Anti-cyclic citrullinated peptides positivity rate in patients with familial Mediterranean fever

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ABSTRACT

Objectives. *To investigate the prevalence and levels of anti-cyclic citrullinated peptide antibodies (anti-CCP) in patients with familial Mediterranean fever (FMF) with and without arthritis.*

Materials and methods. *Eighty-three patients with FMF and 43 healthy controls were included in the study. Thirty seven FMF patients had a history of arthritis, and 46 patients did not. Serum antibodies directed to the anti-CCP were assessed with a commercial enzyme-linked immunosorbent assay (ELISA) kit. Values <20U were considered negative, between 20 and 39U low, 40–99U moderate, and >100U high positive.*

Results. *Positivity rate of anti-CCP in the whole FMF group (14.5%) was three-fold higher than the control group (4.7%). However, the difference failed to achieve a statistically significant level (p=0.09). Anti-CCP levels were 21±30.1 in patients with arthritis and 13.1±10.3 in the non arthritic group (p<0.05). Anti-CCP positivity rates were 10/37 (27%) in patients with arthritis and 2/46 (4.3%) in patients without arthritis (p<0.005). Five FMF patients with arthritis (13.5%) had moderate-high anti-CCP levels (>40U/ml). Anti-CCP levels were between 20–39U/ml in 2FMF patients without arthritis and in 2 healthy controls. Anti-CCP positivity rate is higher in FMF patients with arthritis (27%) than healthy controls (4.7%) (p<0.005).*

Conclusion. *Anti-CCP prevalence is higher in FMF patients with arthritis than without arthritis, and that a significant proportion of FMF patients with arthritis (13.5%) had moderate-high titers of anti-CCP. Therefore, anti-CCP antibodies may not be a reliable indicator to differentiate between FMF arthritis and rheumatoid arthritis.*

Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive genetic disease affecting mainly Turks, non-Ashkenazi Jews, Armenians, and Arabs. It is characterised by recurrent attacks of fever (38–40°C) and painful episodes of sterile poliserositis, typically involving the peritoneum, pleura and synovia and less frequently a rash, described as erysipelas-like erythema (1-3).

Articular attack is a common clinical manifestation of FMF, with a changing prevalence between 30–70% in different populations and is the presenting symptom approximately in 20% of these patients (2, 4, 5, 6). The incidence of arthritis in Turks, Arabs, and Armenians is significantly lower than that reported in Jews (4). It usually presents as non-erosive monoarthritis affecting most frequently the large joints of the lower extremities. However, the poliarticular involvement and protracted arthritis (longer than one month) may occur in 5–10% of arthritic FMF patients (5, 7, 8). In most cases, arthritis recovers completely, although some protracted cases may suffer destructive arthritis leading to disability and joint replacement, especially in the hip and rarely in other joints (4). The arthritis in FMF may be seen in six different clinical patterns: 1) monoarticular; 2) bilateral symmetric arthritis of two joints; 3) symmetric polyarticular arthritis; 4) asymmetric oligoarticular arthritis; 5) recurrent arthritis involving small joints of hand; 6) sacroiliac joint involvement (9).

Standard laboratory tests of FMF patients are non-informative. Leucocytosis and elevation of acute phase reactants, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen may be seen during the attacks (4). Moreover, there is sustained inflammation in attack-free FMF patients (10). Therefore, new laboratory methods are required to diagnose patients without the typical clinical manifestations and having only joint related findings.

Anti-cyclic citrullinated peptide antibodies (anti-CCP) are auto-antibodies which are being increasingly used to aid in early diagnosis of rheumatoid arthritis (RA) and more specific test than the rheumatoid factor in diagnosis of RA (11, 12). It has been shown that anti-CCP positivity can predict the onset of RA in healthy individuals, and also that it is associated with joint destruction (12, 13).

The aim of this study is to investigate the positivity of anti-CCP in patients with FMF and to detect the relationship between anti-CCP and joint involvement.

Materials and methods

This study was conducted in the outpatient nephrology unit of the Ankara Education and Research Hospital. Eighty-three patients with newly or previously diagnosed FMF who met the diagnostic criteria suggested by Livneh *et al*. (14) and 43 healthy participants of similar ages and gender for the control groups were included in the study. FMF patients were further evaluated in two groups. Group I: patient who had a history of arthritis on the course of FMF and Group II: no history of arthritis. The study was reviewed by the ethics committee, and informed consents were obtained from all patients. Detailed medical histories were obtained and physical examinations were performed. Patients with FMF attack, renal failure, known systemic disease and other collagen tissue disease, and pregnant or breast-feeding were excluded from this study.

Morning fasting venous blood samples were collected from both the patients group and the control group, and stored at -20ºC until assayed. Serum antibodies directed to the anti-CCP were assessed with a commercial enzyme-linked immunosorbent assay (ELISA) kits (INOVA diagnostic, Inc., San Diego, USA), and it was considered as positive if the antibody titer was greater than the cut-off value of the kit (20U/ml). According to the manufacturer, the anti-CCP ELISA had a sensitivity of 76% for clinically confirmed RA patients and a specificity of 99% for healthy controls. Values <20U were considered negative, between 20 and 39U low, 40–99U moderate, and >100U high positive. High sensitive CRP and RF levels were measured by the immunonephelometric method and additionally ESR, fibrinogen, white blood cell (WBC) count were performed by routine laboratory methods.

All statistical analyses were carried out with the SPSS version 15.0. The data were presented as mean±SD. The frequencies were calculated for each group and comparisons for categorical variables were made using the Chi-square test. Numerical data were compared by using the Mann-Whitney U test. Pearson's correlation test was used to evaluate possible correlations between quantitative variables. *P*-values <0.05 were considered as statistically significant.

Table I. Demographic and laboratory characteristics of FMF patients and control subjects.

	FMF group (n=83)	Control group (n=43)	*p*-value
Age (year)	30.6 ± 10.7	32.5 ± 9.9	> 0.05
Gender (Male/Female)	30/53	14/29	> 0.05
ESR (mm/h)	18.1 ± 16.4	9.2 ± 9.4	**< 0.005**
HsCRP	8.4 ± 13.3	5.2 ± 7.8	**< 0.05**
RF levels	2.1 ± 12.7	2.1 ± 6.6	> 0.05
RF positivity rate	4/83	4/43	> 0.05
Anti-CCP levels	14.8 ± 21.1	13.1 ± 10.3	> 0.05
Anti-CCP positivity rate	12/83	2/43	> 0.05
White blood count (x10^3/mm^3)	7.9 ± 2.4 x10^3	7 ± 1.8 x10^3	**< 0.05**
Fibrinogen	377.5 ± 90.7	327.3 ± 84.5	> 0.05

Table II. Demographic, clinical and laboratory findings of patients with arthritis and patients without arthritis.

	FMF with arthritis (n=37)	FMF without arthritis (n=46)	*p*-value
Age (year)	30.8 ± 8.5	32.5 ± 9.9	> 0.05
Gender (Male/Female)	15/22	15/31	> 0.05
Disease duration (month)	54 ± 56.5	49.5 ± 73.7	> 0.05
Abdominal pain	27/37	41/46	> 0.05
Pleuritis	8/37	8/46	> 0.05
Skin findings	2/37	2/46	> 0.05
Gene mutation positivity rate	34/37	43/46	> 0.05
ESR (mm/h)	19.3 ± 13.5	17.2 ± 18.4	> 0.05
HsCRP	10.5 ± 17.4	6.8 ± 8.8	> 0.05
RF levels	3.5 ± 18.3	1 ± 5	> 0.05
RF positivity rate	2/37	2/46	> 0.05
Anti-CCP levels	21 ± 30.1	9.8 ± 6	**< 0.05**
Anti-CCP positivity rate	10/37	2/46	**< 0.005**
White blood count (x10^3/mm^3)	7.9 ± 2.8 x10^3	7.9 ± 2.1 x10^3	> 0.05
Fibrinogen	383.7 ± 94.6	372.4 ± 88.1	> 0.05

Results

The demographic and laboratory characteristics of the FMF and control groups were presented in Table I. Two groups were similar on the base of age and gender. Erythrocyte sedimentation rate (ESR), high sensitive CRP levels and white blood count (WBC) were significantly higher in the FMF group than in the control group ($p<0.001$; $p<0.05$ and $p<0.05$, respectively). Fibrinogen levels were similar in those groups. Positivity rate of anti-CCP in the whole FMF group (14.5%) was three-fold higher than the control group (4.7%). However, the difference failed to achieve a statistically significant level ($p=0.09$). Rheumatoid factor (RF) levels and positivity rates of RF were similar between the two groups ($p>0.05$).

Arthritis was assigned in 37/83 (44%) of patients with FMF. One patient had chronic arthritis of the hip joint. Although his abdominal attacks improved after colchicine, his articular symptoms continued, and he eventually underwent hip replacement surgery. The remaining 36 patients in the arthritis group had attacks of monoarthritis involving knee, shoulder, elbow, or wrist. Those arthritis attacks spontaneously recovered within a few days without sequela,

and no arthritis attack in those patients persisted for more than a week. None of those patients experienced severe arthritis attack after colchicine treatment. The demographic, clinical and laboratory characteristics of patients with and without arthritis was presented in Table II. Age, gender, disease duration and rate of clinical findings of subjects were similar between FMF patients with arthritis and without arthritis. Anti-CCP levels were 21±30.1 in patients with arthritis and 13.1±10.3 in non arthritic group (p<0.05). Anti-CCP positivity rates were 10/37 (27%) in patients with arthritis and 2/46 (4.3%) in patients without arthritis (p<0.005). Anti-CCP levels were between 20–39U/ml in 5 patients, between 40–99U/L in 3 patients, and were higher than 100U/ml in 2 patients with arthritis. Five FMF patients with arthritis (13.5%) had moderate-high anti-CCP levels (>40U/ml). Anti-CCP levels were between 20-39U/ml in 2 FMF patients without arthritis and in 2 healthy controls (Table III). The above mentioned patient with chronic hip arthritis was negative for anti-CCP. FMF gene mutations were investigated in 77 of 83 patients with FMF. 24/77 (31.1%) patients have been homozygote gene mutations and 53/77 (68.9%) were heterozygote. Anti-CCP positivity rates were 10/53 (18.8%) in heterozygote group and 2/24 (8.3%) in homozygote group. However, statistical significance was not obtained (p>0.05).

There were no correlations between RF, ESR, HsCRP and anti-CCP levels (p>0.05 for each comparison). Additionally, no association was observed between anti-CCP positivity and presence of clinical features of FMF (p>0.05). Anti-CCP positivity rate is higher in FMF patients with arthritis (27%) than healthy controls (4.7%) (p<0.005). However, there are no difference in anti-CCP levels between patients with arthritis and control subjects (p>0.05). Additionally, ESR and HsCRP levels were higher in patients with arthritis than control group (p<0.001 and p<0.01, respectively).

Table III. Anti-CCP levels in FMF patients with arthritis and without arthritis.

	FMF with arthritis (n=37)	FMF without arthritis (n=46)	Controls (n=43)
Anti-CCP between 20-39 U/ml	5	2	2
Anti-CCP between 40-99 U/ml	3	0	0
Anti-CCP >100 U/ml	2	0	0

Discussion

In this study we investigated anti-CCP levels in FMF patients with or without arthritis. There were no statistically significant differences regarding anti-CCP levels and anti-CCP positivity rates between whole FMF patients and the control group. However, anti-CCP levels and anti-CCP positivity rates were significantly higher in FMF patients with arthritis than patients without arthritis. Additionally, moderate-high levels of anti-CCP were present in 13.5% of FMF patients with arthritis.

Anti-CCP has been described recently as a novel autoantibody with high sensitivity and specificity for the diagnosis of RA. There are many studies that anti-CCP antibodies may serve as a powerful serologic marker for early diagnosis of RA and prognostic prediction of joint destruction (11-13). Furthermore, anti-CCP antibody has been shown to have high specificity (91–98%) for RA (15) and to be a reliable serological marker for the differentiation of RA from other rheumatologic diseases. Moreover, recent studies suggested a relationship between anti-CCP antibody and erosive arthritis, and high titer of anti-CCP antibodies might will be used as a predictor for complication of erosive arthritis in those patients (17, 18). In a recent study, Syversen *et al*. suggested high levels of anti-CCP were better diagnostic for RA and predict better long term progression than low or mild titers of anti-CCP (18).

Anti-CCP levels have been studied in other systemic diseases that may cause arthritis other than RA. Although Damián-Abrego *et al*. found that anti-CCP levels were normal in systemic lupus erythematosus (SLE), Qing *et al*. demonstrated that anti-CCP prevalence (cut-off value: 5U/mL) was 42.1% in a study of 159 (SLE) patients with arthritis (16, 17). Anti-CCP may be detected in patients with Behcet's disease (BD) and psoriatic arthritis (PsA). Koca *et al*. found that anti-CCP was positive in 2.2% of BD patients and RF in 6.5% and they suggested anti-CCP antibody is not associated with BD (19). The prevalence of anti-CCP positivity was observed in approximately 15% percent in patients of PsA with arthritis (20, 21). Furthermore, anti-CCP antibodies found significantly related to erosive, symmetric, and multiple joint involvements in these patients (21).

Recurrent arthritis is a common feature of FMF. Some FMF patients present solely with arthritis attacks. Differential diagnosis of arthritis in FMF includes RA, acute rheumatic fever, ankylosing spondilitis, and adults onset Still's disease (4). Moreover, FMF patients who experience arthritis despite 2mg/day colchicine should be re-evaluated regarding the presence of other hereditary periodic fevers, including tumour necrosis factor-receptor associated periodic syndrome, granulomatous autoinflammatory syndromes (4, 22-24). One of our patients had chronic arthritis of the hip joint. The remaining patients in the arthritis group had attacks of monoarthritis involving knee, shoulder, elbow, or wrist, which spontaneously recovered within a few days without sequela. Furthermore, none of those patients experienced severe arthritis attack after colchicine treatment. Therefore, we concluded that the articular symptoms of those patients were arthritis attacks of FMF.

There are limited studies which investigated relationship between anti-CCP and FMF patients with arthritis in the literature, which reported conflicting results (25, 26). In the first study, the authors reported that 7 of 20 FMF patients with arthritis was positive for anti-CCP (35%), while only 1 of 35 FMF patients without arthritis (2.9%) were positive for anti-CCP (p<0.05). Moreover, they also demonstrated that the levels of anti-CCP antibodies were higher in patients with arthritis. Four patients were weak positive (anti-CCP

values between 20–39 U, and 4 patients had moderate positive (anti-CCP values between 40–59 U) (22). On the other hand, Karatay *et al*. investigated 49 FMF patients (23 with arthritis and 26 without arthritis) reported that no FMF patients with or without arthritis had positive anti-CCP result (23). Since both studies were performed in the same region of Turkey, those conflicting results necessitated a new trial regarding this topic.

In our study, we evaluated higher number of participants with FMF (37 FMF patients with arthritis, 46 patients without arthritis). Anti-CCP positivity rates were higher in patients with arthritis (10/37) than patients without arthritis (2/46) and control group (2/43). Five patients with arthritis had moderate-high anti-CCP levels, while all of the 4 patients without arthritis or controls had low weak anti-CCP positivity. Therefore, we confirmed the results of Uyanik *et al*. in a higher number of participants. Moreover, we demonstrated that significant proportion of FMF patients with arthritis had moderate-high positive values for anti-CCP. The reason for those conflicting results demonstrated by Karatay *et al*. remains to be established. Karatay *et al*. investigated anti-CCP antibody levels by using ELISA of Euro-Diagnostica, Netherlands, and reported their results as either positive (anti-CCP antibody titer was greater than 25UI/ml) or negative, without any data regarding the titer, while we and Uyanik *et al*. used ELISA kits of INOVA diagnostic, Inc., San Diego, USA. Hence, different commertial kits might be a potential explanation for those conflicting results.

In conclusion, we demonstrated that anti-CCP prevalence is higher in FMF patients with arthritis than without arthritis, and that a significant proportion of FMF patients with arthritis (13.5%) had moderate-high titers of anti-CCP. Therefore, anti-CCP antibodies may not be a reliable indicator to differentiate between FMF arthritis and rheumatoid arthritis. All anti-CCP positive patients in the arthritis group had attacks of monoarthritis of the big joints, which spontaneously recovered within a few days without sequela, and all of them responded to colchicine. Hence, the diagnosis of rheumatoid arthritis is unlikely for our patients. On the other hand, attacks of arthritis together with positive anti-CCP might predict the development of rheumatoid arthritis in the future. Therefore, prospective studies with long term follow up of anti-CCP positive FMF patients with arthritis are needed to draw more dependable conclusions.

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