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CLINICAL STUDY

# Do neutrophil gelatinase-associated lipocalin and interleukin-18 predict renal dysfunction in patients with familial Mediterranean fever and amyloidosis?

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**Abstract**

*Background:* The aim of this study was to evaluate whether neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18) predict renal disfunction in patients with familial Mediterranean fever (FMF). *Methods:* This prospective study consisted of 102 patients with FMF in attack-free period, and 40 matched healthy controls. Of the patients, nine were diagnosed as amyloidosis. The patients were divided into two groups according to eGFR as below 120 mL per minute and above 120 mL per minute. Also, patients were divided into three groups according to the degree of urinary albumin excretion as normoalbuminuric, microalbuminuric, and macroalbuminuric. The serum levels of IL-18 (sIL-18) and NGAL (sNGAL), and urinary levels of IL-18 (uIL-18) and NGAL (uNGAL) were measured by using ELISA kits. *Results:* The levels of sIL-18, sNGAL, uIL-18, and uNGAL were detected significantly higher in FMF patients, particularly in patients with amyloidosis, when compared to controls. sNGAL, uIL-18, and uNGAL were significantly higher in patients with eGFR $<$ 120 mL per minute than in patients with eGFR $\geq$ 120 mL per minute. sNGAL, uIL-18, and uNGAL were correlated significantly with urinary albumin excretion, additionally, were inverse correlated with eGFR. The most remarkable findings of this study are of the higher values of sIL-18, sNGAL, uIL-18, and uNGAL in both normoalbuminuric FMF patients and patients with eGFR $\geq$ 120 mL per minute. *Conclusions:* The results of this study suggest that sIL-18, uIL-18, sNGAL, and uNGAL are reliable markers of early renal disfunction in FMF patients, and may let us take measures from the early stage of renal involvement.

**Keywords**

Amyloidosis, estimated glomerular filtration rate, familial Mediterranean fever, interleukin-18, neutrophil gelatinase-associated lipocalin

**History**

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## Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive hereditary disease which is characterized by recurrent attacks of fever and peritonitis, pleuritis, arthritis, or erysipelas-like skin disease.[1] The major complication of FMF is the development of secondary amyloidosis (AA).[2] Amyloidosis primarily manifests as a nephropathy characterized by proteinuria of nephrotic range, uremia and finally end-stage renal failure necessitating chronic dialysis and renal transplantation.[3] Previous studies have suggested that a proinflammatory period is present in attacks, and subclinical inflammation continues in attack-free periods of FMF.[3,4] Amyloidosis, representing the major renal involvement in FMF, is a consequence of longstanding inflammation. Routine urinalysis in detecting early albuminuria is imperative in the care of affected individuals, as it appears in the early course of renal involvement.[4] Once the proteinuria is confirmed the accurate diagnosis of amyloidosis should be confirmed by renal biopsy, rectal biopsy, bone marrow biopsy, abdominal fat aspiration or gingival biopsy.[5] Although albuminuria is generally considered the earliest non-invasive marker for the development of nephropathy, it is widely accepted that more sensitive and specific markers are needed to understand the development and progression of nephropathy of FMF patients.

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family that is expressed at low levels in several human tissues and rapidly released from renal tubular cells in response to various injuries to the kidney.[6,7] Recently, NGAL has been implicated as an early predictive biological marker of acute kidney injury (AKI).[8] On the other

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hand, several recent studies have also defined the role of NGAL in chronic kidney diseases (CKD), and showed increased serum and urinary NGAL levels in cases with CKD.[9–11]

Interleukin-18 (IL-18) is a pro-inflammatory cytokine which increases in endogenous inflammation process. It is synthesized in an inactive form by several tissues including monocytes, macrophages, and proximal tubular epithelial cells. IL-1 beta (IL-1β) and IL-18 induce mesenchymal markers in tubular epithelial cells in a dose-dependent manner.[12,13] In humans, IL-18 and caspase-1 are expressed in renal tubular epithelium and patients with CKD or the nephrotic syndrome exhibit elevated levels of IL-18.[14–17]

The early detection of renal disorder in patients with FMF could optimize and improve patient outcomes. The aim of this study was to evaluate the association between serum and urinary levels of NGAL and IL-18 with eGFR and MAU in more accurately predicting renal function of patients with FMF.

## Patients and methods

The study was conducted in a prospective manner in Cumhuriyet University Medical School between 2010 December and February 2012. The departments of Rheumatology, Internal Medicine, Hematology and Biochemistry have contributed to this study. All patients and healthy controls provided their written consent for the participation in this study. The study was approved by the local Ethics Committees and was in accordance with the Declaration of Helsinki.

A total of 142 people were participated in the study. The participants were designated as follows: 93 patients with FMF in attack-free period, 9 patients with FMF related amyloidosis, and 40 matched individuals as a healthy control group. The patients were divided into 2 groups according to eGFR as below 120 mL per minute and above 120 mL per minute. And also, patients were divided into three groups according to the degree of urinary albumin excretion as normoalbuminuric, microalbuminuric, and macroalbuminuric. The macroalbuminuric group consisted of 11 patients. Of these 11 patients, 9 were diagnosed as amyloidosis, but 2 were not diagnosed as amyloidosis by rectal biopsy. These two patients had only macroalbuminuria.

The diagnosis of FMF was established according to the Tel-Hashomer criteria.[18] All FMF patients were under colchicines (1–2 mg/day) therapy during the study. Attack-free period was defined as being free of attacks for at least 2 weeks based on clinical (fever, abdominal pain, and arthritis) and laboratory findings [high sensitive C-reactive protein (hs-CRP), fibrinogen, and white blood cell (WBC) count]. Amyloidosis was diagnosed by histopathological examination of rectal biopsy. Exclusion criterias were as follows: presence of other systemic diseases (including chronic renal failure, diabetes mellitus, ischemic heart disease, and malignancy), trauma, heavy exercise, smoking, and use of drugs with potential effects on biochemical parameters except colchicine. In order to adequately assess the renal function, estimated glomerular filtration rate (eGFR) was calculated by using Cockcroft–Gault equation.[19]

Totally, a sample of 8 mL fasting venous blood was taken in the morning from all participants. The serum was obtained by centrifugation of blood samples at 3000 rpm for 15 min at 4 °C. Afterwards, serum samples were stored at −80 °C until analysis. Urinary samples were evaluated in spot urine.

Hs-CRP, fibrinogen, and WBC counts were measured within less than 1 h after the sampling. Hs-CRP levels were determined by the nephelometric method (Beckman Array 360 Protein System, Minnesota, Brea). Urinary albumin and creatine levels were evaluated spectrophometricly via Beckman Coulter LX-20 (Beckman Coulter, Inc., Fullerton, CA). Then, the ratio of urinary albumin to urinary creatinine was calculated. Fibrinogen levels were measured by the clotting time method (Beckman Coulter, Inc., Fullerton, CA), and leukocytes were determined with an automatic hematology analyzer (Beckman Coulter, Inc., Fullerton, CA). The levels of sIL-18, uIL-18, sNGAL, and uNGAL levels were measured by using ELİSA kits (Boster Biological Technology Co., Ltd., Wuhan, China). ELİSA kits have an inter-assay coefficient of variances (CV) of 4.56% and 5.27% for IL-18 and NGAL measurements, respectively.

### Statistical analyses

All statistical analyses were performed via the Statistical Package for the Social Sciences (SPSS) 14.0 Package (SPSS Inc., Chicago, IL). Descriptive statistics were presented as arithmetic mean ± standard deviation. Results which did not follow normal distribution are expressed as median (range) values. For the tests of normality, we used the Kolmogorov–Smirnov test. As serum fibrinogen was normally distributed, it was handled with Student's *t*-test and analysis of variance supplemented with *post-hoc* Bonferroni test when a statistically significant difference between the subgroups was noted. The other tests were not normally distributed thus Mann–Whitney *U* test and Kruskall–Wallis test with *post-hoc* Dunn's test were used for comparisons among subgroups. Depending on normality of variables, Pearson's or Spearman's test was used for bivariate correlations. Receiver-operating characteristic (ROC) curves were constructed to determine the optimum cutoff for tests. The area under the curve (AUC) was calculated to quantify sensitivity and specificity. *p* Values of less than 0.05 were regarded as significant.

## Results

A total of 102 patients [35 (34.4%) male, 67 (65.6%) female] participated in the study. There were no significant differences between patients and healthy controls in terms of age and gender distribution. Demographic and clinical characteristics of participants are exhibited in Table 1.

There were no differences between patients with eGFR $< 120$ mL per minute and patients with eGFR $\geq 120$ mL per minute in terms of age and gender distribution, duration of disease, number of attacks per month, and colchine dosage. And also, there were no differences between patients with eGFR $< 120$ mL per minute and patients with eGFR $\geq 120$ mL per minute in terms of Hs-CRP levels, and the other acute phase reactants (WBC and fibrinogen). The levels of urinary albumin, sIL-18, uIL-18, sNGAL, and uNGAL were significantly higher in patients

Table 1. The clinical and laboratory characteristics of patients with familial Mediterranean fever (FMF) and healthy controls (HC), [median (range)].

| | Patients with FMF (eGFR < 120) $n = 51$ | Patients with FMF (eGFR ≥ 120) $n = 51$ | Healty control $n = 40$ |
|---|---|---|---|
| Gender (male/female) | 17/34 | 18/33 | 17/23 |
| Age (years) | $36.53 \pm 12.53$* | $26.18 \pm 10.73$* | $32.69 \pm 9.88$* |
| Disease duration (years) | 7.20 (1–30) | 7.47 (1–30) | – |
| Number of attacks per month | $2.16 \pm 1.16$* | $2.04 \pm 1.10$* | – |
| Colchicine dose (mg/day) | $1.69 \pm 0.79$* | $1.75 \pm 0.75$* | – |
| Urinary albumin (mg/g Cr) | 16.50 (1.00–34609.00)[d] | 12.00 (1.00–2687.00)[d] | 8.00 (3.00–30.00) |
| Serum NGAL (ng/mL) | $3.89 \pm 1.51$[a,c]* | $2.76 \pm 1.67$[d]* | $1.95 \pm 1.39$* |
| Serum IL-18 (pg/mL) | 6.33 (1.11–43.30)[c] | 5.94 (0.60–65.65)[c] | 2.98 (0.40–14.34) |
| Urinary NGAL (ng/mg uCr) | 16.32 (2.36–86.35)[b,c] | 7.75 (0.89–40.92)[c] | 1.84 (0.08–9.23) |
| Urinary IL-18 (pg/mg uCr) | 36.50 (5.0–273.8)[b,c] | 19.50 (3.70–167.0)[d] | 11.90 (1.60–41.60) |

Notes: eGFR, estimated glomerular filtration rate (mL per minute); NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18.
*Mean ± SD.
[a]Significantly different from eGFR ≥ 120 mL per minute at $p < 0.001$ level.
[b]Significantly different from eGFR ≥ 120 mL per minute at $p < 0.05$ level.
[c]Significantly different from HC at $p < 0.001$ level.
[d]Significantly different from HC at $p < 0.05$ level.

Table 2. The relationship between kidney injury markers and the degree of urinary albumin excretion in both patients with familial Mediterranean fever (FMF) and healthy controls (HC), [median (range)].

| | Patients with FMF (normoalbuminuria) $n = 72$ | Patients with FMF (microalbuminuria) $n = 19$ | Patients with FMF (macroalbuminuria) $n = 11$ | Healty control $n = 40$ |
|---|---|---|---|---|
| Serum NGAL (ng/mL) | 2.47 (0.71–5.82)[b,d,e] | 4.49 (0.57–6.82)[a,c] | 5.74 (4.25–6.21)[a] | 1.54 (0.17–5.28) |
| Serum IL-18 (pg/mL) | 4.60 (0.60–19.54)[a,f] | 5.39 (1.10–22.83)[b,c] | 9.62 (2.94–12.91)[a] | 2.98 (0.4–14.34) |
| Urinary NGAL (ng/mg uCr) | 8.81 (0.89–31.85)[a,f] | 12.07 (1.40–24.68)[a,c] | 23.71 (6.31–36.07)[a] | 1.84 (0.08–9.23) |
| Urinary IL-18 (pg/mg uCr) | 18.00 (3.70–125.70)[b,f] | 25.20 (4.60–136.00)[b,c] | 73.25 (17.20–273.80)[a] | 11.90 (1.60–41.60) |

Notes: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18.
[a]Significantly different from HC at $p < 0.001$ level.
[b]Significantly different from HC at $p < 0.05$ level.
[c]Significantly different from FMF (microalbuminuria) at $p < 0.001$ level.
[d]Significantly different from FMF (microalbuminuria) at $p < 0.05$ level.
[e]Significantly different from FMF (macroalbuminuria) at $p < 0.001$ level.
[f]Significantly different from FMF (macroalbuminuria) at $p < 0.05$ level.

with FMF when compared to controls. The most important finding was the higher values of sIL-18, uIL-18, sNGAL, and uNGAL in patients with eGFR ≥ 120 mL per minute with statistical significance when compared to controls. The detailed results are summarized in Table 1. And also, sIL-18, sNGAL, uIL-18, and uNGAL were significantly higher in patients with amyloidosis when compared to controls ($sNGAL = 5.43 \pm 0.79$, $sIL\text{-}18 = 7.36 \pm 2.14$, $uNGAL = 16.90 \pm 10.25$, $uIL\text{-}18 = 99.61 \pm 84.71$; $p < 0.001$).

There were significant differences between normoalbuminuric, microalbuminuric, macroalbuminuric patients and healthy controls in terms of levels of sIL-18, uIL-18, sNGAL, and uNGAL (Table 2). But, the most important finding was the higher values of sIL-18, uIL-18, sNGAL, and uNGAL with statistical significance in normoalbuminuric patients when compared to controls. We obtained a correlation between sNGAL, uIL-18, uNGAL and urinary albumin excretion (Table 3). And, there was an opposite correlation between sNGAL, uIL-18, uNGAL and eGFR (Table 3). The most important findings of this study were the higher values of sIL-18, sNGAL, uIL-18, and uNGAL in both normoalbuminuric FMF patients and patients with FMF with eGFR ≥ 120 mL per minute.

Table 3. The relationship between kidney injury markers and eGFR and urinary albumin excretion in patients with familial FMF.

| | eGFR | | Urinary albumin excretion | |
|---|---|---|---|---|
| | Correlation coefficient[a] | $p$ Value | Correlation coefficient[a] | $p$ Value |
| Serum NGAL (ng/mL) | −0.436 | <0.001 | 0.360 | <0.001 |
| Serum IL-18 (pg/mL) | −0.035 | 0.754 | 0.147 | 0.177 |
| Urinary NGAL (ng/mg uCr) | −0.234 | 0.023 | 0.273 | 0.007 |
| Urinary IL-18 (pg/mg uCr) | −0.302 | 0.005 | 0.445 | <0.001 |

Notes: eGFR, estimated glomerular filtration rate (mL per minute); NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18.
[a]Spearman's correlation analysis.

The cutoff, AUC, sensitivity and 1-specificity of kidney injury markers of patients with FMF are exhibited in Table 4. ROC analyses were performed to define the diagnostic profile of sIL-18, uIL-18, sNGAL, uNGAL, and urinary albumin for detecting renal involvement among subjects with FMF (Figure 1).

Table 4. The cutoff, area under the curve (AUC), sensitivity and 1-specificity of kidney injury markers in predicting renal involvement of patients with familial Mediterranean fever.

| | AUC (95% CI) | *p* Value | Cutoff | Sensitivity (95% CI) | 1-specificity (95% CI) |
|---|---|---|---|---|---|
| Serum NGAL (ng/mL) | 0.939 (0.887–0.990) | <0.001 | 4.61 | 88.9 | 19.6 |
| Serum IL-18 (pg/mL) | 0.721 (0.599–0.842) | 0.028 | 5.34 | 77.8 | 31.8 |
| Urinary NGAL (ng/mg uCr) | 0.843 (0.736–0.950) | 0.018 | 10.83 | 77.8 | 22.4 |
| Urinary IL-18 (pg/mg uCr) | 0.886 (0.791–0.981) | <0.001 | 26.4 | 88.9 | 23.4 |
| Urinary albumin (mg/g Cr) | 0.831 (0.636–1.026) | 0.001 | 46.60 | 75.0 | 18.8 |

Notes: NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18.

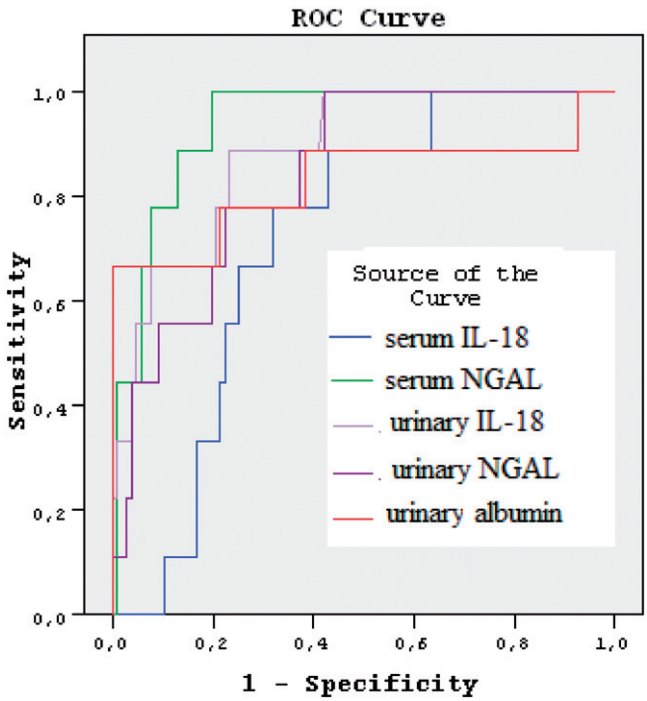



Figure 1. The comparison of ROC curves of neutrophil gelatinase-associated lipocalin (NGAL) and interleukin-18 (IL-18) in predicting renal involvement of patients with familial Mediterranean fever.

## Discussion

FMF is an inherited autosomal recessive disorder, characterized by acute episodes of serosal membrane inflammation and increased risk of renal amyloidosis.[1] Amyloidosis, occurring in up to 50–60% of untreated patients,[20] primarily manifests as a nephropathy that passes through consecutive stages of proteinuria, nephrotic syndrome, uremia, end-stage renal failure and death.[21–23] Routine urinalysis in detecting early albuminuria is imperative in the care of affected individuals, as it appears early in the course of renal involvement.[8] Once the proteinuria is obtained, the diagnosis of amyloidosis should be confirmed by biopsy.[24–26] Due to widespread use of colchicine, only a minority of FMF patients now present with amyloidosis. Recent studies documented amyloidosis in only 7–13% of Turkish patients with FMF.[27] In our study, 9 (8.8%) patients had amyloidosis by rectal biopsy.

The traditional blood markers such as blood urea nitrogen and creatinine and kidney injury markers such as casts, and fractional excretion of sodium have been used for decades in clinical studies for diagnosis and prognosis of kidney disease. But, these are non-specific and do not directly reflect injury to kidney cells.[28] Namely, most of these markers are functional consequences of the injury. Today, easily quantifiable and sensitive injury biomarkers are warranted. In clinical medicine, eGFR provides the best index of overall kidney function, and proteinuria adds additional information on prognosis. Although albuminuria is generally considered as the earliest non-invasive marker for the development of nephropathy, it is widely accepted that more sensitive and specific markers are needed to better understand the development and progression of nephropathy of FMF patients.

Novel biomarkers of tubular injury such as NGAL and IL-18 may enable the early detection of kidney injury before or in the absence of a change in GFR.[29] So, the early detection of renal disorder could optimize and improve patient outcomes.[30] Therefore, we conducted this study to evaluate the association between serum and urinary levels of NGAL and IL-18 with eGFR and MAU in more accurately predicting renal function of patients with FMF either individually or in combination.

The diagnostic approach to renal disease is important in patients with FMF. As it is well known, FMF is an auto inflammatory disease and previous studies have suggested that a proinflammatory period is present in attacks, and subclinical inflammation continues in attack-free periods of FMF.[3,4] Amyloidosis, representing the major renal involvement in FMF, is a consequence of longstanding inflammation. Amyloid can be found anywhere in the kidney, but glomerular deposition typically predominates. Amyloid deposition in the tubulointerstitium produces tubular atrophy and interstitial fibrosis, and in a small proportion of patients, glomerular deposition is scant or absent and the amyloid is confined to the tubulointerstitium or vasculature.[31] When amyloid is confined to the tubulointerstitium or vasculature, proteinuria is minimal and reduced GFR is the principal clinical manifestation.[31] Renal impairment tends to progress less rapidly when tubulointerstitial rather than glomerular deposition predominates. Clear relationships between the extent of amyloid deposition evident by kidney biopsy and severity of clinical manifestations have not been demonstrated. Urinary protein excretion or rate of GFR decline cannot be predicted on the basis of biopsy findings. So, kidney injury markers such as NGAL and IL-18 may give important information to solve this dilemma. In our study, the levels of sIL-18, uIL-18, sNGAL, and uNGAL were significantly higher in patients with FMF when compared to controls. One of the most striking finding of our study was the higher values of sIL-18, uIL-18, sNGAL, and uNGAL in patients with eGFR $\geq$ 120 mL per minute with statistical significance when compared to controls. The other one was the higher values of sIL-18, uIL-18, sNGAL, and uNGAL with

statistical significance in normoalbuminuric FMF patients when compared to controls.

NGAL is a member of the lipocalin superfamily of proteins that has been extensively studied.[23] Although NGAL has originally been found in neutrophils, it has subsequently been shown that NGAL is also released by many epithelial cell types including renal tubular cells following various injuries such as ischemic or nephrotoxic.[6,7,32] Recent studies showed that serum and urinary NGAL represents a novel, sensitive, specific biomarker for early detection of AKI, because NGAL is rapidly induced and released from renal tubular cells in response to injuries to the kidney.[8] On the other hand, several recent studies have also defined the role of NGAL in CKD, and showed increased serum and urinary NGAL levels in cases with CKD.[9–11] Furthermore, uNGAL concentrations were significantly correlated with GFR and proteinuria.[21] Our results were in compatible with these findings. Because, sNGAL and uNGAL concentrations were correlated with albuminuria and eGFR decline in our study. Mori and Nakao[23] suggested that the increase in NGAL is not just the passive consequence of a reduced renal clearance. This hypothesis, named the ''forest fire theory'', assumes that the increase in NGAL in CKD (''forest fire'') is the consequence of a sustained production by ''inflamed'' but vital tubular cells, whereas the rise in serum creatinine and the contraction of GFR are the mere passive result of a general loss of functional cells or nephrons.[11] This is interesting as it indicates that elevated uNGAL was not a result of increased sNGAL.[33] According to murine experiments, tubular reabsorption impairment after glomerular filtration of NGAL is most likely to have caused elevated uNGAL in the presence of diminished plasma concentrations.[34] In addition to, these results suggest that increased uNGAL may result from receptor saturation.[24] In our study, no patients had uremia or no patients were in end stage renal failure necessitating chronic dialysis and/or renal transplantation. We found that both serum and urine NGAL levels were increased when compared with GFR decline. Our results suggest that increased levels of uNGAL found in patients with FMF may be related to increased plasma levels of the substance. Namely, our results were not compatible with the finding of Mori and Nakao.[23]

IL-18 is primarily a macrophage-derived proinflammatory cytokine; however, its expression has been reported in a wide range of cells, including those of bone marrow origin and of kidney cells of the healthy human kidney.[25,26] The sIL-18 levels are known to be increased in various pathologic conditions, such as inflammatory arthritis, inflammatory bowel disease, and systemic lupus erythematosus.[35] Numerous reports indicate that CKD patients have elevated serum or urine levels of IL-18 and its correlation with decreased renal function.[15,36,37] Calvani et al.[38] reported increased glomerular IL-18 expression in a limited number of renal biopsy specimens from patients with WHO class IV and V lupus nephritis. Tubular IL-18 expression is upregulated in murine models of lupus nephritis and renal ischemia.[39,40] The increase in the levels of uIL-18 in FMF patients might be due to systemic inflammation, and also might be due to renal tubular over expression of the cytokine. It is also described that patients with CKD, especially those who had a decrease in GFR, have higher serum concentrations of IL-18 than the general population.[17,41,42] In our study, the levels of sIL-18 and uIL-18 were significantly higher in patients with FMF when compared to controls. Especially, uIL-18 levels of FMF patients were increased when compared with GFR decline.

The results of this study are subjected to some limitations. First, this study was not based on longitudinal observations but was conducted with a cross-sectional design. Second, it is a single-center study with a relatively small sample size, which might underestimate or overestimate the relationship between biomarkers and renal involvement due to FMF. Third, because of high CV% values due to the methodology used for the detection of NGAL and IL-18 levels.

In conclusion, glomerular dysfunction as a result of amyloidosis in FMF is a major factor for the development and progression of nephropathy, however our results suggest that tubulointerstitial damage might also play an important role in the pathogenesis of the nephropathy. Identifying the sensitive biomarkers that can predict the microalbuminuria or nephropathy in the early stage of FMF might provide not only meaningful information regarding early pathophysiology, but also an earlier clinical approach to the diagnosis and treatment of nephropathy of this patient population. In this connection, the most striking findings of this study were of the higher values of sIL-18, sNGAL, uIL-18, and uNGAL in both normoalbuminuric FMF patients and patients with FMF with eGFR $\geq$ 120 mL per minute. Namely, findings from the present study clearly indicate that sIL-18, sNGAL, uIL-18, and uNGAL are predictive markers of very early nephropathy of FMF patients, and these may predict nephropathy progression beyond the information provided by eGFR and MAU. But, further investigation is necessary in order to determine the exact role of these markers in predicting early nephropathy in this patient population. So, more specifically designed prospective studies are needed to externally cross-validate our findings in a larger cohort of FMF patients.

## Declaration of interest

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