

Tubular functions in familial Mediterranean fever

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SUMMARY: Akkuş S, Çalışkan S, Kasapçopur Ö. Tubular functions in familial Mediterranean fever. *Turk J Pediatr* 2002, 44: 317-320.

In this study, we aimed to evaluate renal tubular function in familial Mediterranean fever (FMF). Urinary N-acetyl- β -D glucosaminidase (U-NAG, β_2 -microglobulin (U- β_2 M) and microalbumin (Ua) levels were measured in children with different clinical stages of FMF (58 patients with FMF, 9 patients with amyloidosis secondary to (FMF). Control groups were healthy children (n=21), children with upper respiratory tract infection (URTI) (n=21) and with steroid sensitive nephrotic syndrome (SSNS) (n=18). U-NAG was significantly increased in patients with a recent diagnosis of FMF compared to patients with FMF on colchicine and to healthy controls. In patients with recently diagnosed FMF, a marked decrease in U-NAG, U- β_2 M and Ua were determined after three months on colchicine therapy. On the other hand, U-NAG and U- β_2 M levels were increased in patients with FMF during attacks and then decreased in the post-attack period. U- β_2 M in patients with FMF during attacks was significantly different from patients with URTI. Finally, U-NAG and U- β_2 M were increased significantly in patients with FMF-amyloidosis and SSNS when compared with other FMF groups and healthy controls, respectively. In conclusion, the high U-NAG value in newly diagnosed patients compared to that of patients taking colchicine and the decline of U-NAG and U- β_2 M levels after attack to the levels observed in colchicine users (without a significant change in Ua value) suggest that the renal injury early in the course of FMF might be dominantly at the level of the tubuli.

Key words: familial Mediterranean fever, renal tubular functions.

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and polyserositis. The disease typically affects certain ethnic groups living in the Middle East and around the Mediterranean basin; mainly Sephardic Jews, Armenians, Turks and Arabs¹⁻⁴. Colchicine is used for the treatment of this disease. In some cases that are not treated with colchicine, renal amyloidosis and proteinuria develop. Renal amyloidosis starting with proteinuria may progress into nephrotic syndrome and may finally result in chronic renal failure¹⁻⁶. Additionally, in some cases, Fanconi's syndrome may develop as a consequence of amyloidosis¹⁻⁶.

Two previous studies demonstrated the presence of transient microalbuminuria and tubular proteinuria that became more prominent during attacks⁷⁻⁸. Therefore it may be suggested that microalbuminuria, tubular

proteinuria and enzymuria can predict renal involvement in FMF, as is the case for some other renal diseases. In order to test this hypothesis, a prospective trial involving FMF cases was conducted.

Material and Methods

The patient enrollment started on the 1st of January 1998 and was terminated on the 1st of January 1999. Children with different clinical stages of FMF were enrolled into the study (58 patients with FMF and 9 patients with amyloidosis secondary to FMF). The study groups are summarized below.

A) Familial Mediterranean Fever Group

The diagnosis of FMF was based on Tel-Hashomer criteria in all children with FMF. The children with FMF were further divided into subgroups according to the clinical conditions.

1. *FMF-Attack Free, Under Colchicine Treatment*: This group consisted of children with FMF who were all attack-free, under colchicine treatment. The mean duration of colchicine use was three months.

2. *Newly Diagnosed FMF Patients (FMF-ND)*:

2 a) *Newly Diagnosed FMF Patients (FMF-ND) Pre-Colchicine Treatment*: This group consisted of newly diagnosed patients who had not used colchicine before and who later responded to colchicine use under follow up. Urine specimens were taken from all 23 subjects before initiation of treatment.

2 b) *Newly Diagnosed FMF Patients After Three Months' Colchicine Treatment*: After three months from the initiation of colchicine treatment, urine specimens were recollected from some of the group 2A subjects.

3) *FMF Subjects Who Experienced Attacks Under Colchicine Treatment*:

3 a) *FMF Patients with Attack*: The FMF patients were seen at the time of the attack. All 14 children had fever and abdominal pain.

3 b) *FMF Patients in Post-Attack Period*: Urine specimens were recollected 15 days after the attack from the 3A subjects.

4) *FMF Patients with Amyloidosis*: Nine patients who had amyloidosis confirmed by either rectal or renal biopsy were recruited in this group. All subjects were in different stages of amyloidosis.

B) *Control Groups*

1. *Healthy Children (HC)*: Twenty-one healthy children seen in the Pediatric Outpatient Department of Cerrahpaşa Medical Faculty, who

were between 5 and 15 years of age, were recruited as healthy controls.

2. *Children with Upper Respiratory Tract Infections (URTI)*: This group was designed to be the control group of the FMF subjects at the time of the attack. Twenty children who were seen at the Pediatric Outpatient Department with signs of URTI and who had fever above 38°C were recruited in this group. Urine specimens were collected from the children in the febrile period.

3. *Children with Steroid Sensitive Nephrotic Syndrome (SSNS)*: This group was included in the study as the control group of FMF subjects with amyloidosis. All subjects were under the follow up of the Pediatric Outpatient Department. This group consisted of 18 SSNS patients who had normal C₃ levels and no macroscopic hematuria. None of the children had proteinuria at the time of the study.

The demographic variables for the groups and the clinical stages of FMF cases are shown in Table I.

The second morning urine was collected from both the control and patient groups. Each urine sample was dispensed into three different test tubes and kept at +4°C [for albumin (Ua) measurement] and -20°C for N-acetyl-β-D glucosaminidase (NAG) and β₂-microglobulin (β₂M) measurements] for one month at most. NAG measurements were performed spectrophotometrically (Boehringer-Mannheim) with the use of krezolfonfleilein-glucosaminidase as the substrate. β₂-microglobulin levels were measured with radioimmunoassay (DSL. Beta-2M kit). Micro albumin levels were assessed with radioimmunoassay technique "double antibody" kits (BDPS). Creatinine (c)

Table I. Demographic Characteristics and Clinical States of the Study Groups

| Group | N | Male/female | Mean age at investigation (years) | Mean age at disease onset (years) |
|--|-----|-------------|-----------------------------------|-----------------------------------|
| FMF-attack free C (+) | 21 | 11/10 | 9.8±3.2 | 4.8±3.2 |
| FMF-new diagnosis C (-) | 23 | 12/11 | 8.7±4 | 6.1±4.2 |
| FMF-new diagnosis after 3 months' colchicine treatment C (+) | 11 | 6/5 | | |
| FMF-attack C (+) | 14 | 7/7 | 8.4±3.3 | 4.1±2.9 |
| FMF-post attack C (+) | 11 | 4/7 | | |
| FMF-amyloidosis C (+) | 9 | 3/6 | 15.4±1.7 | 5.3±3.6 |
| Healthy children | 21 | 13/8 | 9.7±2.7 | |
| Upper respiratory tract infection | 20 | 10/10 | 7.4±2.1 | |
| Steroid sensitive nephrotic syndrome | 18 | 11/7 | 6.2±4.4 | |
| Total | 148 | 77/71 | 9.06±3.9 | |

* C=on colchicine treatment; FMF: familial mediterranean fever.

levels were determined by Jaffe method. Urinary NAG, β_2 -M and micro albumin levels were proportionate to creatinine in order to adjust for differences arising from urinary flow.

The weight, height, age at the time of study, gender, and the results of urinalysis were recorded for all children.

Statistical Methods: Student's t, Mann-Whitney U and Wilcoxon tests were used in the statistical analysis.

Results

The results of the study are summarized in Table II. No urinary tract infection was detected in any of the study subjects.

Comparison of Attack-Free Children and Healthy Children: The U-NAG/c, U β_2 M/c and Ua/c ratios in attack-free children who were using colchicine were not significantly different from ratios determined in healthy controls.

Assessment of the Effect of Colchicine: In newly diagnosed children who had never used colchicine, the control values for U-NAG/c, U- β_2 M/c and Ua/c ratios after the use of

colchicine for three months were lower than the basal values; however, the difference between the two groups was not statistically significant. On the other hand, the newly diagnosed children who were randomised to the no colchicine treatment group had significantly higher ratios of U-NAG/c compared to asymptomatic patients treated with colchicine ($p=0.029$); the differences in U- β_2 M/c and Ua/c ratios were not significant.

The Assessment of the Effect of FMF Attack: The patients with an FMF attack had statistically higher U-NAG/c ($p=0.0064$), U- β_2 M/c ($p=0.0001$) and Ua/c ($p=0.036$) ratios compared to patients who were using colchicine but not experiencing an attack. Tubular markers (U-NAG/c and U- β_2 M/c) were higher in patients suffering from an attack than in URTI patients; however, only the difference in U- β_2 M/c ratio was statistically significant ($p=0.046$). The tubular markers (U-NAG/c and U- β_2 M/c) in the post-attack phase were lower than in the attack phase ($p=0.05$ and $p=0.0008$, respectively).

The Assessment of the Effect of Amyloidosis: The values detected in children with amyloidosis were significantly higher than the values

Table II. Results of the FMF and Control Groups [median (range)]

| Group | N | UNAG/c (U/L) | U β_2 M/c (μ gr/gr) | Ua/c (μ gr/mg) |
|--|----|------------------------|-----------------------------------|------------------------|
| FMF-attack free C (+) | 21 | 2.8 (0.9-168) | 4.96 (1.4-65.1) | 0.11 (0.02-0.37) |
| FMF-new diagnosis C (-) | 23 | 4.9 (0.96-25) | 6.8 (0-56.2) | 0.12 (0.04-1.42) |
| FMF-new diagnosis after 3 months' colchicine treatment | 11 | 3.6 (1.32-8.8) | 4.1 (1.01-520.8) | 0.11 (0.09-0.94) |
| FMF-attack C (+) | 14 | 10.8 (0.12-86.3) | 75.9 (9.8-404.04) | 0.2 (0.07-0.6) |
| FMF-post attack C (+) | 11 | 5.1 (1.22-16.8) | 4.95 (1.22-225.8) | 0.1 (0.07-0.06) |
| FMF-amyloidosis C (+) | 9 | 89.9 (18.99-441.02) | 236.9 (7.59-2498.7) | 4.6 (0.2-44.52) |
| Healthy children | 21 | 3.5 (1.05-7.88) | 5.9 (1.36-108.5) | 0.08 (0.0007-0.89) |
| Upper respiratory tract infection | 20 | 3.5 (0-85.71) | 16.4 (0.666) | 0.2 (0.08-4.35) |
| Steroid sensitive nephrotic syndrome | 18 | 45.05 (8.82-285.5) | 333.4 (18.7-1373.6) | 2.1 (1.02-3043) |

* C=on colchicine treatment; FMF: Familial Mediterranean fever; UNAG: urinary N-acetyl- β -D glucosaminidase; c: creatinine; U β_2 M: urinary β_2 -microglobulin; Ua: urinary microalbumin.

detected in all other FMF groups. There was no statistically significant difference between the group with amyloidosis and the group with SSNS with regard to laboratory values.

Discussion

There were no statistically significant difference between the FMF group treated with colchicine and the healthy controls, with regard to U-NAG/c and U- β_2 M/c values. However, the U-NAG/c ratio in the newly diagnosed cases who were not receiving colchicine treatment was significantly higher than in cases on colchicine treatment. Additionally, there was a trend toward a decline in tubular markers after three months of colchicine treatment. This suggests that tubular involvement is possible in FMF patients, and colchicine may have a protective effect on tubular function.

Patients experiencing an attack had statistically higher ratios of U-NAG/c and U- β_2 M/c compared to asymptomatic patients treated with colchicine, and there was a significant decline in these values after the attack. These findings suggest a transient tubular impairment. Saatçi et al.⁸ detected similar increases in β_2 microglobulinuria during attack. Detection of significantly higher levels of U- β_2 M/c during attacks compared to URTI cases with fever suggests that this finding cannot be explained on the basis of high fever and inflammation alone and that additional factors can have a role as well. Oren et al.⁷ detected microalbuminuria in the first morning urine samples of some adult FMF patients receiving colchicine. In our study, the Ua/c ratios in FMF cases receiving colchicine treatment and in healthy children were not different. In newly diagnosed cases of FMF, there were no statistically significant changes after three months of colchicine treatment. These findings suggest that there is no early glomerular injury in FMF. On the other hand, the Ua/c ratio during attacks was significantly higher than the ratio detected in healthy children. Similarly Saatçi et al.⁸ described a higher Ua/c ratio during attacks. One possible explanation for the increase in this ratio is fever. Accordingly, there were no significant differences between the values detected in URTI cases. Another possibility is the development of microalbuminuria secondary to tubular involvement during attacks.

In the group with amyloidosis, there was a marked increase in tubular markers, similar to the one observed in SSNS. The absence of a difference between the groups suggests that proteinuria is responsible for this increase.

In conclusion, the high U-NAG value in newly diagnosed patients compared to that of patients taking colchicine, and the decline of U-NAG and U- β_2 M levels after attack to the levels observed in colchicine users (without a significant change in Ua value) suggest that the renal injury early in the course of FMF might be dominantly at the level of the tubuli. These findings are not enough to support the hypothesis that proteinuria and enzymuria have predictive value in FMF cases. A prospective trial is warranted in cases who are not receiving colchicine, but that does not seem feasible for ethical reasons.

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