chi-square(x2) test to compare classified data. When the anticipated frequency is less than 5, the CExact test was used in its place. The Spearman rank correlation equation for non-normal variables and non-linear monotonic relation was used to correlate different variables. Statistical significance was defined as two-sided p-value results less than 0.05. IBM SPSS (Statistical Package for the Social Science; IBM Corp., Armonk, NY, USA) release 22 for Microsoft Windows was used to perform all statistical computations. Receiver operator characteristic (ROC) curve was performed to detect the importance of Chemerin in the diagnosis of FMF.

RESULTS

The age of FMF patients ranged from 5 years to eighteen years (12.34±3.6). Male to female ratio was 1:1. Positive consanguinity was detected in 41.3%, while positive family history was present in 39.7%. The anthropometric measurements showed that all absolute and z-scores are within normal ranges (Table 1). Our results showed that FMF patients have significant higher levels of serum Chemerin, serum amyloid A and serum lipids (cholesterol, triglycerides, and low-density lipoprotein) compared to controls (Table 2). The current study indicated that Chemerin positively significantly correlated with total cholesterol and significantly inversely correlated with the high-density lipoprotein (Table 3). This work showed that Chemerin significantly directly correlated with ESR and serum amyloid A. While it significantly inversely correlated with vitamin D. But there was no correlation between Chemerin and Colchicine intake (Table 4).

Genetic analysis of *MEFV* gene in our FMF patients revealed that 39 cases showed *M6941* mutations, 26 cases had *M6801* mutations, and one case only showed *V726A* mutation. There was no correlation between Chemerin, and gene mutations except that Chemerin had a high level in the case with *V726A* mutation, but it was only one case (Table 5). Receiver Operator Characteristic (ROC) analysis was done to determine the best cut off value of Chemerin to diagnose FMF. The best cut off selected was 58.9 that achieved 100% sensitivity and 55% specificity of Chemerin in the diagnosis of FMF (Figure 1).

Table1: The anthropometric findings of the FMF patients

Parameters	FMF
Weight (kg)	25.5±10.1
Weight-Z score	-0.38±0.03
Height (cm)	122.4±21.2
Height -Z score	-0.144±0.01
Head circumference (cm)	51.4±2.5
Head circumference Z score	-0.21±0.49
BMI (kg/m^2)	17.53±8.62
BMI-Z score	-0.26±0.01

Table 2: Laboratory data among the cohort

	FMF (n=66)	Controls (n=60)	p-value
Chemerin (nmol/L)	76.3±16.9	56.2±5.4	0.05
Serum Amyloid A (µmol/L)	35.2±22.2	10.3±17.4	0.01
Vitamin D (nmol/L)	21.2±2.1	24.8±1.7	0.01
Cholesterol (mmol/L)	114.8±14.7	103.4±8.4	0.1
Triglycerides (mmol/L)	113.6±34.5	78.8±9.5	0.01
High density lipoprotein (HDL) (mmol/L)	40.9±6.7	55.4±5.7	0.01
Low density lipoprotein (LDL) (mmol/L)	50.4±13.9	18.9±6.9	0.01

p-value < 0.05 significant

Table 3: The correlation between the Chemerin level and lipid profile

Correlations			Chol	TGs	HDL	LDL
Spearman's rho	Chemerin	Correlation Coefficient	-0.32	-0.16	-0.27	0.14
		p value	0.01	0.21	0.03	0.25
		N	66	66	66	66

p-value < 0.05 significant

Table 4: Correlation between Chemerin and other markers

Vit. D	Correlation Coefficient	-0.24
	p value	0.05
Colchicine	Correlation Coefficient	-0.21
	p value	0.11
Chemerin		
ESR	Correlation Coefficient	0.3
	p value	0.02
Serum Amyloid A	Correlation Coefficient	0.37
	p value	0.002

p-value < 0.05 significant

Table 5: *MEFV* gene mutations in FMF cohort patients

			Number of Mutations				
Mutation	**Variant**	**Patient Number**	**Mean**	**Std Dev**	**Median**	**Min**	**Max**
M680I	*2040 G>C & 2040 G>A*	26	86.65	14.01	88	67	110
M694I	*2082 G>A*	39	82.26	16.72	78	59	120
V726A	*2177 T>C*	1	112	-	112	112	112

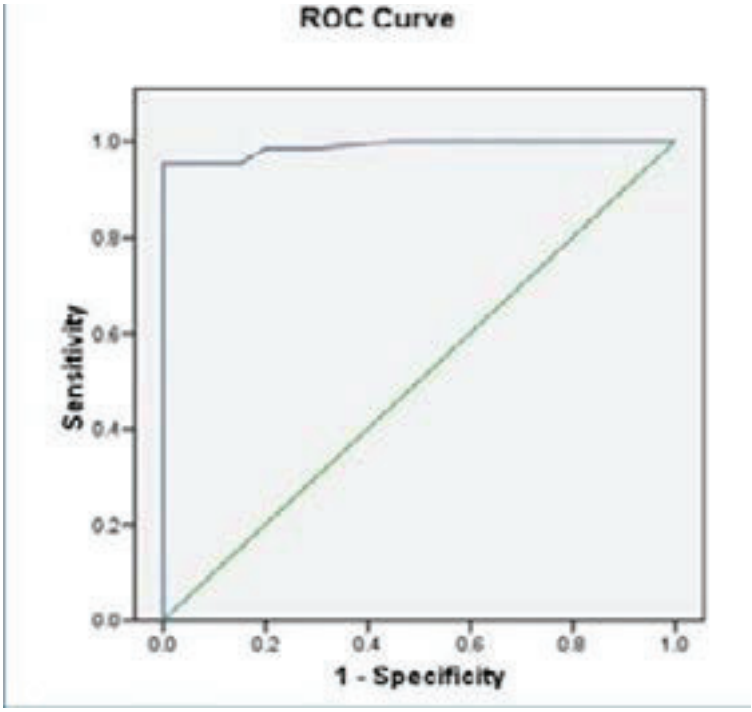

Figure 1: Receiver Operator Characteristic (ROC) analysis showed a good sensitivity and specificity of Chemerin in the diagnosis of FMF, area under the curve = 0.95, sensitivity: 100%, Specificity:55%, P = 0.006.

DISCUSSION

Inflammation in FMF is related to endothelial dysfunction, platelet hyperactivation and increased atherosclerotic burden (13). Our study verified that there was a highly positive correlation between the atherogenic factors in the form of total cholesterol, total triglycerides and low-density lipoprotein in FMF patients, while there was an inverse correlation of the high-density lipoprotein amongst the FMF patients compared to normal controls. Our findings are consistent with earlier research (1, 14). While Tasliyurt et al. did not detect an increase in the lipid profile in their patients (15). Chemerin is significant in influencing both physiologic and pathophysiologic processes, according to recent investigations (16, 6). Chemerin and its receptor CMKLR1 form a complex which is implicated in the regulation of immune response and encourages the development and differentiation of pre-adipocytes (17). Additionally, Chemerin contributes to the early stages of acute inflammation (18). Therefore, it is markedly elevated in a number of inflammatory disorders (19, 20). In this report, the Chemerin level showed positive correlation with the ESR and serum amyloid A level amongst the FMF patients, which is similar to former reports (21, 22). The increased concentration of Chemerin in adipose tissue causes recruitment of immune cells leading to elevation of inflammatory indices; ESR and serum amyloid A (23). Moreover, Chemerin highly positively correlated to total cholesterol and negatively correlated with HDL in the FMF patients (24). Similarly in our study, there was a significantly positive correlation of lipid profile and Chemerin in the form total cholesterol, triglyceride and LDL and negative correlation with HDL. The HDL function reverses the cholesterol transmission and prevents the LDL oxidation accordingly diminishing its atherogenic activity (25). Chemerin is suggested to play a role in the regulation of the enzyme responsible for lipid metabolism by diminishing the gathering of adenosine cyclic monophosphate (CAMP) and stimulates the calcium release in the adipocytes (6). The current research revealed no correlation between Chemerin and colchicine intake. Moreover, no association was detected between the Chemerin level and *M6941* and *M6801* mutations while a high level of Chemerin was reported in the patient with *V726A* mutation. Similarly, earlier research found that high inflammatory markers were more frequent in *V726A* allele (26). While a previous study reported that patients with *V726A* mutations were related to the mild form of the disease, this is in contrary to our results, but this may be due to the presence of only one patient with *V726A* mutation (27). Furthermore, low vitamin D levels were determined among the FMF patients. This is similar to the findings of previous investigators (28, 29). Therefore, we recommend vitamin D supplementation to these patients.

In addition, the ROC analysis was done to determine the best cut off values of Chemerin to diagnose the FMF. The best cut off selected was 58.9 that achieved 100% sensitivity and 55% specificity. Consequently, our study showed a good sensitively and specificity of Chemerin in the diagnosis of FMF. The study's limitation is the small sample size and only one case showed *V726A* mutation.

CONCLUSION

Chemerin that acts as a pro-inflammatory adipokine could be considered as a biomarker reflecting the chronic proinflammatory status in the FMF patients and may facilitate the development of Chemerin as a new therapeutic modality in the treatment of FMF. It is recommended that a follow-up study be conducted to investigate its efficacy as a potential FMF treatment by keeping Chemerin levels in FMF patients low. In addition, vitamin D supplementation is recommended for FMF patients.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. Mahmoud SSE, Ismail NA, Farag YM, et al. Intima media thickness as an early predictor of atherosclerosis in Egyptian children with familial Mediterranean fever. A*rch Med Sci Atheroscler Dis* 2018; 3: e106-e111.
2. Skendros P, Papagoras C, Mitroulis I, et al. Autoinflammation: lessons from the study of familial mediterranean fever. *J Autoimmun* 2019; 104: 102305.
3. Lachmann HJ, Sengül B, Yavuzşen TU, et al. Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. *Rheumatology (Oxford)* 2006; 45(6): 746-750.
4. Bakogiannis C, Sachse M, Stamatelopoulos K, et al. Platelet-derived chemokines in inflammation and atherosclerosis. *Cytokine* 2019; 122: 154157.
5. Zekry ME, Sallam AM, AbdelHamid SG, et al. Genetic and Epigenetic Regulation of MEFV gene and their impact on clinical outcome in auto-inflammatory familial mediterranean fever patients. *Curr Issues Mol Biol* 2023; 45: 721–737.
6. Su X, Cheng Y, Zhang G, et al. Chemerin in inflammatory diseases. *Clin Chim Acta* 2021; 517: 41-47.
7. Lehrke M, Becker A, Greif M, et al. Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis. *Eur J Endocrinol* 2009; 161(2): 339-344.
8. Sun JX, Zhang C, Cheng ZB, et al. Chemerin in atherosclerosis. *Clin Chim Acta* 2021; 520: 8-15.
9. Leite Portella D, Arruda M, Gómez-Campos R, et al. Physical growth and biological maturation of children and adolescents: proposed reference curves. *Ann Nutr Metab* 2017; 70(4): 329-337.
10. Lotfy HM, Marzouk H, Farag Y, et al. Serum vitamin D level in Egyptian children with familial mediterranean fever. *Immunol Lett* 2017; 185: 74-78.
11. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin*

Chem 1972; 18(6): 499-502.
12. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* 2005; 5: 13.
13. Ozalper V, Kara M, Tanoglu A, et al. Evaluation of endothelial dysfunction in patients with familial mediterranean fever: the relationship between the levels of asymmetric dimethylarginine and endocan with carotid intima-media thickness and endothelium-dependent vasodilation. *Clin Rheumatol* 2017; 36(9): 2071-2077.
14. Mohamed R, El-Bassyouni HT, Elwan HS, et al. Carotid intima-media thickness, lipid profile, serum amyloid A and vitamin D status in children with familial mediterranean fever. *Egyptian Rheumatologist* 2020; 42(3): 237-240.
15. Tasliyurt, T, Gokce, E, Sahin, S, et al. Prevalence of nonalcoholic fatty liver disease in familial mediterranean fever. *Endocr Metab Immune Disord Drug Targets* 2020; 20: 952-958.
16. Li J, Lu Y, Li N, et al. Chemerin: a potential regulator of inflammation and metabolism for chronic obstructive pulmonary disease and pulmonary rehabilitation. *Biomed Res Int* 2020; 2020: 4574509.
17. Fischer TF, Czerniak AS, Weiß T, et al. Ligand-binding and -scavenging of the Chemerin receptor GPR1. *Cell Mol Life Sci* 2021; 78(17-18): 6265-6281.
18. Acewicz M, Kasacka I. Chemerin activity in selected pathological states of human body - a systematic review. *Adv Med Sci* 2021; 66(2): 270-278.
19. Gonzalez-Ponce F, Gamez-Nava JI, Perez-Guerrero EE, et al. Serum Chemerin levels: a potential biomarker of joint inflammation in women with rheumatoid arthritis. *PLoS ONE* 2021; 16(9): e0255854.
20. Macvanin MT, Rizzo M, Radovanovic J, et al. Role of Chemerin in cardiovascular diseases. *Biomedicines* 2022; 10(11): 2970.
21. Vazquez-Villegas ML, Gamez-Nava JI, Saldaña-Cruz AM, et al. Functional disability is related to serum Chemerin levels in rheumatoid arthritis. *Sci Rep* 2021; 11(1): 8360.
22. Kolahdouzi S, Baghadam M, Kani-Golzar FA, et al. Progressive circuit resistance training improves inflammatory biomarkers and insulin resistance in obese men. *Physiol Behav* 2019; 205: 15-21.
23. Szpakowicz A, Szpakowicz M, Lapinska M, et al. Serum Chemerin concentration is associated with proinflammatory status in chronic coronary syndrome. *Biomolecules* 2021; 11(8): 1149.
24. Huang CL, Xiao LL, Xu M, et al. Chemerin deficiency regulates adipogenesis is depot different through TIMP1. *Genes Dis* 2020; 8(5): 698-708.
25. Duan H, Song P, Li R, et al. Attenuating lipid metabolism in atherosclerosis: the potential role of anti-oxidative effects on low-density lipoprotein of herbal medicines. *Front Pharmacol* 2023; 14: 1161657.
26. Lofty HM, Marzouk H, Farag Y, et al. Serum amyloid a level in Egyptian children with familial mediterranean fever. *Int J Rheumatol* 2016; 2016: 7354018.
27. Maggio MC, Corsello G. FMF is not always “fever”: from clinical presentation to “treat to target”. *Ital J Pediatr* 2020; 46(1): 7.
28. Kazem Y, Zarouk WA, Hamed K, et al. The effect of anti-inflammatory diet and vitamin D supplementation on the amelioration of the clinical status and cognitive functions of familial mediterranean fever patients. *Kobe J Med Sci* 2020; 66: E159-E165.
29. Abdallah HR, Thomas MM, Abuelhamd WA, et al. The influence of vitamin D administration on the clinical presentation, body mass index, and osteoprotegerin (OPG) level in a sample of Egyptian children with familial Mediterranean fever. *Egypt Pediatric Association Gazette* 2021; 69: 44.