

Autoantibodies are not associated with familial mediterranean fever

Emel Guler¹, Ece Kaptanoglu², Ozlem Sahin³, Ferhan Candan⁴, Emrullah Hayta³, Hasan Elden⁵

ACTA REUMATOL PORT. 2012;37:144-148

ABSTRACT

Objective: It has been suggested that Mediterranean fever (MEFV) gene mutations are also seen in certain autoimmune diseases and are related to severity of the disease activity. As most of the clinical symptoms of these inflammatory diseases are related to autoantibody positivity, we assessed autoantibody prevalence in patients with Familial Mediterranean fever (FMF) and investigated the relationship between clinical involvement of FMF and the autoantibodies. There are a few studies on this subject with conflicting results.

Patients and Methods: Fifty patients with FMF without attack and 27 healthy controls were enrolled to the study. Clinical characteristics of the patient group were questioned. Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) values, Fluorescent antinuclear antibody (ANA), extractable nuclear antigen (ENA) profile was studied in both groups.

Results: No statistically significant difference was found in ANA, ENA profile, anti-CCP, and RF positivity between the groups ($p > 0.05$). There was no relationship between the autoantibodies and the clinical status in patients with FMF. MEFV gene mutations were identified in 98% of the FMF patients.

Conclusion: In conclusion, autoantibody positivity is similar to the healthy population in FMF. Although MEFV mutations affect clinical course in other autoantibody mediated diseases, it is not related to autoantibody formation in FMF.

Keywords: Anti-CCP; ENA profile; Familial Mediterranean fever; ANA; RF

INTRODUCTION

Familial Mediterranean Fever (FMF) is a systemic, autoinflammatory and autosomal recessive disease characterized by recurrent abdominal, chest and joint pain accompanied by fever¹. It is prevalent in the eastern Mediterranean society especially among the Jews, Armenians, Arabians and Turkish people².

In 1997, Mediterranean fever (MEFV) gene in the short arm of the 16th chromosome was described to be responsible from the disease³. Owing to the mutations in this gene, the resultant defect in pyrin/marenostrin protein which plays an important role in cytokine activation and regulation of apoptosis increases the predisposition to inflammation, proinflammatory state and besides exposes the patient to a subclinical inflammation between the attacks⁴.

Mutations in the MEFV gene have also been detected in various autoimmune diseases and were reported to be responsible from the clinical activity and progress of the disease⁵⁻⁷. It is also well known that certain autoantibodies are pathognomonic for some autoimmune disease and are closely related to specific clinical involvements⁸⁻¹¹.

Despite many researches, the pathogenesis of FMF is still not clear. FMF is considered an auto-inflammatory disease; on the other hand, an autoimmune etiology has been suggested based on the multisystem manifestations of the disease. Accompanying hypergammaglobulinemia, vasculitic rash, and a good clinical response to corticosteroids in some colchicine-resistant cases suggest an autoimmune ground in the pathogenesis¹²⁻¹⁴. Also the overlapping clinical involvement with some autoimmune diseases¹⁴⁻¹⁷ have directed research towards possible autoimmune origins in disease however conflicting results have been obtained in recent studies¹⁸⁻²².

1. Specialist in Physical Medicine and Rehabilitation of Kayseri Education and Research Hospital, Kayseri, Turkey

2. Professor of Cumhuriyet University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Sivas, Turkey

3. Assistant Professor of Cumhuriyet University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Sivas, Turkey

4. Professor of Cumhuriyet University, Faculty of Medicine, Department of Internal Medicine, Sivas, Turkey

5. Professor of Cumhuriyet University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Sivas, Turkey

OBJECTIVES

For these set out intriguing suggestions we intended to search the prevalence of autoantibodies including rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), florescent antinuclear antibody (ANA), and extractable nuclear antigen (ENA) antibody profile and to evaluate the possible effects of these antibodies on the clinical involvement in FMF.

PATIENTS AND METHODS

Fifty attack free patients with FMF classified according to Tell-Hashomer's criteria² referred to the rheumatology outpatient department were included in the study during January-June 2010. Twenty-seven healthy voluntary subjects matched with FMF patients regarding age, sex, were included as the control group. Patients or healthy controls with history or physical and laboratory signs and symptoms of conditions which cause autoantibody positivity like connective tissue diseases, infections, and malign diseases were excluded from the study. This study was approved and reviewed by the Local Ethics Committee. Informed consent was obtained from each subject and the study was performed in accordance with the principles of the Declaration of Helsinki.

Five milliliters of blood was obtained from all of the patients and controls and stored at 4°C in vacuum jelled biochemistry tubes until it is conveyed to the laboratory within two hours for RF, anti-CCP, ANA and ENA antibody profile (ds-DNA, ssDNA, Histon, ribAMA m2, PM-Scl, Jo-1, Ro-52, Scl-70, Nucleosomes, SS-A, SS-B, Sm, n-RNP/ Sm, CENP B, PCNA) levels.

The age, sex, and the type of clinical involvement (abdominal pain, chest pain, fever, arthritis, arthralgia, erysipelas like rash and amyloidosis) of the patients were recorded. To evaluate the disease activity, erythrocyte sedimentation rate (ESR) and C - reactive protein (CRP) were used.

LABORATORY PARAMETERS

RF: RF was studied with Becman Coulter USA kit (instrument: Becman coulter image, USA), by nephelometry. Cutoff value was accepted as 20 IU/mL.

Anti-CCP: Anti-CCP was detected automatically by Aeskulisa test kit (Germany) with the instrument Triturus (Italy). Cutoff value was accepted as 12 U/mL.

ANA: Immunofluorescence assay is used. ANAs

were tested on HEp-2 cells. Characterization of fluorescence pattern was documented.

ENA Profile: Detected by immunoblotting method using EUROIMMUN test kit with the instrument Euroblot Master.

CRP: CRP was studied automatically by Becman Coulter (USA) test kit with the Becman coulter image instrument by nephelometry. Cutoff value was accepted as 8 mg/L.

ESR: ESR was studied automatically by Becton Dicson test kit, with the BD Sedisystem (USA) instrumen

STATISTICAL ANALYSIS

Data were first tested to see whether they were normally distributed (Kolmogorov- Smirnov Test). Data from the groups were compared using independent Student's t-test and Mann-Whitney U test. Autoantibody positivity was compared by Pearson chi-square test and Fisher's exact test between groups. Statistical significance was assumed at $p < 0.05$.

RESULTS

Demographic features and laboratory variables of the patients and controls are given in Table I and the clinical involvements of the patient group are given in Table II.

Mutation frequency was found to be 98% in the present study and there was only one patient where mutation was not detected. This patient was a 37-years-old female. She had fever, abdominal pain, arthritis and arthralgia. RF, anti-CCP, ANA and ENA profile were all negative.

Out of 50 patients with FMF, RF was positive in 2 (4%), FANA was positive in 3 (6%), anti-dsDNA was positive in 3 (6%), anti-ssDNA was positive in 1 (2%) and anti-nucleosome was positive in 1 (2%) patient. Other autoantibodies evaluated under ENA profile were negative. In the control group, there was only anti-ssDNA positivity in 1 (3.3%) control, all other autoantibodies were negative. There was not a significant difference between the groups regarding autoantibody positivity ($p=0.539$ for RF, $p=0.548$ for anti-dsDNA, $p=1.000$ for anti-ssDNA, $p=1.000$ for anti-nucleosome). The demographic features of the patients and the control which had autoantibody positivity are given in Table III. Anti-CCP was negative in all of the patients and the controls. There was not a significant difference between the groups regarding median anti-

-CCP values (p=0.138). The pattern and titration of the ANA in patients with FMF is shown in Table III. The ENA profiles were all negative in these three patients.

Though the median values of CRP and ESR were slightly higher in the patient group, the difference was not significant (p=0.392, p=0.072, respectively).

The autoantibody positivity (RF, anti-dsDNA, anti-ssDNA, anti-nucleosome) was compared in the FMF patients with and without fever, abdominal pain, chest pain, arthralgia, arthritis, erysipelas like rash, and amyloidosis. In only the patients with and without abdominal pain there was a significant difference regarding RF positivity which was frequent in the patients without abdominal pain (p=0.040). There was not a significant difference between the patients with

and without abdominal pain in other autoantibodies (p=0.060 for anti dsDNA, p=1.000 for anti-ssDNA, p=1.000 for anti-nucleosome). There was also not a significant difference regarding presence of other clinical parameters; fever (p=0.228 for RF, p=0.324 for anti-dsDNA, and p=1.000 for anti-ssDNA and anti-nucleosome), chest pain (p=0.189 for RF, p=1.000 for anti-dsDNA and anti-ssDNA, p=0.440 for anti-nucleosome), arthritis (p=1.000 for RF, anti-dsDNA, anti-ssDNA, and anti-nucleosome), arthralgia (p=0.189 for RF, p=0.079 for anti-dsDNA, p=0.440 for anti-ssDNA and anti-nucleosome), amyloidosis (p=1.000 for RF, anti-dsDNA, anti-ssDNA, and anti-nucleosome) and erysipelas like rash (p=1.000 for RF, anti-dsDNA, anti-ssDNA, and anti-nucleosome). Anti-CCP was negative in all of the patients.

TABLE I. DEMOGRAPHIC AND LABORATORY FEATURES OF THE PATIENT AND THE CONTROL GROUPS [MEDIAN (MINIMUM-MAXIMUM)]

	Patient Group n=50	Control Group n=27	P value
Age (years)	31.5 (18-62)	37 (18-47)	0.071
Gender F/M (n)	38/12	17/10	0.227
CRP (mg/L)	4 (1-64)	3.4 (1.4-8)	0.392
ESR (mm/h)	11 (2-50)	6 (1-32)	0.072

F: Female, M: Male, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

TABLE II. THE CLINICAL INVOLVEMENTS OF THE PATIENTS

Type of involvement	Number (%) n=50
Fever	44 (88%)
Abdominal pain	49 (98%)
Chest pain	28 (56%)
Arthralgia	43 (86%)
Arthritis	28 (56%)
Amiloidosis	3 (6%)
Erysipela	4 (8%)

TABLE III. THE DEMOGRAPHIC FEATURES OF THE PATIENTS AND CONTROLS WITH AUTOANTIBODY POSITIVITY

	Gender	Age	ANA	Anti-dsDNA	Anti-ssDNA	Anti-nucleosome	RF
Patients							
FY	F	19	1/320 centromer	-	-	-	-
MG	M	32	-	-	-	-	+
NA	F	62	-	+	-	-	+
SI	F	41	1/320 centromer				
I/160 homogeneous	-	-	-	-			
SG	F	58	1/160 centromer	-	-	-	-
HT	F	54	-	+	-	-	-
HY	F	18	-	+	+	-	-
FS	F	36	-	-	-	+	-
Controls							
ZD	F	46	-	-	+	-	-

ANA: Florescent Anti-nuclear antibody, Anti-dsDNA: Double-stranded DNA antibody, Anti-ssDNA: Single-stranded DNA antibody, RF: Rheumatoid factor, F: Female, M: Male

DISCUSSION

According to the results of the present study there is not a difference between the patients with FMF and healthy controls regarding autoantibody positivity. Data on observation of MEFV gene mutations in various autoimmune diseases besides FMF and on the possible effects of these mutations on disease severity has been increased recently⁵⁻⁷. On the other hand it is well known that a subclinical inflammation takes place between the attacks in FMF²³. This continuous inflammation may give rise to new autoantigens and so autoantibodies resulting from tissue destruction. Besides similarities in some clinical involvements of FMF with autoimmune diseases, some factors like abnormalities of suppressor T lymphocytes²⁴, and insufficiency of C5a inhibitor activity²⁵ are suggested to be mechanisms involved in etiopathogenesis of FMF.

Recently various autoantibodies are studied in FMF to search autoimmune etiology in FMF however the results are conflicting^{18-22,26}. Flatau et al¹⁹ have searched anti-dsDNA and anti-ssDNA in 18 patients with FMF and 72 healthy controls. Though the anti-dsDNA (n=4) positivity was comparable between the groups, anti-ssDNA positivity (n=6) was higher than the control group. Moreover when they excluded attack free patients, both the anti-dsDNA and anti-ssDNA were found to be higher in the patient group. In another study searching anti-dsDNA prevalence in FMF in a larger cohort, there was a contradictory result, Ben-Chetrit and Levy²¹ did not find an increase in anti-dsDNA positivity. Furthermore, no difference was found between the patients with active disease or quiescent disease. This result was confirmed by the study of Konca et al²⁰. They have also investigated anti-dsDNA prevalence in 21 FMF patients with attack, 19 patients with systemic lupus erythematosus (SLE) and 36 healthy controls. Anti-dsDNA was positive in only one patient with FMF whereas it was positive in 16 patients with SLE. Anti-dsDNA was found to be negative in all of the healthy control cases. Swissa et al²² had included the biggest series of patients with FMF in their study searching autoantibodies. They have investigated the anti-ssDNA, anti-dsDNA, anti-RNP and anti-SSA in 168 patients with FMF, which revealed that the positivity of these autoantibodies were not different from that of the controls. The relation of these autoantibodies with attacks could not be supported contrary to Flatau et al¹⁹. In the present study, we found

that positivity of FANA and ENA profile of patients with FMF were not different from that of the healthy controls and moreover these autoantibodies do not seem to have any effects on clinical involvement in FMF. Our methods and case numbers are relatively good among the other similar studies in the literature, which would prevent bias also. Unfortunately we included only attack free patients in our study, which prevents us from making comments on autoantibody positivity of patients within the attack period.

Anti-CCP is high sensitive and specific autoantibody in the diagnosis of RA. It is predictive in estimating the probability of radiological destruction in the early arthritis²⁷. Uyanik et al¹⁸ have investigated 55 attack free patients with FMF for anti-CCP and RF positivity. The RF positivity was not different from that of the controls. However anti-CCP was positive in 8 patients with FMF whereas it was negative in all of the controls. Unfortunately this result could not be supported yet. Moreover in a study of Karatay et al²⁶ with a similar design, contradictory results were found. One of the interesting aspects of these two studies is that both studies were made in the same population. Karatay et al²⁶ investigated RF and anti-CCP positivity in 49 attack free patients with FMF and 30 healthy controls where 23 of the patients had history of arthritis. They have found anti-CCP negative in all cases both in the patient and control groups. In the present study, we confirm the results of Karatay et al²⁶, RF and anti-CCP positivity was not different in the patients with FMF and healthy controls.

The relation of autoantibodies with clinical involvement of FMF was not adequately searched in literature. There is only study of Uyanik et al¹⁸ searching the relation of anti-CCP positivity and arthritis. They have reported that anti-CCP levels were found to be higher in those FMF patients with arthritis. In the present study, we documented all clinical involvements and searched the relation of autoantibody positivity and these involvements in FMF. Our results did not support Uyanik et al. suggestion of arthritis in FMF and accompanying anti-CCP positivity. On the other hand we observed that RF positivity was significantly increased in FMF patients without abdominal pain, and anti-CCP levels were higher in patients without chest pain. Unfortunately though statistically significant, we believe that it would not be fair to speculate on these results as they could be merely accidental as the numbers of the patients with autoantibody positivity was really low.

CONCLUSION

The results of the present study revealed that the positivity of autoantibodies including ANA, ENA profile, RF, anti-CCP is neither increased nor related to the clinical involvement in FMF. These results exclude doubt about the possible role of autoantibodies in etiopathogenesis of FMF.

CORRESPONDENCE TO

Ozlem Sahin
 Cumhuriyet University, Faculty of Medicine,
 Department of Physical Medicine and Rehabilitation
 58141 Sivas/Turkey
 Phone: 00 90 346 2580682
 Fax: 00 90 346 2581305
 E-mail: sahinfr@yahoo.com

REFERENCES

- Ben-Chetrit E, Levy M. Familial Mediterranean fever. *Lancet* 1998;351(9103):659-664.
- Livneh A, Langevitz P, Zemer D, et al. Criteria for the diagnosis of familial Mediterranean fever. *Arthritis Rheum* 1997;40:1879-1885.
- Gershoni-Baruch R, Shinawi M, Leah K, Badarnah K, Brik R. Familial Mediterranean fever: prevalence, penetrance and genetic drift. *Eur J Hum Genet* 2001;9:3-7.
- Stanjov S, Kastner DL. Familial autoinflammatory disease: genetics, pathogenesis and treatment. *Curr Opin Rheumatol* 2005;17:586-599.
- Giaglis S, Mimidis K, Papadopoulos V, et al. Increased frequency of mutations in the gene responsible for familial Mediterranean fever (MEFV) in a cohort of patients with ulcerative colitis: evidence for a potential disease-modifying effect? *Dig Dis Sci* 2006;51:687-692.
- Rabinovich E, Livneh A, Langevitz P, et al. Severe disease in patients with rheumatoid arthritis carrying a mutation in the Mediterranean fever gene. *Ann Rheum Dis* 2005;64:1009-1014.
- Durmus D, Alayli G, Cengiz K, Yigit S, Canturk F, Bagci H. Clinical significance of MEFV mutations in ankylosing spondylitis. *Joint Bone Spine* 2009;76:260-264.
- Tipping PG, Buchanan RC, Riglar AG, Dimech WJ, Littlejohn GO, Holdsworth SR. Measurement of anti-DNA antibodies by ELISA: a comparative study with Crithidia and a Farr assay. *Pathology* 1991;23:21-24.
- Tzioufas AG, Terzoglou C, Stavropoulos ED, Athanasiadou S, Moutsopoulos HM. Determination of anti-ds-DNA antibodies by three different methods: comparison of sensitivity, specificity and correlation with lupus activity index (LAI). *Clin Rheumatol* 1990;9:186-192.
- Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. *Clin Chem* 2001;47:1089-1093.
- Chan MT, Owen P, Dunphy J, et al. Associations of erosive arthritis with anti-cyclic citrullinated peptide antibodies and MHC Class II alleles in systemic lupus erythematosus. *J Rheumatol* 2008;35:77-83.
- Erken E, Ozer HT, Bozkurt B, Gunesacar R, Erken EG, Dinkci S. Early suppression of familial Mediterranean fever attacks by single medium dose methyl-prednisolone infusion. *Joint Bone Spine* 2008;75:370-372.
- Ozturk MA, Kanbay M, Kasapoglu B, et al. Therapeutic approach to familial Mediterranean fever: a review update. *Clin Exp Rheumatol* 2011;29:577-86.
- Flatau E, Kohn D, Schiller D, Lurie M, Levy E. Schönlein-Henoch syndrome in patients with familial Mediterranean fever. *Arthritis Rheum* 1982;25:42-47.
- Given WP, Edelson HS, Kaplan HB, Aisen P, Weissmann G, Abramson SB. Generation of C5-derived peptides and other immune reactants in the sera of patients with systemic lupus erythematosus. *Arthritis Rheum* 1984;27:631-637.
- Levy M, Ehrenfeld M, Levo Y, Fischel R, Zlotnick A, Eliakim M. Circulating immune complexes in recurrent polyserositis. (Familial mediterranean fever, periodic disease). *J Rheumatol* 1980;7:886-890.
- Ilfeld D, Weil S, Kuperman O. Immunoregulatory abnormalities in familial Mediterranean fever. *Clin Immunol Immunopathol* 1981;18:261-267.
- Uyanik A, Albayrak F, Uyanik MH, Dursun H, Keles M, Cetinkaya R. Antibodies directed to cyclic citrullinated peptides in familial Mediterranean fever. *Rheumatol Int* 2010;30:467-471.
- Flatau E, Shneyour A, Hadad N, Shimoni Z. Determination by ELISA anti-DNA antibodies in patients with familial Mediterranean fever. *Isr J Med Sci* 1989;25:553-556.
- Konca K, Erken E. Antinuclear antibodies in familial Mediterranean fever. *Journal of Islamic Academy of Sciences* 1994;7:201-204.
- Ben-Chetrit E, Levy M. Autoantibodies in familial Mediterranean fever (recurrent polyserositis) *Br J Rheumatol* 1990;29:459-461.
- Swissa M, Schul V, Korish S, Livneh A, Pras M, Shoenfeld Y. Determination of autoantibodies in patients with familial Mediterranean fever and their first degree relatives. *J Rheumatol* 1991;18:606-608.
- Ozen S, Bakaloglu A, Yilmaz E, et al. Mutations in the gene for familial Mediterranean fever: do they predispose to inflammation? *J Rheumatol* 2003;30:2014-2018.
- Ilfeld D, Weil S, Kuperman O. Immunoregulatory abnormalities in familial Mediterranean fever. *Clin Immunol Immunopathol* 1981;18:261-267.
- Özel A, Demirtürk L, Yazgan Y. Familial Mediterranean Fever. A review of the disease and clinical and laboratory findings in 105 patients. *Dig Liver Dis* 2000;32:504-509.
- Karatay S, Yildirim K, Erdal A, Uzkeser H, Erdem FH, Yanmaz V. Anti-cyclic citrullinated peptide antibodies are not associated with familial Mediterranean fever. *J Back Musculoskelet Rehabil* 2010;23:21-23.
- Riedemann JP, Munoz S, Kavanaugh A. The use of second generation anti-CCP antibody (anti-CCP2) testing in rheumatoid arthritis-a systematic review. *Clin Exp Rheumatol* 2005;23:69-76.