ORIGINAL ARTICLE

The role of neutrophil gelatinase-associated lipocalin and IL-1β in early prediction of renal dysfunction in patients with familial Mediterranean fever

Hala T. El-Bassyouni, Wafaa G. Shousha, Fateheya M. Metwally, Khaled Hamed, Randa S. Lotfy, Zeinab H. Korany, Shimaa S. Ramadan

ABSTRACT

Background: Familial Mediterranean fever (FMF) is a monogenic, autosomal recessive autoinflammatory disease. Neutrophil gelatinase-associated lipocalin (NGAL) is considered as a good marker of kidney disorders. In addition, Interleukin-1β (IL-1β) is the major cytokine involved in both FMF pathogenesis and inflammatory appearance. The study aimed to assess the diagnostic and prognostic roles of NGAL and IL-1β in FMF patients and elucidate their roles in the early prediction of kidney dysfunction.

Methods: The study included 56 FMF patients who were chosen based on clinical criteria as well as 58 healthy age and sex-matched controls. Serum IL-1β and lipocalin-2 (NGAL) were quantitatively determined using solid-phase enzyme-linked immunosorbent assay (ELISA). Furthermore, the levels of urea, creatinine, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), estimated glomerular filtration rate (eGFR), white blood cell count (WBC) and haemoglobin were determined.

Results: NGAL and IL-1β levels were significantly increased in FMF patients compared to the healthy controls (p=0.003 and p=0.001 respectively). NGAL significantly correlated with eGFR (p=0.046). Besides, IL-1β significantly correlated with the severity of the disease (p=0.003). This study indicated that NGAL and IL-1β could be good diagnostic biomarkers for FMF disease (AUC =0.740 and 0.781 respectively). Moreover, the current study delineated that serum IL-1β significantly correlated with serum lipocalin-2 (NGAL) (p < 0.000).

Conclusion: The current study highlighted the role of serum NGAL and IL-1β in the diagnosis of FMF, progression of the disease and early prediction of renal dysfunction in FMF patients.

Keywords: Familial Mediterranean fever, Lipocalin-2 (NGAL), IL-1β, renal dysfunction

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INTRODUCTION

Familial Mediterranean fever (FMF) is a monogenic, autosomal recessive and autoinflammatory disease (1). It is characterized by self-limited inflammatory attacks (short crisis lasting for several days) of recurrent fever and painful serositis mediated by inflammatory cytokines (2,3). FMF commonly occurs among people of the Mediterranean basin such as Jews, Turks, Armenians, and Arabs but patients from various ethnicities (such as Italy, Japan, America) are being described (4). FMF occurs due to MEFV gene mutations (5). The diagnostic criteria and classifications of FMF include: the Tel-Hashomer criteria proposed for adult patients (6), revised by Livneh et al (7) and the Turkish paediatric criteria (8). The diagnosis relies mainly on the clinical criteria, then it is confirmed by genetic testing (9,10). Ethnic origin and family history are supportive tools in FMF diagnosis (11). The prevalence of the disease in Turks, non-Ashkenazi Jews, Armenians and Arabs is with a carrier rate of 1:5, 1:5, 1:7 and 1:16 respectively (12). The development of amyloidosis is the most severe complication of FMF which initially affects the kidneys and is characterized by proteinuria developing to nephrotic and renal damage and finally to end-stage kidney failure needing dialysis and renal transplantation (2,13). Colchicine is the drug of treatment of FMF as it is safe and well-tolerated, the maximum advisable daily doses are 2 mg for children and 3 mg for adults (14).

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein which is linked to the family of lipocalin related to the low level in renal tubular cells and responds to different acute injuries in the kidney (15). NGAL was found in the urine or blood earlier than serum creatinine level elevation and it is an early predictive biomarker of acute kidney injury (AKI) (16). The plasma/serum concentrations of NGAL are elevated throughout the AKI and the serum level of NGAL correlates with the AKI severity. Accordingly, it is a good marker of acute kidney injuries (17). It is suggested that NGAL plays a role in chronic kidney diseases (CKD) (18). Interleukin-1β (IL-1β) is a member of the IL-1 family and is defined as the main cytokine influencing the disease pathogenesis (19). The inflammation in FMF might be caused by the elevated quantities of IL-1β (20). There is a great demand for new biomarkers to help in diagnosis and prognosis of familial Mediterranean fever (FMF). Therefore, the study aimed to unveil the diagnostic and prognostic roles of neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-1β (IL-1β) in FMF patients and identify their roles in the early prediction of kidney dysfunction.

SUBJECTS AND METHODS

A total of 56 patients with FMF in the attack-free period (AFP), 26 (46.4%) males and 30 (53.5%) females in a ratio of 1:1.15 were enrolled in this study. The control group consisted of 58 healthy age and sex-matched individuals without a history of any health problems [24 (41.4%) males and 34 (58.6%) females]. The patients were recruited from the Clinical Genetics Department, National Research Centre, Egypt. Ethical approval was obtained from the Ethics Committee of the National Research Centre. A written informed consent from all patients' guardians and controls were obtained according to the Helsinki Declaration 1983, and the Institutional Review Board of the National Research Centre. The diagnosis of FMF was dependent on the Tell-Hashomer criteria (7). Most of the patients were administrating colchicine during blood sampling. The participants did not have other systemic diseases (including diabetes mellitus, chronic renal failure, malignancy, and ischemic heart disease) nor administered drugs other than colchicine. The disease severity was estimated using the criteria of F-SS-1 (21). The appropriate estimation of the renal function, glomerular filtration rate (eGFR) was calculated using bedside Schwartz (22).

Mutation analysis

The DNA was extracted from 2ml of peripheral blood into EDTA anticoagulated tubes, using the QIAamp DNA Blood Isolation Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The DNA concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). For MEFV single nucleotide polymorphisms (SNPs), genotyping was performed using commercial kits (DNA technology, Moscow, Russia) and the Light-Cycler 480 II Real-Time PCR System (Roche Diagnostics, Mannheim, Germany) according to the manufacturers' protocols.

Fasting venous blood samples from all subjects were obtained. Centrifugation was done at 3000 g for 15 min at 4°C, sera were obtained and stored at -80°C till analysis. Serum urea and serum creatinine were measured using biosystem urea kit, Spain & biosystem creatinine kit, Spain; respectively. CRP was measured using Spin react CRP kit, Spain. Determination of ESR was based on the Westergren method. WBCs count and haemoglobin were assessed automatically by Biotech HL-3125. Serum lipocalin-2 was evaluated using solid-phase enzyme-linked immunosorbent assay kit (Catalog No: DLCN20, SLCN20, PDLCN20 human Lipocalin-2(NGAL) R and

D Systems, Bio-Techne Ltd, Middle East, Africa). Serum Il-1β was measured using solid-phase enzyme-linked immunosorbent assay kit (Catalog No: DLB50, SLB50, PDLB50 human Interleukin 1 beta (IL-1β) R and D Systems, Bio-Techne Ltd, Middle East, Africa). This assay uses the quantitative sandwich enzyme immunoassay method. A specific monoclonal antibody for the marker has been precoated onto a microplate. Standards and samples were added into the wells and any quantity of the marker present was linked by the immobilized antibody. Following the removal of any unattached substance, a marker specific enzyme-linked polyclonal antibody was added to the wells. After elimination of any unattached antibody-enzyme reagent *via* washing, addition of a substrate solution was done, and the colour produced was in proportion to the quantity of the marker attached in the first step. Then the colour development was ceased, and its intensity was identified. The absorbance measurement was performed at 450nm. Standard curves were constructed by plotting the absorbance of each standard against its concentration. From these standard curves, the concentrations of IL-1β and NGAL (lipocalin-2) for patients and controls were obtained.

Statistical analysis

The collected data were statistically analysed using the MedCalc statistical program version 11. Data were presented as mean ± SEM. The comparison of quantitative variables between the two groups of the present study was done using the independent samples t-test. The differentiation of parameters within the same group according to clinical data was established using One-way ANOVA. Pearson's correlation coefficient (r) was calculated to determine the correlations between the different parameters. The ROC curve analysis was performed and from which the cut-off value for each parameter was determined according to the best discrimination between patients and controls regarding optimal values of sensitivity and specificity. In addition, the area under curve (AUC) was determined from these ROC curves. All p values were two-sided and p-values less than 0.05 were considered statistically significant.

RESULTS

The demographic data of the patients are presented in Table 1 which explained that the patients ranged in age from 2.5 to 17 years with a mean of 8.53 years. The age of the control group ranged from 4.58 to 16 years with a mean of 10.1 years. The male/female ratio of FMF patients and controls was M/F = 26/30, M/F = 24/34; respectively. It was also shown that in the patients with FMF the mean duration of the disease was 4.1 years, the mean number of attacks per month was 8.9 and the mean dose of colchicine was 1.4mg/day. 53.57% of the patients had heterozygous M694I mutation. However, the heterozygous of E148Q, M680I and compound heterozygous of V726A/M694I were in 7.14% of patients and for the homozygous M680I, M694I, M694V, heterozygous V726A, compound heterozygous P706P/V726A, P706P/E148Q and heterozygous K671M the percent was 3.57% for each one individually.

Table 2 displayed the laboratory analyses of patients with FMF and healthy control. The haemoglobin showed a significant decrease in patients with FMF compared to the healthy control (98.0g/L, 128.0g/L, respectively, p <0.001). Furthermore, WBCs count indicated a significant increase in patients with FMF compared to healthy control (11.53x103/ mm^3, 7.75x103/mm^3; respectively, p <0.001). Moreover, the serum level of urea was higher in patients (2.07mmol/L) compared to the control (2.01mmol/L) but this elevation was not statistically significant. In addition, serum level of creatinine showed a significant increase in FMF patients compared to that of the healthy control (75.14 and 56.58µmol/L respectively, p=0.004). Moreover, CRP indicated a significant increase in patients with FMF compared to healthy control (6.11mg/L and 2.56mg/L respectively, p<0.001). ESR showed a significant increase in FMF patients compared to the healthy control (42.43mm\h and 11.2mm\h respectively, p<0.001). eGFR illustrated a significant decrease in patients with FMF compared to healthy control (66.93mL/min/1.73m^2 and 89.3mL/min/1.73m^2 respectively, p<0.001). Both serum levels of NGAL and IL-1β indicated significant increase in patients with FMF (1.91pg/mL and 3.31ng/mL; respectively) compared to the healthy control (1.47pg/mL, p=0.001 and 1.43ng/mL, p= 0.003; respectively).

Table 3 showed the mean level of NGAL was increased with increasing the severity from mild to severe, but this elevation wasn't statistically significant (1.64 ng /mL and 3.7 ng/mL; respectively). Besides, the mean level of serum IL-1β in the FMF patients was significantly increased with increasing the severity of disease (1.44 pg/mL and 1.85 pg/mL; respectively, p=0.003). Nevertheless, there is a statistically significant increase in the mean serum level of IL-1β among FMF patients with heterozygous mutations K671M, P706P/E148Q and P706P/V726A (2.95 ±0.05 pg/ml, 2.95 ±0.05 pg/mL and 2.85 ±0.05 pg/mL respectively) if compared to the remaining mutations (p <0.001). While the mean serum level of Lipocalin-2 is significantly increased among FMF patients with heterozygous mutation K671M (9.15±0.05 ng/mL) and decreased significantly among FMF patients with homozygous mutation M694V, heterozygous mutation M680I and E148Q (1.05±0.05ng/mL, 1.1±0.04ng/mL & 1.4±0.16ng/mL respectively) comparing to the remaining mutations (p <0.001).

The coefficients of correlations between the biochemical parameters and the clinical data of the FMF patients are shown in Table 4 illustrated that there was a significant positive correlation of WBCs count and CRP levels with disease duration (r=0.362, p=0.008 and r=0.396, p=0.004 respectively). The colchicine dose showed significant positive correlations with both serum IL-1β and serum urea levels (r=0.360, p=0.019 and r=0.327, p=0.034 respectively).

The coefficients of correlation between the biochemical parameters in the FMF patients are shown in Table 5. The data indicated the presence of significant negative correlation of haemoglobin versus ESR (r= -0.630, p<0.001). There was a significant positive correlation between WBCs and ESR (r=0.909, p<0.001). The data also indicated the presence of significant positive correlations of serum urea level versus serum IL-1β (r=0.286, p=0.036). Serum creatinine disclosed significant negative correlation with eGFR (r=-0.412, p=0.002). Serum creatinine revealed also a significant positive correlation with serum CRP level (r=0.308, p=0.024). Interestingly, a significant positive correlation was found between serum IL-1β and serum NGAL (r=0.692, p<0.001). A significant positive correlation was also found between serum NGAL and eGFR (r=0.278, p=0.046).

The receiver operating characteristic (ROC) curve was designed for IL-1β and lipocalin-2 (NGAL). The ROC curve for IL-1β was illustrated in Figure 1 and for Lipocalin-2 (NGAL) in Figure 2. Sensitivity, specificity, cut off value and the area under curve (AUC) for each parameter were depicted in Table 6. The data showed that the area under curve (AUC) for IL-1β and lipocalin-2(NGAL) was 0.781 and 0.740; respectively.

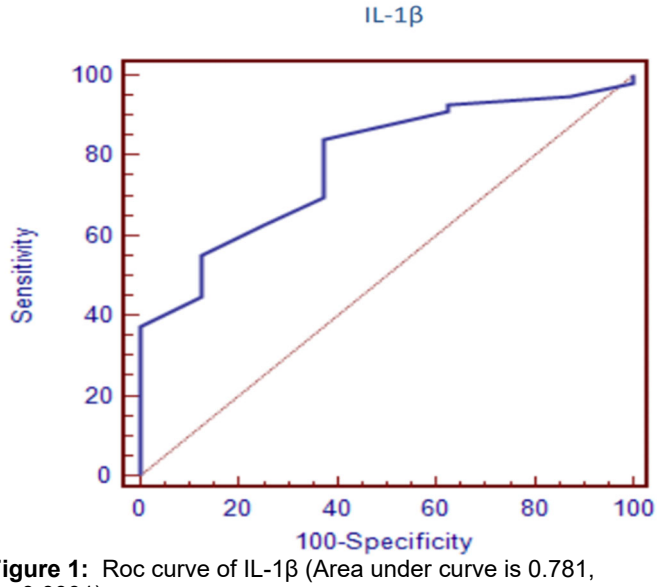

Figure 1: Roc curve of IL-1β (Area under curve is 0.781, p =0.0001)

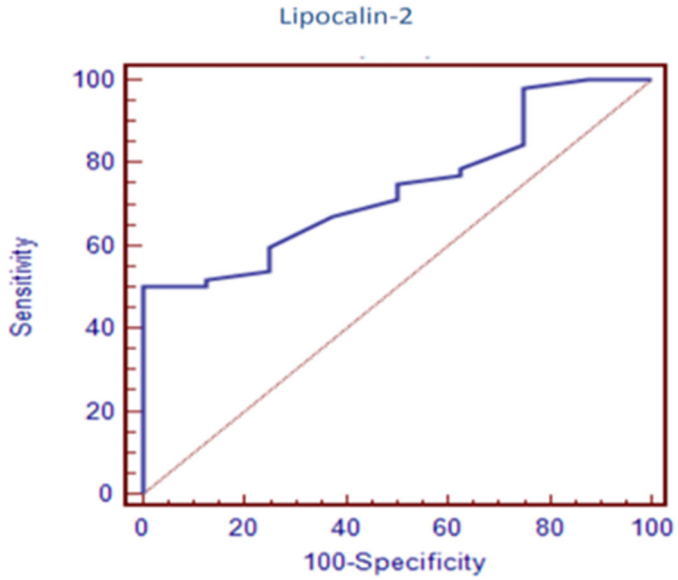

Figure 2: Roc curve of lipocalin-2 (NGAL) (Area under the curve is 0.740, p =0.0002)

Table 1: Clinical characteristics of patients with familial Mediterranean fever and healthy control

	Patients with FMF (n=56)	**Healthy Control** (n=58)
Age *(years)*	8.53±0.51	10.1±0.9
Gender *(male/female)*	26/30	4/14
Disease duration *(years)*	4.1±0.41	—
Number of attacks per month	8.90±1.73	—
Colchicine dose *(mg/day)*	1.4±0.1	—
MEFV gene mutation, % (n)		
Heterozygous E148Q	7.14 (4)	—
Heterozygous K671M	3.57 (2)	—
Heterozygous M680I	7.14 (4)	—
Heterozygous M694I	53.57 (30)	—
Heterozygous V726A	3.57 (2)	—
Heterozygous P706P/V726A	3.57 (2)	—
Heterozygous P706P/E148Q	3.57 (2)	—
Heterozygous V726A / M694I	7.14 (4)	—
Homozygous M680I	3.57 (2)	—
Homozygous M694I	3.57 (2)	—
Homozygous M694V	3.57 (2)	—

Data were expressed as mean ± standard error of mean.

Table 2: Laboratory investigations of FMF patients and controls

	Patients with FMF (n=56)	**Healthy Control** (n=58)	***P* value**
Haemoglobin (g/L)	98.0 ±2.0	128.0±1.4	<0.001**
WBCs (x10^3/mm^3)	11.53±0.5	7.75±0.3	<0.001**
Urea (mmol/L)	2.07±0.05	2.01± 0.09	0.335
Creatinine (mmol/L)	75.14±3.54	56.58± 2.65	0.004**
CRP (mg/L)	6.11±0.53	2.56±0.14	<0.001**
ESR (mm\h)	42.43±3.2	11.2±0.7	<0.001**
Serum IL-1β (pg/mL)	1.91±0.07	1.47±0.06	0.001**
Serum lipocalin-2 (NGAL) (ng/mL)	3.31±0.34	1.43±0.17	0.003**
$_e$GFR (Bedside Schwartz) (mL/min/1.73 m2)	66.93±2.6	89.3±3.91	<0.001**

Data were expressed as mean ± standard error of mean, *Significant change at p< 0.05, **Highly significant change at p< 0.001

Table 3: Mean serum IL-1β and Lipocalin-2 (NGAL) in FMF patients by potential categorical variables

Variables	**Serum IL-1β** (pg/mL)	**Serum Lipocalin-2 (NGAL)** (ng/mL)
Gender		
Female	1.90±0.11	2.83±0.42
Male	1.91±0.08	3.87±0.53
p value	0.974	0.129
Severity		
Mild	1.44±0.04	1.64±0.04
Moderate	2.85±0.05	5.75±0.05
Severe	1.85±0.08	3.7±0.5
p value	0.003*	0.125
MEFV gene mutations		
Heterozygous E148Q	1.6±0.08	1.4±0.16
Heterozygous K671M	2.95±0.05	9.15±0.05
Heterozygous M680I	1.7±0.17	1.1±0.04
Heterozygous M694I	1.8±0.08	3.0±0.36
Heterozygous V726A	1.65±0.05	5.95±0.05
Heterozygous P706P/V726A	2.85±0.05	5.75±0.05
Heterozygous P706P/E148Q	2.95±0.05	
Heterozygous V726A / M694I	2.05±0.18	4.5±2.1
Homozygous M680I	1.93±0.05	5.05±0.05
Homozygous M694I	1.45±0.05	3.25±0.05
Homozygous M694V	1.85±0.05	1.05±0.05
p value	<0.001**	<0.001**
Family history		
Yes	1.87±0.11	3.2±0.42
No	1.94±0.08	3.4±0.52
p value	0.598	0.827

Data were expressed as mean ± standard error of mean, * Significant change at $p< 0.05$, ** Highly significant change at $p< 0.001$ Comparison done using ANOVA test.

Table 4: Correlation between the biochemical parameters and the clinical data of FMF patients

	Disease duration (years)		**Number of attacks** (per month)		**Colchicine dose** (mg/day)	
	Correlation coefficient	P value	Correlation coefficient	P value	Correlation coefficient	P value
Haemoglobin(g/L)	-0.064	0.652	-0.164	0.311	0.050	0.753
WBCs(x10^3/mm^3)	0.362**	0.008	0.092	0.574	0.108	0.497
Urea(mmol/L)	0.127	0.379	0.251	0.128	0.327*	0.034
Creatinine(mmol/L)	0.146	0.302	-0.103	0.528	0.141	0.372
CRP (mg/L)	0.396**	0.004	0.237	0.151	0.076	0.631
ESR (mm\h)	0.135	0.340	0.178	0.272	0.007	0.966
IL-1β(pg/mL)	0.272	0.051	-0.339*	0.032	0.360*	0.019
Lipocalin-2 (NGAL) (ng/mL)	0.164	0.266	-0.194	0.244	0.211	0.190
$_e$GFR (Bedside Schwartz) (mL/min/1.73 m2)	0.187	0.185	-0.003	0.986	0.056	0.727

* Significant change at $p< 0.05$, ** Highly significant change at $p< 0.001$

Table 5: Coefficients of correlations between the biochemical parameters in FMF patients

	Hemoglobin	WBCs	Urea	Creatinine	CRP	ESR	IL-1β	Lipocalin-2
WBCs (x10^3/mm^3)	r=-0.622** P=<0.001							
Urea (mmol/L)	r=0.057 P=0.684	r=0.140 P=0.311						
Creatinine mmol/L	r=0.003 P=0.985	r=-0.131 P=0.337	r=-0.242 P=0.078					
CRP (mg/L)	r=-0.042 P=0.765	r=0.178 P=0.198	r=-0.194 P=0.160	r=0.308* P=0.024				
ESR (mm\h)	r=-0.630** P=<0.001	r=0.909** P=<0.001	r=0.123 P=0.377	r=-0.129 P=0.344	r=0.096 P=0.489			
Serum IL-1β (pg/ mL)	r=0.326* P=0.014	r=0.037 P=0.787	r=0.286* P=0.036	r=-0.086 P=0.527	r=-0.190 P=0.168	r=-0.110 P=0.42		
Serum lipocalin-2 (NGAL) (ng/mL)	r=0.275* P=0.048	r=-0.063 P=0.659	r=0.171 P=0.236	r=0.161 P=0.254	r=-0.114 P=0.432	r=-0.138 P=0.328	r=0.692** P=<0.001	
GFR (Bedside Schwartz) (mL/min/1.73 m2)	r=-0.125- P =0.360	r=0.113 P=0.408	r=0.352** P=0.009	r=-0.412** P=0.002	r=-0.010 P=0.945	r=0.070 P=0.609	r=0.414** P=0.002	r=0.278* P=0.046

* Correlation is significant at p< 0.05, **Correlation is highly significant at p< 0.01

Table 6: The sensitivity, specificity, cut off value and AUC for IL-1β, lipocalin-2 in FMF patients

Parameters	Cut-off value	Sensitivity (%)	Specificity (%)	Area under curve
IL-1β	>1.4 (pg/mL)	83.9	62.5	0.781
lipocalin2 (NGAL)	>2.4(ng/mL)	50.0	100.0	0.740

DISCUSSION

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease associated with mutation in pyrin inflammasome. This leads to increase in the production of interleukin-1β (IL-1β) which initiates the progress of inflammation and influences the disease pathogenesis (19).

In this study the IL-1β was significantly elevated in FMF patients compared to healthy control (p=0.001). This coincides with previous studies that detected increased serum level of IL-1β in FMF patients compared to controls (23-26). This result elucidates that the autoinflammation observed in patients with *MEFV* gene is mediated by IL-1β. In contrast, other studies noticed that the serum IL-1β level did not record significant differences between FMF patients and the healthy individuals (27,28). Aforementioned study identified that the serum level of cytokines was basically decreased after colchicine treatment (29). Similarly, this study found a significant correlation of colchicine dose with both serum Il-1β and serum urea levels (Il-1β p=0.019 and urea p=0.034), that may help in the prediction of the dysfunction in the filtration of the kidney and the progression of the disease. According to our results, there was a significant increase in the mean level of IL-1β in the FMF patients which correlated with the severity of the disease (p=0.003). This revealed the role of IL-1β in the prediction of the progression of the disease and is inconsistence with the report of Ibrahim et al. (30). Although IL-6 is the most significant for differentiating between FMF and healthy controls also the combined measurement of IL-18, IL-17 and IL-6 had the ability to distinguish between FMF patients and the healthy controls with high accuracy (specificity 100%, sensitivity 89.2%, and accuracy 95.5%) (27,31). In our study, IL-1 β revealed a good diagnostic value in FMF patients (sensitivity 83.9%, specificity 62.5%, AUC was 0.781, p =0.0001).

In this report, a statistically significant increase in the level of IL-1β among FMF patients was noted especially in FMF patients with heterozygous mutations K671M, P706P/E148Q and P706P/V726A. Similarly, a previous study by Sharma et al. (32) reported high IL-1 β levels in patients with V726A genotype. A previous study stated that NGAL gene expression is strongly induced in human epithelial cells by interleukin (IL)-1β (33). The current study displayed agreement with the previous study as serum IL-1β drastically correlated with serum lipocalin-2(sNGAL) (r=0.692, p<0.001).

CONCLUSION

The measurement of both serum NGAL and IL-1β may be helpful for following up the progression of FMF and may aid in the early prediction of renal dysfunction in FMF patients.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest

AUTHOR INFORMATION

Hala T. El-Bassyouni, PhD, Prof. of Clinical Genetics[1]
Wafaa G. Shousha, PhD, Professor of Biochemistry[2]
Fateheya M. Metwally, PhD, Profession of Environmental and Occupational Medicine[3]
Khaled Hamed, PhD, Professor of Clinical Genetics[1]
Randa S. Lotfy, PhD, Researcher[4]
Zeinab H. Korany, MSc[2]
Shimaa S. Ramadan, PhD, Associate Professor of Biochemistry[2]

[1] Clinical Genetics Department, human Genetics and Genome Research Institute, National Research Centre, Egypt
[2] Faculty of Science, Helwan University, Egypt
[3]Occupational Medicine Department, National Research Center, Egypt
[4] Molecular Genetics and Enzymology Department, National Research Centre, Egypt

Corresponding Author: Dr. Hala T. El- Bassyouni
Email: halabassyouni@yahoo.com

REFERENCES

1. Tajika M, Takeuchi Y, Arai M, et al. Remarkable effectiveness of tocilizumab in familial Mediterranean fever exon 3 variant with severe abdominal pain and PFAPA-like symptoms: a child case report and review of the literature. *Mod Rheumatol Case Reports* 2019; 3:179-185.
2. Kukuy O, Livneh A, Ben-David A, et al. Familial Mediterranean fever (FMF) with proteinuria: clinical features, histology, predictors, and prognosis in a cohort of 25 patients. *J Rheumatol* 2013; 40: 2083-2087.
3. Zarouk WA, El-Bassyouni HT, Ramadan A, et al. Screening of the most common MEFV mutations in a large cohort of Egyptian patients with familial Mediterranean fever. *Gene Reports* 2018; 11:23–28.
4. Touitou I. The spectrum of familial mediterranean fever (FMF) mutations. *Eur J Hum Genet* 2001; 9: 473-83.
5. Giancane G, Ter Haar NM, Wulffraat N, et al. Evidence-based recommendations for genetic diagnosis of familial Mediterranean fever. *Ann Rheum Dis* 2015; 74:635–641.
6. Sohar, E., Gafni, J., Pras, M. Heller, H. Familial Mediterranean fever: a survey of 470 cases and review of the literature. *Am J Med* 1967; 43: 227-253.
7. Livneh A, Langevitz P, Zemer D, Zaks N, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997; 40: 1879-1885.
8. Yalçınkaya, F, Özen S, Özçakar ZB, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. *Rheumatology* 2009; 48: 395–398.
9. Ben-zvi I, Herskovizh C, Kukuy, O, et al. Familial Mediterranean fever without MEFV mutations : a case – control study. *Orphanet Journal Rare Dis* 2015; 10: 34.
10. Kilinc M, Ganiyusufoglu E, Sager H, et al. The report of sequence analysis on familial Mediterranean fever gene (MEFV) in South-eastern Mediterranean region (Kahramanmaraş) of Turkey. *Rheumatology Int* 2016; 36: 25–31.
11. Berkun Y, Eisenstein EM. Diagnostic criteria of familial Mediterranean fever. *Autoimmun Rev* 2014; 13:388–390.
12. Tunca M, Akar S, Onen F, et al. Familial Mediterranean fever (FMF) in Turkey: results of a nationwide multicenter study. *Medicine* (Baltimore) 2005; 84: 1–11.
13. Anton PA, Targan SR, Vigna SR, et al. Enhanced neutrophil chemiluminescence in familial Mediterranean fever. *J Clin Immunol* 1988; 8:148–156.
14. Alghamdi M. Familial Mediterranean fever, review of the literature. *Clin Rheumatol* 2017; 36:1707–1713.
15. Huelin P, Solà E, Elia C, et al. Neutrophil Gelatinase-Associated Lipocalin for Assessment of Acute Kidney Injury in Cirrhosis: A Prospective Study. *Hepatology* 2019; 70:319–333.
16. Siligato R, Gembillo G, Calabrese V, et al. Amyloidosis and Glomerular Diseases in Familial Mediterranean Fever. *Medicina (Kaunas)* 2021; 1;57:1049.
17. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol* 2008; 3:665–673.
18. Oksay SC, Dursun H, Neijmann ST, Hatipoglu S. Using urinary neutrophile gelatinase-associated lipocalin for prognosticate renal dysfunction in children with familial Mediterranean fever the study design: a pilot study. *Adv Rheumatol* 2021; 1,61:20.
19. Hentgen V, Vinit C, Fayand A, Georgin-Lavialle S The Use of Interleukine-1 Inhibitors in Familial Mediterranean Fever Patients: A Narrative Review. *Front Immunol* 2020; 28, 11:971.
20. Soriano A, Verecchia E, Afeltra A, et al. IL-1β biological treatment of familial Mediterranean fever. *Clin Rev Allergy Immuno*l 2013; 45:117–130.
21. Demirkaya E,Acikel C, Hashkes P, et al. Development and initial validation of international severity scoring system forfamilial Mediterranean fever (ISSF). *Ann Rheum Dis* 2016; 75: 1051-1056.
22. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009; 20:629-637.
23. Srisawat N, Murugan R, Kellum JA. Repair or progression after AKI: A role for biomarkers?. *Nephron Clin Pract* 2014; 127(1-4):185-189.
24. Direskeneli H, Ozdogan H, Korkmaz C, et al. Serum soluble intercellular adhesion molecule 1 and interleukin 8 levels in familial Mediterranean fever. *J Rheumatol* 1999; 26:1983–1986.
25. Poland DCW, Drenth JPH, Rabinovitz E, et al. Specific glycosylation of α1-acid glycoprotein characterises patients with familial Mediterranean fever and obligatory carriers of MEFV. *Ann Rheum Dis* 2001; 60:777–780.
26. Yildrim K, Uzkeser H, Keles M, et al. Relationship between serum interleukin-1β levels and acute phase response proteins in patients with familial Mediterranean fever. *Biochem Med* 2012; 22:109–113.
27. Koga T, Migita K, Sato S, et al. Multiple serum cytokine profiling to identify combinational diagnostic biomarkers in attacks of familial Mediterranean fever. *Medicine (Baltimore)* 2016; 95:e3449.
28. Yamazaki T, Shigemura T, Kobayashi N, et al. IL-18 serum concentration is markedly elevated in typical familial Mediterranean fever with M694I mutation and can distinguish it from atypical type. *Mod Rheumatol* 2016; 26:315–317.
29. Kiraz S, Ertenli I, Arici M, et al. Effects of colchicine on inflammatory cytokines and selectins in familial Mediterranean fever. *Clin Exp Rheumatol* 1998; 16:721–724.
30. Ibrahim JN, Chouery E, Lecron JC, et al. Study of the association of IL-1β and IL-1RA gene polymorphisms with occurrence and severity of Familial Mediterranean fever. *Eur J Med Genet* 2015; 58(12):668-673.
31. Gang N, Drenth JP, Langevitz P, et al. Activation of the cytokine network in familial Mediterranean fever. *J Rheumatol* 1999; 26:890-897.
32. Sharma D, Sharma BR, Vogel P, et al. IL-1β and Caspase-1 Drive Autoinflammatory Disease Independently of IL-1α or Caspase-8 in a Mouse Model of Familial Mediterranean Fever. *Am J Pathol*, 2017; 187: 236–244.
33. Nasioudis D, Witkin SS. Neutrophil gelatinase-associated lipocalin and innate immune responses to bacterial infections. *Med Micro Immunol* 2015; 204: 471–479.