Relation between microalbuminuria and gene mutations in familial Mediterranean fever

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We aimed to investigate the urinary microalbumin level, which is a sensitive marker of glomerular function for establishing probable renal involvement in early stages of the disease in patients with familial Mediterranean fever (FMF), and to determine the relation between gene mutations of these cases and urinary microalbumin levels.

Fifty patients with FMF who were admitted to our department and had been followed up in the pediatric rheumatology outpatient clinic for five years were included in the study. Diagnosis was based on Tel-Hashomer criteria. Gene mutations (M694V, V726A, M680I) and acute phase reactants were determined as supportive findings. Routine renal function tests with 24 hour urinary microalbumin levels and urinary microalbumin/creatinine ratios were evaluated.

There was a statistically significant difference between the study and control groups in terms of microalbumin/creatinine ratios, whereas no difference was observed with respect to the other parameters. Comparison of subgroups (gene mutations) in terms of all parameters (age, age at diagnosis, duration of delay in treatment, glomerular filtration rate, tubular reabsorption of phosphorus, and microalbumin/creatinine ratios) showed no difference.

We suggest measurement of urinary microalbumin levels at regular intervals in order to establish renal injury early and decrease related complications.

Key words: familial Mediterranean fever, gene mutation, microalbumin.

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by episodes of fever and polyserositis, and is primarily seen in Jews, Armenians, Turks and Arabs[1-3]. Diagnosis is based on Tel-Hashomer criteria (Table I). The most important complication of FMF that affects prognosis is amyloidosis, and renal amyloidosis is the most frequent form. Amyloidosis process occurs in four stages: preclinic, proteinuric, nephrotic and uremic. Proteinuria may regress and even improves with colchicine treatment. However, no response may be seen in some cases[4-9].

Microalbuminuria is the loss of albumin by urine at a level of 30-300 mg/day, which can not be established by routine methods. Albumin excretion increases in all of the diseases that damage glomerular basement membrane (GBM). Microalbuminuria is an important marker of early glomerular injury[10,11].

In our study, we aimed to investigate the urinary microalbumin level in FMF patients, which is a sensitive marker of glomerular function for establishing probable renal involvement in early stages of the disease, and to determine the relation between gene mutations of these cases and urinary microalbumin levels.

Material and Methods

This study was carried out on 50 patients with FMF who were admitted to our department and had been followed up in the pediatric rheumatology outpatient clinic for five years. Of the 50 patients, 27 were males and 23 were females and the mean age was 10.24±3.98 years (min: 3 years, max: 19 years). Diagnosis was based on Tel-Hashomer criteria. Gene mutations (M694V, V726A, M680I) and acute phase reactants were determined as supportive findings. Patients who were

Table I. Tel-Hashomer Criteria†

Major
1. Recurrent episodes of fever accompanied by peritonitis, synovitis or pleuritis
2. AA type amyloidosis without a predisposing disease
3. Improvement with colchicine treatment
Minor
1. Recurrent episodes of fever
2. Erysipelas-like erythema
3. FMF history in 1st degree relatives

†Diagnosis: 2 major or 1 major and 2 minor criteria.

given colchicine for at least one year, whose frequency and severity of FMF attacks were decreased with colchicine treatment and who had no renal, endocrine, metabolic, cardiac, neurologic, gastroenterologic, hematologic and rheumatologic disease other than FMF were included in the study. Age at onset of the disease, age at diagnosis (age at the beginning of colchicine treatment) and duration of delay in treatment (period between onset of symptoms and beginning of treatment) were calculated. Routine renal function tests, such as blood urea nitrogen (BUN), creatinine (Cr), glomerular filtration rate (GFR) and tubular reabsorption of phosphorus (TRP), 24 hour (hr) urine microalbumin levels, and urinary microalbumin/creatinine ratios were evaluated. GFR was measured via both 24 hr urine collection and Schwartz formula. This formula was used to prevent mistakes that result from collection of urine and to minimize the statistical errors.

The control group was composed of 20 (11 males, 9 females) healthy children whose ages ranged between 3 and 17 years. Biochemical parameters, routine renal function tests, 24 hr urine microalbumin levels and urinary microalbumin/creatinine ratios were evaluated in the control group as well. Normal ranges of urinary microalbumin/creatinine ratio were based on the findings of Akkus et al.'s[10] study, which was carried out on healthy Turkish children and in which ranges were found as 0.00007-0.89 μg/mg. Urinary microalbumin/creatinine ratios of FMF and control groups were compared for statistical significance. Patients with FMF were divided into subgroups according to the gene mutations (homozygote, heterozygote, compound heterozygote and negative) and subgroups were also compared. Urinary microalbumin levels were measured via turbidimetric immunoassay method by Beckmann Coulter LX30 autoanalyzer.

In the selection of control and study groups, each child's parents were informed about the study and a written approval was obtained from them. Children in the control group were of similar age and gender with the study group and were selected among the patients who presented to our hospital with upper respiratory tract infection. Patients who had fever were excluded to rule out proteinuria caused by fever. The study was approved by our hospital's ethical committee.

In statistical analysis, in addition to determinative statistical methods (mean, standard deviation), Kruskal-Wallis test for comparison of groups, Mann-Whitney-U test for comparison of dual groups, and $\chi 2$ test for comparison of qualitative data were used in evaluation of results. Statistical significance was determined as $p<0.05$.

Results

Fifty children with FMF who were followed up in our pediatric rheumatology outpatient clinic were included in the study. Twenty-seven (54%) of the patients were males and 23 (46%) were females, with male/female ratio of 1.17. Mean age was 10.24±3.98 years (min: 3 years, max: 19 years).

In the family history, siblings of 8 (16%) of the patients had FMF and parents of 8 were relatives. Symptoms of the cases were verified. Forty-five (90%) of the patients had abdominal pain, 35 (70%) had fever, 7 (14%) had arthritis, 10 (20%) had erysipelas-like erythema and 10 (20%) had chest pain (Fig. 1). Five of the 7 patients with arthritis had monoarticular, while 2 had oligoarticular involvement.

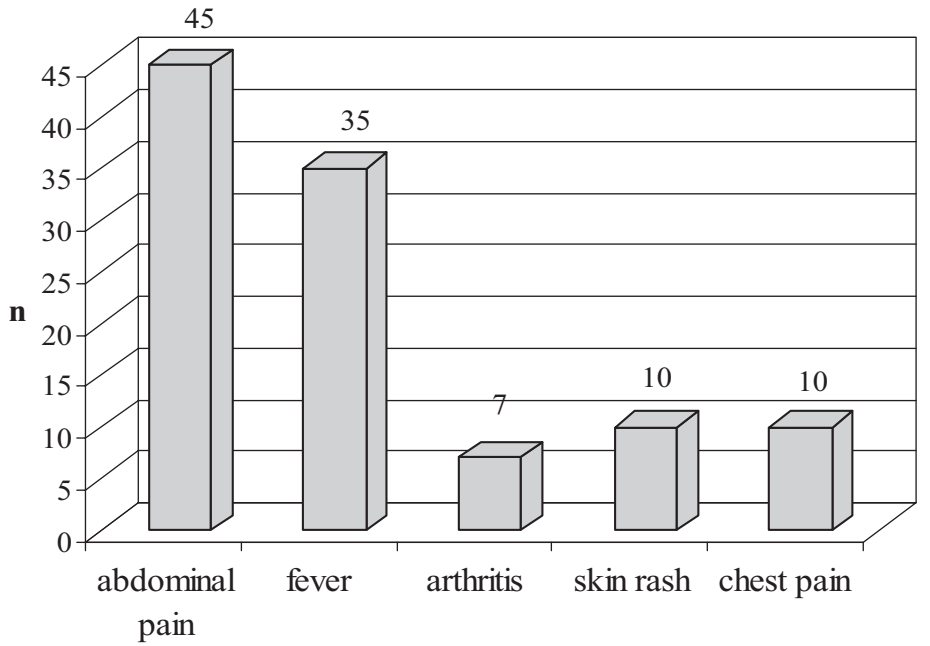

Fig. 1: Symptoms of the patients.

Investigation of gene mutations revealed homozygote in 20 (40%), heterozygote in 17 (34%) and compound heterozygote in 9 (18%) of the cases. Four (8%) of the patients were mutation-negative (-) (Table II).

Kruskal-Wallis test did not show a statistical difference between subgroups (gene mutations) in terms of all parameters (age, age at diagnosis, duration of delay in treatment, GFR, TRP and microalbumin/creatinine ratios). Chi-square test revealed no statistical significance in comparison of mutation-positive (homozygote, heterozygote, compound heterozygote) and mutation-negative subgroups with respect to GFR, TRP and microalbumin/creatinine ratios ($p>0.05$).

Table II. Gene Mutations of the Patients

	n	%
M694V/M694V homozygote	19	38
M694V/-heterozygote	16	32
M694V/V726A compound heterozygote	4	8
M694V/M680I compound heterozygote	5	10
V726A/-heterozygote	1	2
M680I/M680I homozygote	1	2
-/-(mutation-negative)	4	8

Microalbumin/creatinine ratio, BUN, Cr, TRP and GFR levels were compared between FMF and control groups by using Mann-Whitney U test. There was a statistically significant difference between the two groups in terms of microalbumin/creatinine ratios (p: 0.01), whereas no difference was observed with respect to the other parameters ($p>0.05$) (Table III). In the study group, a significant relation was shown between duration of delay in treatment (mean 2.01 ± 1.41 y) and microalbumin/creatinine ratios, where an increase in duration of delay in treatment was associated with increase in microalbuminuria (p: 0.01).

Discussion

Familial Mediterranean fever gene was mapped on the short arm of the 16th chromosome. This gene was cloned in 1997 and was shown to be composed of 10 exons, of which the 10th is important for mutations. M694V, M680I and V726A mutations on this exon constitute 85% of

Table III. Comparison of the Two Groups in Terms of BUN, Cr, TRP and Urinary Microalbumin (Mean±SD)

Group	n	Urinary microalbumin (µg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	TRP (%)
FMF	50	1.309±1.153	25.46±7.014	0.694±0.147	90.96±3.226
Control	20	0.252±0.219	24.25±4.141	0.885±0.159	82.45±5.853

BUN: Blood urea nitrogen. Cr: Creatinine. TRP: Tubular reabsorption of phosphorus.

the mutations on the affected chromosome[12-15]. Gene mutations are important as a supportive finding in FMF diagnosis even if they are not accepted as diagnostic criteria.

Early glomerular pathology can not be detected in children whose renal function tests (BUN, Cr, GFR, TRP) are normal in routine measurements. It was emphasized in certain studies that cases with FMF who would have renal amyloidosis could be established beforehand via measurement of urinary microalbumin levels[10,11]. In our study, urinary microalbumin/creatinine ratio was adopted as a marker of early glomerular injury. Oren et al.[11] stated that early glomerular injury could be established via microalbuminuria in early-morning urine specimen in some of the patients with FMF who were treated with colchicine and were without symptoms. Akkuş et al.[10] measured microalbumin/creatinine ratio in 17 patients with FMF in the attack period in 1999 and it was found to be significantly high. In our study, we assessed the levels of urinary microalbumin in patients with FMF on colchicine treatment. Although measurement of BUN, Cr, sodium (Na), potassium (K), calcium (Ca), GFR and TRP revealed normal values, there was a significant difference between microalbumin/creatinine ratios in our study group. We thus proceeded to measure microalbumin levels at regular intervals on visits. We emphasize that we had to monitor the compliance of the patients with the treatment (whether or not they regularly used the medication with appropriate doses).

In our study, mean age, male/female ratio and clinical findings of the patients were similar to other studies[1-3,8]. Diagnosis of our patients with FMF was based on Tel-Hashomer criteria with the help of supportive findings (gene mutations and acute phase reactants). With the encouragement of recent studies related to gene mutations, we investigated our patients by means of statistical significance between gene mutations and age at diagnosis, present age, duration of delay in treatment, GFR, TRP and levels of microalbuminuria via Kruskal-Wallis test, and no difference was observed. Cloning of FMF gene led to associated gene mutations and amyloidosis, the most serious complication of FMF[9,16-18]. At first, it was claimed that M694V mutation was widely responsible for amyloidosis and that V726A mutation did not cause amyloidosis and thus glomerular injury. Zemer et al.[9] studied the relation between M694V mutation and amyloidosis, and their study group was composed of Jews. Studies among various ethnical groups showed that not only M694V mutation but also V726A and M680I mutations led to amyloidosis; however, the high frequency of the M694V mutation in Jews with amyloidosis, as shown in the studies of Shohat et al.[17], should be taken into consideration. Our study does not point out a significant relation between gene mutations and urinary microalbumin/creatinine ratios. On the other hand, urinary microalbumin/creatinine ratios were significantly higher in the patient group than controls.

Mann-Whitney U test showed a statistically significant relation in that patients with longer duration of delay in colchicine treatment had higher urinary microalbumin/creatinine ratios. Greater delay in treatment resulted in more glomerular injury. In our opinion, if there is an increase in urinary microalbumin levels of patients on colchicine treatment, the dosage, compliance and duration of delay in treatment have to be reviewed. Microalbumin/creatinine ratio analysis is important in early diagnosis of glomerular injury, which can be prevented with early treatment.

In conclusion, as renal amyloidosis is the most important factor regarding prognosis, we suggest measurement of urinary microalbumin levels at regular intervals. Early establishment of renal injury will decrease related complications. However, data related to gene mutations and early markers of renal injury are limited and more studies are needed to shed light on early diagnosis and treatment of FMF.

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