Rheumatol Int (2009) 30:223–227
DOI 10.1007/s00296-009-0941-4

ORIGINAL ARTICLE

Association of anti-CCP positivity with serum ferritin and DAS-28

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Received: 7 November 2008 / Accepted: 28 April 2009 / Published online: 15 May 2009

Abstract Antibodies to cyclic citrullinated peptides (anti-CCP) are highly specific for the diagnosis of rheumatoid arthritis (RA) with a marginal increased prediction of the disease. In this study, we aimed to investigate the relation of the presence of anti-CCP with clinical manifestations and disease activity in a cohort of RA patients. A total of 61 RA patients were included in this study. Data of disease-related parameters such as duration of disease, medications, degree of pain (visual analog scale, VAS), disease activity score 28 (DAS-28) and health assessment questionnaire (HAQ) were recorded. Laboratory workup included erythrocyte sedimentation rate (ESR), plasma C-reactive protein (CRP), rheumatoid factor (RF), anti-CCP, complete blood count and anemia parameters. Anti-CCP positivity was associated with higher scores of DAS-28, longer duration of morning stiffness, serum RF positivity and low levels of serum ferritin, while it was not associated with disease duration, VAS, HAQ, ESR, CRP and hemoglobin.

Keywords Rheumatoid arthritis · Anti-CCP antibodies · Ferritin · Disease activity · Rheumatoid factor

Introduction

Rheumatoid arthritis (RA) is the most common autoimmune disease affecting approximately 1% of the world's population [1]. Chronic inflammation of the involved joints results in progressive loss of function, which, together with the extraarticular manifestations and adverse events of therapy, may increase morbidity and mortality of patients with RA [2, 3].

Rheumatoid factor (RF), which is an antibody directed against the Fc region of IgG, has been used as a diagnostic marker of RA. Rheumatoid factor has an acceptable sensitivity, but may be present in healthy elderly persons or in patients with other autoimmune and infectious diseases [4]. Other rheumatoid arthritis-associated autoantibodies include antiperinuclear factor and antikeratin antibodies [5, 6]. The epitopes of their antigens are arginyl residues citrullinated by peptidyl arginine deiminase [7–9]. Some enzyme-linked immunosorbent assays (ELISA) use linear citrulline-containing peptides that have similar sensitivity to and higher specificity than RF for diagnosing rheumatoid arthritis [10]. Assays that use cyclic citrullinated peptide (CCP) that were developed to detect anti-CCP antibody have higher sensitivity [11].

Although the presence of anti-CCP is accepted to be a reliable diagnostic and prognostic tool in RA [12, 13], its association with disease activity and severity remains unclear. In the present study, we have investigated the relation of the presence anti-CCP with clinical manifestations and disease activity in a cohort of patients with established diagnosis of RA.

Patients and methods

A total of 61 patients, attending the clinic of Ankara Physical Medicine and Rehabilitation Training and Research Hospital, fulfilled the American College of Rheumatology criteria for RA [14]. Data regarding disease variables were collected during clinical evaluation of the patient as well as based on information available in the case records. Patients with other connective tissue disease, acute or chronic

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infectious diseases or malignancy were not included in the study to avoid positive anti-CCP results associated with other conditions [15]. All patients gave their informed consent and the study was approved by the local ethics committee at the Ankara Physical Medicine and Rehabilitation Training and Research Hospital.

In addition to physical examination, data of disease-related parameters such as duration of disease, medications, degree of pain (visual analog scale, VAS), disease activity score 28 (DAS-28), health assessment questionnaire (HAQ) were recorded. Laboratory workup included erythrocyte sedimentation rate (ESR > 20 mm/h considered positive), plasma C-reactive protein (CRP > 0.5 mg/dl considered positive), RF (determined by the standard latex agglutination technique, Olympus America Inc.), anti-CCP (Microparticle Enzyme Immunoassay-MEIA, Abbott Laboratories Inc., USA), complete blood count and anemia parameters (serum iron, iron binding capacity, transferin, ferritin, vitamin B12).

Statistics

At study end, patients were divided into anti-CCP positive and anti- CCP negative and comparisons between the two groups were performed in all the above-mentioned characteristics. Constant variables were compared with the use of independent- samples t-test, while in comparisons regarding categorical variables the chi-square test was applied. Logistic regression was used to detect factors associated with anti-CCP positivity. The level of two-sided statistical significance was set at 0.05. All data were analyzed using SPSS, version 16.

Results

The socio-demographic and disease-associated characteristics of the patient with rheumatoid arthritis are summarized in Table 1. Of the 61 patients, 51 (83.6%) were women and 10 (16.4%) were men with a mean age of 53.27 years (±11.26) and the mean duration of disease was 108.5 months (±102.8). As much as 43 (70.5%) patients were positive for IgM RF, and 38 (62.3%) were positive for anti-CCP. Of the 18 RF-negative patients, 6 (33.3%) were anti-CCP positive.

A total of 54 patients were treated with disease-modifying anti-rheumatic drugs (DMARDS). The most common treatment was with methotrexate (68.8%), followed by corticosteroid (44.3%), sulfosalazine (31.1%), hydroxychloroquine (24.6%) and leflunomide (13.1%).

DAS-28 scores (4.82 ± 1.48 and 3.84 ± 1.50; $P = 0.01$), rate of patients with morning stiffness (50 and 21.73%; $P = 0.05$) and serum RF positivity (84.2 and 47.8%; $P = 0.006$) were significantly higher in anti-CCP-positive patients compared to anti-CCP-negative patients. Anti-CCP-positive patients had longer duration of disease than the negative patients (125.71 ± 104.4 and 77.38 ± 94.30; $P = 0.08$), but the difference was not statistically significant. Anti-CCP-positive patients had significantly lower serum ferritin levels than anti-CCP-negative patients (36.74 ± 39.15 and 80.91 ± 71.28; $P = 0.02$). Serum hemoglobin and thrombocyte levels were similar for anti-CCP-positive and negative patients (Table 2).

In multivariate logistic regression analysis, DAS-28 (OR: 2.0, 95% CI: 1.0–3.98, $P = 0.049$) and RF positivity (OR: 9.892, 95% CI: 2.1–47.5, $P = 0.004$) were associated with anti-CCP positivity (Table 3).

Table 1 Socio-demographic and disease-related characteristics of the patients ($n = 61$)

Age, mean ± SD	53.27 ± 11.26
Female	51 (83.6%)
Duration of disease (months), mean ± SD	108.5 ± 102.8
VAS score (cm), mean ± SD	5.55 ± 2.72
DAS28 score	
Inactive	13 (21.3%)
Moderate	26 (42.6%)
Very active	22 (36.0%)
Stanford HAQ score < 0.3	14 (22.9%)
Morning stiffness > 60 min	24 (39.3%)
Treatment	
NSAID	21 (34.4%)
Steroid	27 (44.2%)
DMARD	53 (86.9%)
ESR (mm/h), mean ± SD	28.2 ± 21.9
CRP (mg/L), mean ± SD	1.3 ± 2.06
RF positive	43 (70.5%)
Anti-CCP positive	44 (72.1%)

Except where indicated otherwise, values are the number (%)

Discussion

The present study of a cohort of 61 patients with RA demonstrated the association of anti-CCP positivity with DAS-28, morning stiffness, serum RF positivity and low serum ferritin levels. Anti-CCP positivity was not associated with HAQ, ESR and CRP. DAS-28 was the only clinical independent determinant of anti-CCP positivity.

Inclusion of anti-CCP into standard diagnostic criteria of RA was proposed by some authors [16–19] to increase the diagnostic specificity, but others demonstrated a marginal increase in prediction of the disease [20, 21]. In this study,

Table 2 Comparison of parameters in Anti-CCP-negative and positive patients with RA

	Anti-CCP positive ($n = 38$)	Anti-CCP negative ($n = 23$)	P
Age	53.84 ± 11.08	52.34 ± 11.74	0.61
Male/female ratio	6/32 (18.7%)	4/19 (21.0%)	0.84
Disease duration (months)	125.71 ± 104.4	77.38 ± 94.30	0.08
VAS	5.89 ± 2.67	4.95 ± 2.75	0.19
DAS-28	4.82 ± 1.48	3.84 ± 1.50	0.01
HAQ	1.03 ± 0.79	0.94 ± 0.78	0.67
Morning stiffness > 60 min	19/38 (50%)	5/23 (21.73%)	0.05
ESR (mm/h)	28.81 ± 21.05	27.26 ± 23.55	0.79
CRP (mg/dl)	1.25 ± 1.93	1.39 ± 2.30	0.80
RF (+)	32/38 (84.2%)	11/23 (47.8%)	0.006
Ferritin	36.74 ± 39.15	80.91 ± 71.28	0.02
Hb (g/dl)[a]	12.5 ± 1.83	13.0 ± 1.38	0.26
Anemia rate	9/38 (23.6%)	4/23 (17.39%)	0.79
Thrombocyte	304 ± 83	284 ± 84	0.38

Values are mean ± standard deviation unless mentioned otherwise

VAS visual analog scale, *DA-28* disease activity score, *HAQ* Health Assessment Questionnaire, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *RF* rheumatoid factor, *Hb* hemoglobin

[a] Anemia defined as Hb < 12.0 g/dl for males and <11.0 g/dl for females

Table 3 Logistic regression analysis with anti-CCP positivity as the dependent variable

	OR	95% CI	P
Duration of disease	1.002	0.994–1.010	0.609
Morning stiffness	1.016	0.999–1.032	0.059
DAS-28	2.0	1.004–3.983	0.049
VAS	0.744	0.519–1.066	0.107
ESR	1.003	0.960–1.027	0.681
RF (+)	9.892	2.057–47.556	0.004
Ferritin	0.970	0.939–1.002	0.065

VAS visual analog scale, *DAS-28* disease activity score, *ESR* erythrocyte sedimentation rate, *RF* rheumatoid factor

only 33.3% of RF-negative RA patients were anti-CCP positive. This result is similar with the results of studies where anti-CCP positivity in RF-negative patients were found to be between 20 and 43% [11, 22].

Joint erosions and deformities are the major adverse outcomes. Several studies demonstrated the association of anti-CCP positivity and joint destruction in patients with established RA [18, 21, 23].

Follow-up parameters of disease activity in RA patients are duration of morning stiffness, degree of joint pain, HAQ, DAS-28, CRP, ESR, hemoglobin and serum RF positivity [24–32]. Limited number of studies demonstrated an association between ESR, HAQ and anti-CCP positivity [33, 34]. In this study, HAQ, VAS, CRP, ESR, hemoglobin, anemia rate and platelet levels were not associated with anti-CCP positivity.

DAS-28 is a composite index that measures disease activity in patients with RA [32]. There is considerable disagreement in the relation between anti-CCP positivity and DAS-28 scores in RA patients [17, 33–36]. In this study, DAS-28, serum RF positivity and morning stiffness were found to be associated with anti-CCP positivity. In logistic regression analysis of multiple parameters, higher DAS-28 scores and RF positivity were associated with 2 and 9.89 times greater risk of being anti-CCP positive, respectively.

The reason for anemia in rheumatoid arthritis is multifactorial. The majority of the anemia cases encountered in RA patients were reticuloendothelial iron-replete anemia of chronic disease. Anemia in active RA has been associated with impaired erythropoiesis due to elevated inflammatory cytokines, defective erythropoietin production or response in bone marrow and blockade of iron reticuloendothelial cells leading to erythroblast iron deprivation. Ferritin is an acute phase reactant, which has moderate association with the degree of inflammation and it is also used as a surrogate marker of iron stores in anemia patients [37]. In this study, 21.3% of the patients had anemia, 75.4% of the patients had active disease according to DAS-28 scores, and 73.7% of the patients had elevated serum CRP levels or ESR. Only two (3.3%) patients had elevated serum ferritin levels in this study group; which was similar to previous result [38]. In our study, low levels of ferritin was found to be associated with anti-CCP positivity, which may indicate the negative effects of active RA on iron metabolism. We did not find any association between serum hemoglobin, thrombocyte levels or anemia rate and anti-CCP positivity. A limitation of our study was the insufficient number of patients in whom the association of anti-CCP positivity with anemia markers was investigated. Further studies are needed to investigate the possible association of ferritin and anti-CCP positivity.

Conclusion

Anti-CCP is a candidate marker for routine diagnosis and prognostic evaluation of RA patients. Our study demonstrates an association between clinical activity of the disease and anti-CCP positivity. Further studies are needed to incorporate anti-CCP tests into the routine management of rheumatoid arthritis patients.

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