Anticyclic Citrullinated Peptide Antibody and Rheumatoid Factor in South Tunisian Patients With Rheumatoid Arthritis: Association With Disease Activity and Severity

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Objective: To explore relationships between immunological status, clinical features, radiographic damage, disease activity, and functional disability in Tunisian patients with rheumatoid arthritis (RA). **Materials and Methods:** The study was carried out in 112 patients with RA. Demographic characteristics, disease duration, disease activity score 28 (DAS28), the Health Assessment Questionnaire (HAQ), and the Sharp/van der Heijde score were collected. Anticyclic citrullinated peptide antibody (anti-CCP) and rheumatoid factor (RF) were performed. **Results:** We found that anti-CCP positivity was associated with longer disease duration ($P = 0.001$), presence of RF ($P = 4.89 \times 10^{-8}$), and night pain positivity ($P = 0.025$). Patients with positive RF had more night pain and higher anti-CCP positivity (for all

$P \leq 0.05$). Anti-CCP titer was correlated with disease duration ($P = 0.034$) and Sharp total score ($P = 1.2 \times 10^{-4}$). Moreover, there was a significant correlation between RF and anti-CCP antibodies titers ($P = 0.011$). Indeed, DAS28 correlated with HAQ ($P = 1.8 \times 10^{-7}$) and morning stiffness duration ($P = 0.045$). In multivariate regression analysis, the main factors associated with anti-CCP titers were radiographic damage ($P = 1.625 \times 10^{-4}$) and RF ($P = 0.013$). For DAS28, only HAQ ($P = 2.9 \times 10^{-4}$) was associated. **Conclusion:** These findings suggest that anti-CCP antibodies are associated with RF and more severe joint damage. Moreover, disease activity is associated with functional disability. J. Clin. Lab. Anal. 28:21–26, 2014. © 2013 Wiley Periodicals, Inc.

Key words: rheumatoid arthritis; anti-CCP antibodies; rheumatoid factor; DAS28

INTRODUCTION

Rheumatoid arthritis (RA [MIM 180300]) is the most common systemic autoimmune disease of unknown etiology and pathogenesis (1). It affects approximately 1% of the world's population with a female:male ratio ranging from 2:1 to 4:1 (2, 3). It is also characterized by chronic inflammation of the synovial membrane, which can lead to progressive joint destruction and in many cases, results in permanent disability (patients do not carry out their everyday activities), poor quality of life, and increased morbidity and mortality (4, 5). Several studies have shown that reduced physical function is associated with depression (6, 7); likewise, optimism may have a positive impact on physical health in RA patients (8). In fact, the disease requires adaptation and involves many future uncer-

tainties because it cannot be cured (9). RA patients are also characterized by various types of disease-specific and nondisease-specific autoantibodies (10). The first known autoantibody is the rheumatoid factor (RF), which is an immunoglobulin (IgM) antibody directed against the constant region (Fc) of the IgG (11). It has an acceptable sensitivity (60–80%) but a low specificity (80–90%) due to its presence in patients with other autoimmune and

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Received 5 November 2012; Accepted 15 May 2013

DOI 10.1002/jcla.21638

Published online in Wiley Online Library (wileyonlinelibrary.com).

infectious diseases, as well as in healthy elderly persons (12, 13). In contrast, anticyclic citrullinated protein antibodies (anti-CCP; antibodies directed against citrullinated antigens, especially fibrin, in which arginine has been posttranslationally modified into the nonstandard amino acid citrulline; (14)) have a similar sensitivity of 68–80% but seem to be a reliable diagnostic and prognostic tool in RA (15, 16) due to their higher specificity of 98% (17–20) and their early detection, years before the onset of symptoms (21, 22). It has been shown that RF and anti-CCP are associated with more severe joint destruction and a more aggressive disease course (23–28). Other studies have shown that early diagnosis and treatment reduce joint destruction, preserve function (29), and lead to disease prevention (3). Furthermore, anti-CCP has been incorporated into newly proposed criteria “the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification” for RA (30). The purpose of this study was to determine if the presence and level of anti-CCP and RF antibodies are associated with higher activity and/or more severe joint damage in Tunisian RA patients, and to explore the relationships between immunological status, clinical features, radiographic damage, disease activity, and functional disability.

PATIENTS AND METHODS

Data Collection

This study was carried out in 112 RA patients recruited from the Department of Internal Medicine, University Hospital Hedi Chaker, Sfax, Tunisia. All patients fulfilled the 1987 American College of Rheumatology criteria for RA (31) and provided written informed consent. We also obtained approval from the local ethical committee at the University Hospital Hedi Chaker, Sfax, Tunisia for this study. A rheumatology university physician reviewed all clinical data.

Demographic and clinical variables such as sex, age, disease duration, morning stiffness duration, and night pain were collected. Morning stiffness duration was defined by Hazes et al. (32) as the time from waking to maximum improvement. Disease activity was assessed by the disease activity score, using a 28 joint score (DAS28) that included the number of swollen joints, number of tender joints, erythrocyte sedimentation rate (ESR), and a general health assessment on a visual analogue scale (from 1–100) obtained from the patient on self-evaluated disease severity (33). This total score is a number between 0 and 10, indicating the amount of disease severity. In fact, patients in remission are defined as <2.6 , low disease activity as ≥ 2.6 to <3.2 , moderate disease activity as ≥ 3.2 to ≤ 5.1 , and high disease activity as >5.1 (33, 34). Functional disability

was assessed by the Health Assessment Questionnaire (HAQ), which was developed by Fries et al., and consists of eight categories that represent the activities of daily living. The final score of the questionnaire ranges between 0 and 3 (35).

To assess radiological structural damage, radiographs of hands and feet were scored using the method of Sharp/van der Heijde. Each side of the metatarsophalangeals or interphalangeal joint was scored for erosions from 0 to 5 with a maximum of 10 for both sides of the joint, and for joint space narrowing from 0 to 4 with a maximum of 4 per joint. For the total Sharp/van der Heijde score, the erosions and joint space narrowing scores were added together (36).

Laboratory Markers of RA

ESR (mm/h) was measured using standard laboratory method, RF was measured by nephelometry with a cutoff of 15 UI/ml, and anti-CCP by enzyme-linked immunosorbent assay (ELISA; anti-CCP ELISA (IgG) EUROIMMUN®, Lübeck, Allemagne), and a cutoff of 5 U/ml was established.

Statistical Analysis

Patients' characteristics were presented as means and standard deviation for continuous variables and as frequencies and percentages for categorical variables. The Student's *t*-test for independent samples and chi-square test were used to analyze the significance of the different variables between patients with and without antibodies (anti-CCP and RF). The comparisons of the means of continuous variables in patients with different categories of disease activity were performed by analysis of variances using an ANOVA test for normally distributed variables and a nonparametric analysis (Kruskal–Wallis test) for nonnormally distributed variables. Correlations between levels of autoantibodies, DAS28, and patient's parameters were calculated using Pearson's correlation coefficient. Afterward, we performed multiple regression analysis using anti-CCP antibody level and DAS28 score as dependent variables and only variables with significant *P*-value at the correlations analysis were introduced in each regression model. Results of regression were presented as beta coefficient. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows and *P*-value less than 0.05 was considered significant.

RESULTS

One hundred and twelve patients (93 female and 13 male) with RA were included in our study. The

TABLE 1. Patients' and Disease Characteristics (N = 112)

Female (%)	83
Age, mean $\pm$ SD (years)	51.88 $\pm$ 13.27
Disease duration, mean $\pm$ SD (years)	15.4 $\pm$ 10.66
DAS28, mean $\pm$ SD	4.67 $\pm$ 1.52
DAS28:	
Remission (%)	8.9
Low activity (%)	11.6
Moderate activity (%)	38.4
High activity (%)	41.1
HAQ, mean $\pm$ SD	1.55 $\pm$ 0.84
Sharp total score, mean $\pm$ SD	75.11 $\pm$ 76.1
Morning stiffness duration (min)	37.62 $\pm$ 38.09
Night pain (%)	57.1
Anti-CCP, mean $\pm$ SD (U/ml)	99.22 $\pm$ 126.76
Anti-CCP positive (%)	64.7
RF, mean $\pm$ SD (U/l)	193.64 $\pm$ 224.68
RF positive (%)	75.9

Values are mean $\pm$ standard deviation or percentage.

DAS28, disease activity score; HAQ, Health Assessment Questionnaire; Anti-CCP, anticyclic citrullinated protein; RF, rheumatoid factor.

characteristics of patients are shown in Table 1. The mean age of our patients was 51.88 $\pm$ 13.27 years and the mean disease duration was 15.4 $\pm$ 10.66 years. The mean DAS28 was 4.67 $\pm$ 1.52. Ten patients were in a state of clinical re-

mission, 13 with low activity, 43 with moderate activity, and 46 with high activity. The mean HAQ score was 1.55 $\pm$ 0.84, indicating moderate disability. The mean Sharp/van der Heijde score was 75.11 $\pm$ 76.1. Moreover, 64.7% of patients were positive for anti-CCP and 75.9% were positive for RF. Table 2 shows the difference between patients with and without anti-CCP and RF antibodies. In fact, disease duration (17.29 $\pm$ 11.48 and 11.11 $\pm$ 7.2 years; $P = 0.001$), night pain positivity (43.1 and 15.7%; $P = 0.025$), and serum RF positivity (61 and 16%; $P = 4.89 \times 10^{-8}$) were significantly higher in anti-CCP-positive patients than anti-CCP-negative patients. Patients with positive RF antibody had more night pain (64.6 vs. 38.5%; $P = 0.017$) and anti-CCP antibody positivity (79.2 vs. 17.4%; $P = 4.89 \times 10^{-8}$). Furthermore, Table 3 shows a significant relationship between different DAS28 groups and functional disability (mean of HAQ, $P = 0.04$) on one hand and morning stiffness duration ($P = 0.023$) on the other.

Further, there was a significant correlation between the titer of anti-CCP and disease duration ($r = 0.212$, $P = 0.034$) and Sharp score ($r = 0.353$, $P = 1.2 \times 10^{-4}$). Moreover, there was a significant correlation between the presence of anti-CCP and RF antibodies ($r = 0.295$, $P = 0.011$). Disease activity was correlated significantly with

TABLE 2. Data Stratification According to the Immunological Status

	Anti-CCP (+)	Anti-CCP (–)	<i>P</i> -value	RF (+)	RF (–)	<i>P</i> -value
Female (%)	53.9	29.4	0.616	82.9	80.8	0.504
Age, mean $\pm$ SD (years)	51.56 $\pm$ 13.67	51.08 $\pm$ 12.91	0.638	51.6 $\pm$ 13.44	51.46 $\pm$ 13.32	0.964
Disease duration, mean $\pm$ SD (years)	17.29 $\pm$ 11.48	11.11 $\pm$ 7.2	0.001*	14.93 $\pm$ 10.7	15.92 $\pm$ 10.5	0.677
DAS28, mean $\pm$ SD	4.7 $\pm$ 1.42	4.94 $\pm$ 1.6	0.465	4.72 $\pm$ 1.5	4.636 $\pm$ 1.6	0.8
HAQ, mean $\pm$ SD	1.4 $\pm$ 0.85	1.7 $\pm$ 0.87	0.388	1.54 $\pm$ 0.841	1.57 $\pm$ 0.861	0.087
Sharp total score, mean $\pm$ SD	87.48 $\pm$ 86.12	59.28 $\pm$ 59.13	0.055	80.42 $\pm$ 83.786	57.46 $\pm$ 48.1	0.905
Morning stiffness duration (min)	39.84 $\pm$ 41.25	30.97 $\pm$ 31.32	0.177	41.1 $\pm$ 40.09	27.31 $\pm$ 29.54	0.182
Night pain positive (%)	43.1	15.7	0.025*	64.6	38.5	0.017*
Anti-CCP positive (%)				79.2	17.4	4.89 $\times 10^{-8}$ *
RF positive (%)	61.0	16	4.89 $\times 10^{-8}$ *			

Values are mean $\pm$ standard deviation or percentage.

DAS28, disease activity score; HAQ, Health Assessment Questionnaire; Anti-CCP, anticyclic citrullinated protein; RF, rheumatoid factor.

*Significant at $P \leq 0.05$.

TABLE 3. Clinical and Laboratory Continuous Variables in Relation to Different Activity Disease Level

DAS28	Age [#]	Disease duration [#]	HAQ*	Sharp [#]	Anti-CCP [#]	RF [#]	Morning stiffness duration*
Remission	48.6 $\pm$ 10.81	19.5 $\pm$ 11.96	0.89 $\pm$ 0.85	82.8 $\pm$ 99.23	105.5 $\pm$ 141.13	267.75 $\pm$ 314.26	26.5 $\pm$ 54.87
Low	51.46 $\pm$ 12.7	16 $\pm$ 13.42	0.86 $\pm$ 1.02	78.31 $\pm$ 89.57	111.1 $\pm$ 163.78	85.71 $\pm$ 109.7	17.08 $\pm$ 22.10
Moderate	53.72 $\pm$ 14.45	16.93 $\pm$ 9.56	1.5 $\pm$ 0.71	73.72 $\pm$ 82.32	96.54 $\pm$ 103.18	234.48 $\pm$ 226.12	43.13 $\pm$ 40.67
High	50.98 $\pm$ 12.91	12.91 $\pm$ 10.66	1.78 $\pm$ 0.82	73.8 $\pm$ 61.52	97.63 $\pm$ 137.18	168.48 $\pm$ 231.34	40.67 $\pm$ 32.99

Values are mean $\pm$ standard deviation.

DAS28, disease activity score; HAQ, Health Assessment Questionnaire; Anti-CCP, anticyclic citrullinated protein; RF, rheumatoid factor.

*Significant at $P \leq 0.05$.

[#]Nonsignificant at $P > 0.05$.

TABLE 4. Correlations Between Autoantibody Titers or Disease Activity Score and Disease Parameters

	Anti-CCP		RF		DAS28	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Female	0.026	0.799	0.022	0.846	−0.157	0.099
Age	0.081	0.424	−0.018	0.877	−0.029	0.764
Disease duration	0.212	0.034*	−0.121	0.290	−0.177	0.062
DAS28	0.023	0.823	−0.20	0.861	1	–
HAQ	0.009	0.951	0.051	0.778	0.462	1.8×10^{-7} *
Sharp total score	0.353	1.2×10^{-4} *	−0.010	0.929	0.008	0.934
Morning stiffness duration	0.089	0.389	0.144	0.224	0.194	0.045*
Night pain positive	−0.003	0.977	0.085	0.462	−0.155	0.103
Anti-CCP	1	–	0.295	0.011*	0.023	0.823
RF	0.295	0.011*	1	–	−0.020	0.861

DAS28, disease activity score; HAQ, Health Assessment Questionnaire; Anti-CCP, anticyclic citrullinated protein; RF, rheumatoid factor.

r, Pearson's correlation coefficient.

*Significant at $P \leq 0.05$.

HAQ ($r = 0.462$, $P = 1.8 \times 10^{-7}$) and morning stiffness duration ($r = 0.194$, $P = 0.045$; Table 4). Additionally, the Sharp/van der Heijde score showed a statistically significant correlation with age ($r = 0.239$ and $P = 0.011$) and disease duration ($r = 0.376$ and $P = 3.65 \times 10^{-5}$, data not shown).

The multivariate regression analysis shows a positive relationship between the titer of anti-CCP antibody and radiographic damage (Sharp score, $P = 1.625 \times 10^{-4}$) and RF titer ($P = 0.013$), but disease duration did not significantly modify the relationship (Table 5). Likewise for the disease activity, only functional disability (HAQ, $P = 2.9 \times 10^{-4}$) has shown a significant relationship. Morning stiffness duration did not improve this model (Table 5).

DISCUSSION

In this study, we explored associations between immunological status (anti-CCP and RF antibodies), clinical features, radiographic damage (Sharp score), disease activity (DAS28), and functional disability (HAQ) in definite Tunisian RA patients with longstanding and medium active disease.

In our data, Sharp score was higher in the anti-CCP-positive and RF-positive group, but the differences were not statistically significant. However, a significant correlation was found between the radiological joint damage score and anti-CCP titer, but not with RF titer. In regression analysis, the association was maintained between anti-CCP levels and radiographic damage. Our investigation confirms previous suggestions that anti-CCP may reflect the development of joint damage in RA and that the presence of anti-CCP can be a diagnostic tool and predict radiological damage (27, 37). We have also noticed that mean disease duration in the anti-CCP-positive group was significantly higher than the anti-CCP-negative group, and was also correlated with anti-CCP titer and radiographic damage. In fact, increasing radiological damage with longer disease duration has been described by many other studies (38, 39).

As already reported in most studies, we detected an association between anti-CCP and RF positivity ($P = 4.89 \times 10^{-8}$), their means correlate significantly ($P = 0.011$) and in multiple regression analysis, this association persists ($P = 0.013$). Furthermore, our study does not show an association between anti-CCP positivity or titers and DAS28. These results agree with some studies from Thailand, Greece, and Egypt (40–42) but disagree

TABLE 5. Multiple Regression Analysis Results for Anti-CCP and DAS28

	Anti-CCP			DAS28	
	Beta coefficient	<i>P</i>		Beta coefficient	<i>P</i>
Sharp total score	0.713	1.625×10^{-4} *	HAQ	0.875	2.9×10^{-4} *
RF	0.162	0.013*			
Disease duration	3.68×10^{-2}	0.979	Morning stiffness duration	3.5×10^{-3}	0.449

DAS28, disease activity score; HAQ, Health Assessment Questionnaire; Anti-CCP, anticyclic citrullinated protein; RF, rheumatoid factor.

*Significant at $P \leq 0.05$.

with those reported in Turkish (43) and Moroccan (28) patients with RA. Moreover, we found significant correlations between DAS28 and HAQ ($P = 1.8 \times 10^{-7}$) on the one hand and the morning stiffness duration ($P = 0.045$) on the other hand. These results are in accordance with those reported by Ghosh et al. (44) and Khan et al. (45).

It is worth noting that there were significant declines in levels of both anti-CCP and RF antibodies in RA patients treated with different DMARD (disease modifying anti-rheumatic drugs) (46) or antitumor necrosis factor (anti-TNF) therapy (infliximab, (47)). Yet, the major limitation of our present study is the lack of a treatment strategy. Further studies with treatment modalities such as DMARDs, anti-TNF therapy, and systemic glucocorticoids will be required.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

We thank Professor Paola Migliorini (Immunology Department, University of Pisa, Italy) and Jeffrey Hammer (National Institutes of Health, Bethesda, Maryland 20892–2510, USA) for their critical reading of this study. This work was supported by the Ministry of Higher Education and Scientific Research, Tunisia.

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