ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2014; 13(3):147-156.

Anti-Cyclic Citrullinated Peptide Antibody and Rheumatoid Factor Isotypes in Iranian Patients with Rheumatoid Arthritis: Evaluation of Clinical Value and Association with Disease Activity

Yadollah Shakiba[1], Susan Koopah[1], Ahmad Reza Jamshidi[2], Ali Akbar Amirzargar[1], Ahmad Masoud[1], Amir Kiani[3], Mohammad Hossein Niknam[1], Bahareh Nazari[1], and Behrouz Nikbin[1,4]

[1] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Rheumatology Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[3] Molecular Diagnostic Research Center, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Received: 4 May 2013; Received in revised form: 28 July 2013; Accepted: 29 September 2013

ABSTRACT

In this study we determined the frequency, sensitivity and specificity of anti cyclic citrullinated peptides (anti-CCP) IgG antibody, total rheumatoid factor (RF-T), and RF isotypes in Iranian patients with rheumatoid arthritis (RA) and their association with age, clinical and serological parameters.

Anti-CCP and RF-T and RF isotypes level were measured in 418 patients and 399 healthy controls by enzyme-linked immunosurbant assay (ELISA). Additionally, serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), visual analog scale (VAS) and disease activity score (DAS28) were evaluated in RA patients.

The anti-CCP was positive in 53.1% of RA patients and 4.7% of controls. The frequency of RF-T was 61.87% and 17.66% in RA patients and controls respectively. The prevalence of RF isotypes in RA patients was 46.52% for RF-IgM, 23.47% for RF-IgA and 21.74% for RF-IgG. 31.39% of RA patients were RF-IgM positive without RF-IgA and RF-IgG and 21.9% were positive for all three RF classes. The anti-CCP positive patients showed increased number of swollen joints. On the other hand, RF-T positive patients exhibited a longer disease duration, lower age of onset and also higher ESR, CRP level and increased swollen joints. RF-T titer was significantly higher in RA patients with active disease compared to remission, low and moderate active groups. The sensitivity and specificity were 53.1, 95.3 for anti-CCP antibody and 61.8, 82.3 for RF-T.

Our results support that anti-CCP and RF titer maybe valuable in estimation of disease activity and other inflammatory parameters in RA patients.

Keywords: Anti-CCP; C-Reactive Protein; Disease Activity; Rheumatoid Arthritis; Rheumatoid Factor

Corresponding Author: Behrouz Nikbin, MD, PhD; Department of Immunology, School of Medicine, and Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran. Tel/ Fax: (+98 21) 6641 9536, E-mail: dnik@ams.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune disease that affects about 1% of population worldwide. RA is the most common inflammatory arthritis characterized by synovial hypertrophy or pannus formation.[1] The diagnosis of RA is based on clinical manifestations. Early diagnosis is very important in RA to prevent the reduction of functional capacity and associated morbidity and mortality. Today, serological tests are routinely used for determination of autoantibodies in RA patients. Various autoantibodies can be detected in serum and synovial fluid of RA patients.[2] Rheumatoid factor (RF) has been widely used as a serological marker in diagnosis of RA for years. RF can be detected in up to 70–80% of RA patients and is associated with disease activity, bone erosion and disease outcome.[3] The major RF isotypes is IgM but RF-IgA and RF-IgG are also present in the serum and synovial fluid of patients with RA and may provide additional diagnostic information. Among RF isotypes, RF-IgA has been reported to be more specific in RA diagnosis and also is associated with higher disease activity. RF can be detected in other rheumatologic disorders and increases with age in normal population.[4] Based on this fact, recent studies suggested that RF isotypes including RF-IgM, RF-IgA and RF-IgG may increase accuracy of results and predict the RA associated consequences.[5-7]

In recent years, a number of studies demonstrated that antibodies against cyclic citrullinated peptide (CCP) are more specific (around 98%) and can be used as a predictive and prognostic marker for RA.[8,9] Further investigations proved that anti-CCP antibodies can be detected years before the onset of RA and also they are associated with higher disease activity and joint destruction in RA patients.[10] Anti-CCP antibodies are detected in both shared epitope (SE)-positive and SE- negative RA patients but it seems that having SE alleles is associated with the presence of anti-CCP antibodies.[11] Anti-CCP positivity is added to the new RA diagnosis criteria of the American College of Rheumatology (ACR) in 2010.[12] Altogether many data support using anti-CCP antibodies for distinguishing RA from other similar diseases and also for predicting the prognosis of RA patients. The goal of the present study was to evaluate the presence and clinical value of IgG anti-CCP antibody and RF isotypes in Iranian patients with RA and their associations with disease activity and inflammatory markers.

MATERIALS AND METHODS

418 cases of patients (375 females and 43 males) fulfilling the 1987 ACR classification criteria for rheumatoid arthritis were selected.[13] All patients were known treated cases referred from Iran Rheumatism Center. Mean age of patients was 49.8±12.3 years (range, 20 to 80 years), and median-IQR of disease duration was 9 (5-13) years. The study was approved by ethical board of Tehran University of Medical Sciences. The entire participants were informed about the research and written consent was obtained. The control group consisted of 399 healthy people (383 female and 16 male) with no history of autoimmune diseases. Mean age of control group was 47.6±11.9 years (range, 22 to 82 years). Baseline information including demographic and age of disease onset were collected by face to face interviewing in the first visit. For calculation of disease activity Score of 28 joints (DAS28-CRP), we used the EULAR activity criteria (clinical remission values below 2.6, as low activity between 2.6 to 3.2, moderate activity from 3.2 to 5.1 and high activity values over 5.1).[14] All of patients were under treatment with corticosteroids and methotrexate. Patients receiving other interfering drugs including therapeutic monoclonal antibodies (anti-TNF or anti-CD20) were excluded from the study.

Laboratory Tests

Serum samples were obtained from both patient and control groups, aliquoted and stored at -80°C until assayed. 1 hour ESR was determined by the westergreen method. Serum C-Reactive Protein (CRP) was assayed by an enzyme-linked immunosorbent assay (Elisa) kit (hs-CRP, Monobind inc, USA). Elisa was used for detection of RF-total (RF-T) and RF isotypes (Aesku, Wendelsheim, Germany). According to manufacture instruction, positive test was defined as a level over 24 U/ml for RF-T and over 18 U/ml for RF isotypes (RF-IgM, RF-IgA and RF-IgG). IgG anti-CCP antibodies were also assayed using a commercially available Elisa kit (Genesis Diagnostics, UK). The titer of 6.25 RU/ml was considered as threshold for a positive result.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Statistical Analysis

Statistical analysis was performed using the SPSS 17 for Windows. Data were analyzed first for normality of distribution by using the Kolmogorov–Smirnov test. Results were expressed as mean±SD for normally distributed data, median and interquartile range (IQR) for non-normally distributed data, and percentages for categorical data. Comparison of groups was carried out with Student's t test, Mann–Whitney U test and one-way ANOVA as appropriate. Fisher's exact test was used for comparison of percentages. Correlations were analyzed using Spearman's correlation coefficient. Two-sided *p* values <0.05 were considered significant throughout. Receiver operating characteristic (ROC) curve analysis was done by plotting sensitivity against 1-specificity for various cutoff points of anti-CCP and RF-T. The optimal cutoff point was determined at the maximum value of Youdens's index.

RESULTS

Demographic, Laboratory and Clinical Features of RA Patients

The demographical data, clinical and laboratory features are summarized in table 1. The mean±SD age of the patients was 49.8±12.3 years. The median-IQR of disease duration was 9 (5-13) years. The anti-CCP was positive in 222 (53.21%) patients and negative in 196 (46.89%) patients. The RF-Total was positive in 258 (61.87%) and negative in 169 (38.13%) patients. The median-IQR of the anti-CCP and RF-Total titer was 6.5 (1.3-47.8) RU/ml and 68.9 (18-228) U/ml, respectively. 16.5% of patients had familial history of RA.

The Frequency and Titer of Anti-CCP and RF-T in RA Patients and Controls

The frequency and titer of anti-CCP and RF-T are summarized in table 2. The frequency and titer of Anti-CCP and RF-T were significantly higher in RA patients than controls. Moreover, these data showed the higher odds ratio for anti-CCP antibody compared to RF-T. In comparison to anti-CCP antibody, the frequency of RF-T was higher in both RA and control group which suggested more specificity of anti-CCP antibody in RA diagnosis. RF was present in 191 out of 222 (86%) of RA patients with positive anti-CCP and 67 out of 196

Table 1. Demographic and clinical feature of patients with RA.

Age at visit (years), a	49.8±12.3
Sex (female/male)	375/43
Disease duration (years), b	9 (5-13)
Age of onset (years), a	39.78±12.8
Family History, N (%)	69 (16.5%)
1h Erythrocyte sedimentation rate (mm/h), b	14 (8-24)
C reactive protein (mg/l), b	3.3 (0.65-8.8)
VAS, b	30 (20-50)
DAS28-CRP, a	2.6±1.12
Joint deformity, N (%)	91 (21.77%)
Remission, N (%)	235 (56.2%)
Low activity, N (%)	63 (15.1%)
Moderate activity, N (%)	105 (25.1%)
High activity, N (%)	15 (3.6%)
IgG Anti-CCP (RU/ml), b	6.5 (1.3-47.8)
Total-RF (U/ml), b	68.9 (18-228)
Positive Anti-CCP, N (%)	222 (53.1)
Positive Total RF, N (%)	258 (61.87%)

a=mean±SD, b=median (IQR)

Table 2. The frequency and titer of anti-CCP and RF-T in RA patients and control groups

Topics	RA patients	Controls	
Positive Anti-CCP, N (%)	222 (53.1)	18 (4.7%)	$\chi^2 = 223.21$ $P=5.3 \times 10^{-57}$ OR=22.97
Positive Total RF, N (%)	258 (61.87%)	65 (17.66%)	$\chi^2= 157.76$ $P=7.8\times 10^{-38}$ OR= 7.56
Anti-CCP (RU/ml) a	6.5 (1.3-47.8)	1.6 (0.9-2.6)	$P= 4.4\times 10^{-26}$
Total-RF (U/ml) a	68.9 (18-228)	18.5 (11.9-26.2)	$P= 1.4\times 10^{-35}$

a=median (IQR)

(34.18%) patients with negative anti-CCP. There was significant difference between anti-CCP positive and negative patients for rheumatoid factor (χ^2= 117.5, p value= 1.5×10^{-28}).

The Prevalence of RF Isotypes in RA Patients and Controls

230 RA samples were selected randomly and RF-T positive samples were analyzed for RF isotypes. All RF-T positive samples in control group were analyzed for RF isotypes determination. The odds ratio and relative risk for different RF isotypes are summarized in table 3. Among 3 RF isotypes, RF-IgM was found to be more common in both RA and control group and showed a greater odds ratio and relative risk score. RF-IgG had lower frequency in both RA and control groups.

The Features of Age, Serological and Inflammatory Parameters in the Anti-CCP and RF-T Positive Versus Anti-CCP and RF-T Negative Patients

The analysis of age, clinical and serological parameters among the anti-CCP and RF-T positive and negative patients with rheumatoid arthritis are summarized in table 5. The number of swollen joints was significantly higher in both anti-CCP and RF-T positive patients compared to anti-CCP and RF-T negative patients. RF-T positive patients showed longer disease duration (p=0.02) and lower age of disease onset (p=0.05).

Moreover the ESR, CRP and DAS28-CRP were higher in RF-T positive patients. There was no difference of joints deformity frequency in anti-CCP and RF-T positive and negative patients.

Anti-CCP, RF-T and Disease Activity

The frequency of anti-CCP antibody was significantly higher in RA patients with high active disease versus patients with remission, low active and moderate active disease (p=0.01, 0.002 and 0.009, respectively). Frequency of RF-T was significantly higher in RA patients with high active disease compared to patients in remission and low disease activity groups (p=0.03 and p =0.03, respectively). We analyzed the significance of difference of anti-CCP and RF titers in RA with different disease activity using Kruskal-Wallis test. The significant values were corrected with Bonferroni adjustment. The titer of anti-CCP was not significantly different in RA patients with different disease activity but RF-T titer was higher in high active group compared to remission and low activity groups (p=0.006 and p=0.001, respectively) (Figure 1). These data suggest that anti-CCP antibody

Table 3. The presence of RF isotypes in RA patients and controls

Topics		RA, n=230	Control , n=367)	*P* value	χ^2	OR
RF-IgM, n (%)	Positive	107 (46.52)	11 (3)	5.8×10 - 40	168.9	28.15
	Negative	123 (53.48)	356 (97)			
RF-IgA, n (%)	Positive	54 (23.47)	4 (1.1)	5.3×10^{-20}	80.8	27.84
	Negative	176 (76.52)	363 (98.9)			
RF-IgG, n (%)	Positive	50 (21.74)	2 (0.54)	3×10^{-20}	79.87	50.69
	Negative	180 (78.26)	365 (99.46)			

Table 4. The combination of different patterns of RF-isotypes in RA patients and controls

RA patients	N (%)	Controls	N (%)
IgM+ IgA- IgG-	43 (31.39)	IgM+ IgA- IgG-	10 (15.62)
IgM+ IgA+ IgG+	30 (21.9)	IgM+ IgA+ IgG+	0
IgM+ IgA- IgG+	19 (13.86)	IgM+ IgA- IgG+	1 (1.56)
IgM+ IgA+ IgG-	15 (10.94)	IgM+ IgA+ IgG-	0
IgM- IgA- IgG-	21 (15.32)	IgM- IgA- IgG-	48 (75)
IgM- IgG- IgA+	8 (5.83)	IgM- IgA+ IgG-	4 (6.25)
IgM- IgG+ IgA+	1 (0.72)	IgM- IgA+ IgG+	0
IgM- IgG+ IgA-	0	IgM- IgA- IgG+	1 (1.56)
Total	137 (100)	Total	64 (100)

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

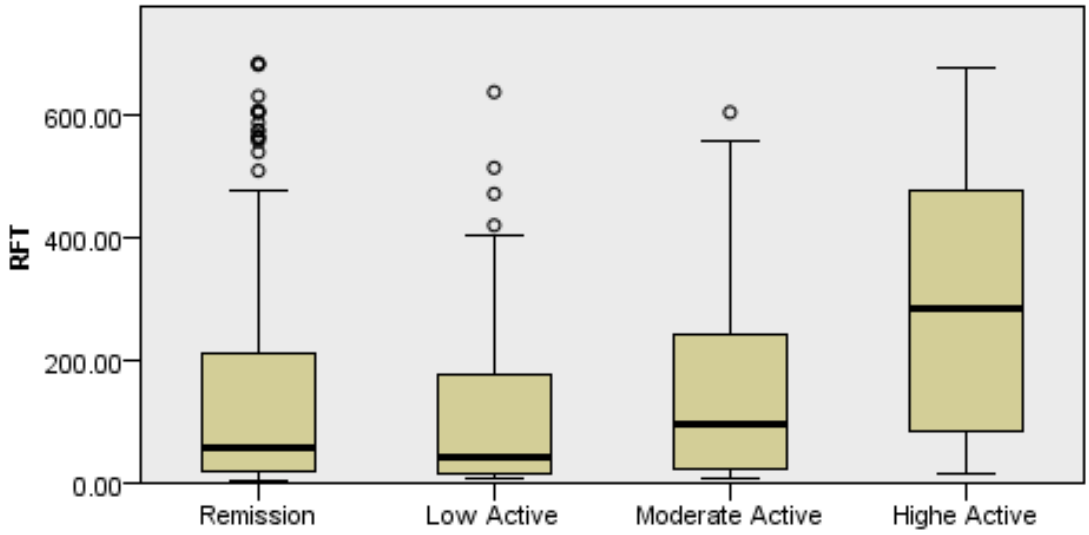

Figure 1. RF-T titer in RA patients with different disease activity

Table 5. Difference of age, clinical and serological markers in the anti-CCP and RF-T positive versus anti-CCP and RF-T negative RA patients. a=mean±SD, b=median (IQR)

Topics	Anti-CCP+	Anti-CCP-	*P*	RF-Total +	RF-Total –	*P*
Age, a	50 (11.9)	49.6 (12.7)	0.73	49.4 (12.3)	50.4 (12)	0.42
Disease duration, b	10 (6-14)	9 (5-13)	0.24	10 (6-15)	8 (4-12)	0.007 *
Age of onset, a	39.7 (12.1)	39.8 (13.5)	0.9	38.7 (12.5)	41.5 (13)	0.02 *
ESR, b	14 (8.7-25)	13.5 (8-22)	0.22	15 (10-26)	10 (7-20)	0.00002 *
CRP, b	3.4 (0.7-9.9)	2.9 (0.6-8.5)	0.13	4 (1-10.7)	2.5(0.3-5.7)	0.001 *
VAS, b	30 (10-50)	40 (20-50)	0.08	30 (15-50)	30 (20-50)	0.32
Tender joints, b	1 (0-4)	1 (0-2)	0.48	1 (0-3)	1 (0-2)	0.44
Swelled joints, b	0 (0-1)	0 (0-1)	0.02 *	0 (0-1)	0 (0-0)	0.005 *
DAS28-CRP, b	2.4 (1.7-3.3)	2.5 (1.7-3.2)	0.7	2.4 (1.7-3.4)	2.4 (1.6-3.2)	0.08
Deformity, n (%)	46 (20.7)	45 (23)	0.33	57 (22.1)	34 (21.4)	0.9

and RF are different markers in RA and maybe considered their roles in RA pathogenesis in separate ways.

The Frequency of RF Isotypes in RA Patients with Different Disease Activity

The frequency of RF isotypes compared in RA patients with different activity is presented in figure 2. There was no different in RF-IgM and RF-IgG frequencies between RA groups. The frequency of RF-IgA was significantly higher in RA patients with high active disease compared to low active group (p=0.05).

The Correlation between Anti-CCP and RF-T with Disease Activity Related Parameters

The analysis of correlation between anti-CCP and RF-T titer and clinical and serological markers of disease activity are summarized in table 6. There was a significant correlation between anti-CCP titer and RF-T, CRP and the number of swollen joints. There was no significant correlation between anti-CCP titer and ESR, number of joint tenderness, visual activity score and DAS28-CRP score. RF-T titer was significantly correlated with ESR, CRP and the number of swollen joints. Also no association was found between RF-T titer and the number of tender joints, VAS and DAS28 score.

Sensitivity and Specificity of Anti-CCP and RF

The sensitivity and specificity of anti-CCP, RF-T and RF isotypes are shown in table 7. The overall diagnostic specificity of anti-CCP, RF-T, RF-IgM, RF-

IgA and RF-IgG was 95.3, 82.3, 97, 98.9 and 99.4, respectively. All RF isotypes had higher specificity than anti-CCP but their sensitivity was lower. Among RF-isotypes, RF-IgM showed a higher sensitivity and similar specificity to other isotypes.

For further comparisons of the diagnostic value of anti-CCP and RF-T, we performed ROC analysis (Figure 3) and calculation the area under curve (AUC). The AUC was 0.72 for anti-CCP and 0.75 for RF-T. Therefore, both markers exhibited comparable diagnostic values. Based on Youdens's index, the optimal cutoff for RF-T was 50.2 U/ml, higher than manufacture recommended cutoff (24.2 U/ml), and 5.6 RU/ml for anti-CCP, lower than manufacture recommended cutoff (6.25 RU/ml).

Sensitivity and Specificity of the Combination of Anti-CCP with RF-T and RF Isotypes

We analyzed the specificity and sensitivity of anti-CCP in combination with RF-T and RF isotypes. The combination of anti-CCP antibodies and RF-T and RF isotypes increased sensitivity and decreased specificity. The combination of CCP and RF-IgG gave the lowest sensitivities (16.9) but the highest specificity (100%) (Table 8).

Table 6. The correlation between anti-CCP and RF-T titer with disease activity related parameters

Topics		CCP	RF-T	ESR	CRP	TJ	SJ	VAS	DAS28-CRP
Anti-CCP	r	1	0.5 **	0.07	0.09 *	0.05	0.13**	-.06	0.05
	P		<0.001	0.1	0.05	0.25	0.008	0.18	0.29
RF-T	r	0.5**	1	0.23**	0.19**	0.3	0.13**	-0.41	0.08
	P	<0.001		<0.001	<0.001	0.53	0.007	0.4	0.1

CCP= cyclic citrullinated peptide, RF-T= total RF, ESR= erythrocyte sedimentation rate, CRP= C-Reactive Protein, TJ=Tender joints, SJ=swollenjoints, VAS=visual analogue scale, DAS28-CRP=disease activity score28-CRP, *= low correlation, **=high corellation

Table 7. The sensitivity and specificity of anti-CCP, RF-T and RF isotypes in patients with RA

Topics	Anti-CCP	RF-Total	RF-IgM	RF-IgA	RF-IgG
Sensitivity, *	53.1 (48.2-57.9)	61.8 (56.9-66.5)	46.5 (39.9-53.1)	23.4 (18.2-29.5)	21.7 (16.7-27.7)
Specificity, *	95.3 (92.5-97.1)	82.3 (77.9-86)	97 (94.5-98.4)	98.9 (97-99.6)	99.4 (97.8-99.9)
PPV, *	92.5 (88.2-95.3)	79.8 (74.9-84)	90.6 (83.5-95)	93.1 (82.4-97.7)	96.1 (85.6-99.3)
NPV, *	65 (60.9-68.9)	65.5 (61-69.8)	74.3 (70.1-78.1)	67.3 (63.1-71.2)	66.9 (62.8-70.8)

*=% (95% CI), PPV=positive predictive value, NPV=negative predictive value

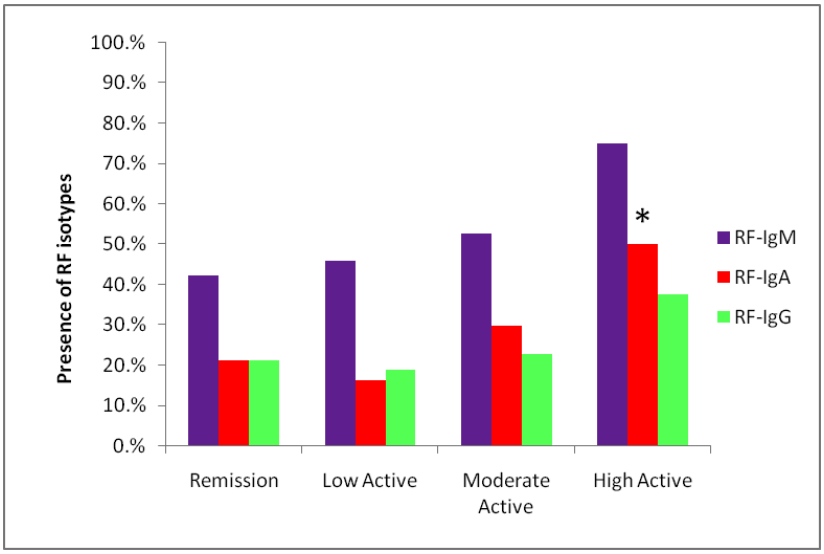

Figure 2. Presence of RF isotypes in RA patients with different disease activity. * The frequency of RF-IgA was significantly higher in RA patients with high active disease compared to low active group (p=0.05).

Table 8. The specificity and sensitivity of anti-CCP in combination with RF-T and RF isotypes

Topics	Anti-CCP+ and RF-T+	Anti-CCP + and RF-IgM+	Anti-CCP + and RF-IgA+	Anti-CCP + and RF-IgG+
Sensitivity, a	45.8 (40.9-50.7)	33.9 (27.8-40.4)	17.8 (13.2-23.5)	16.9 (12.4-22.5)
Specificity, a	98.6 (96.6-99.5)	99.7 (98.2-99.9)	99.7 (98.2-99.9)	100 (98.7-100)
PPV, a	97.4 (93.8-99)	98.7 (92.1-99.9)	97.6 (85.9-99.8)	100 (88.8-100)
NPV, a	61.6 (57.5-65.5)	70.6 (66.4-74.5)	65.9 (61.8-69.8)	65.7 (61.6-69.6)

a=% (95% CI), PPV=positive predictive value, NPV=negative predictive value

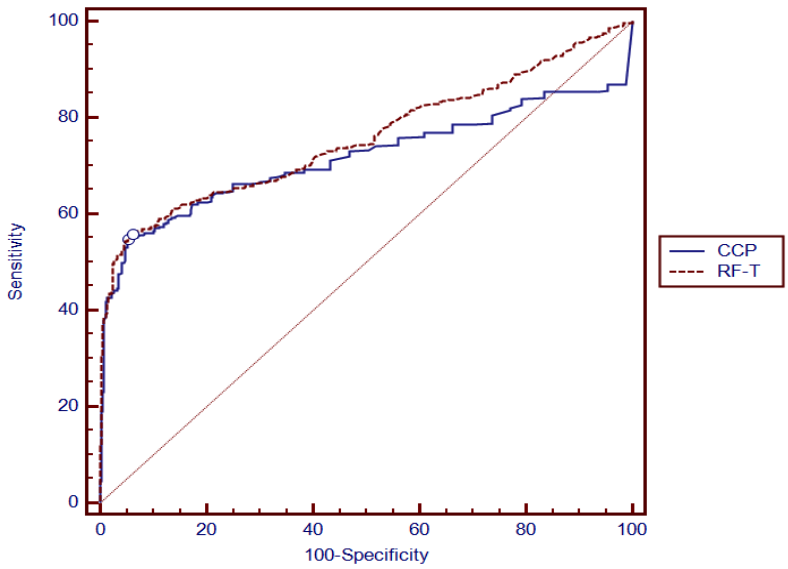

Figure 3. Receiver operating characteristic curve (ROC) of anti-CCP and RF-T

DISCUSSION

Diagnosis of RA is a clinical decision based on several diagnostic criteria. Since RA is a progressive and disabling disease, an early diagnosis provides a crucial role in choosing suitable treatment and reducing morbidities. Despite the progress in serological detection of RA by anti-CCP antibodies, the prevalence and clinical value of RF isotypes have not been fully revealed. Rheumatoid factor usually refers as an RF-IgM but other isotypes also may be detected in RA patients. In the present study we analyzed the frequency and clinical value of anti-CCP, RF-T and RF isotypes in a large number of Iranian patients with RA and evaluated their relationship with inflammatory parameters and disease activity.

The female/male ratio in RA patients was 8.7/1. Most of the studies, mentioned the 3/1 ratio of female/male in RA patients.[15] The mean age of disease onset was 39.87±12.8 years which showed that RA affecting Iranian people in third and forth decades of life.

Most of the researchers, evaluated the anti-CCP and RF-IgM in RA patients. In the present study, we assayed RF-T and also its isotypes in addition to anti-CCP antibody in a large number of RA patients. RF-T detects all RF isotypes together and reduces the risk of missing patients with uncommon RF isotypes. Anti-CCP antibody and RF-T were positive in 53.2% and 61.87% of patients, respectively. In control group, anti-CCP was positive in 4.7% and RF-T was positive in 17.66% of cases. Higher frequency of RF-T in control group indicated its higher sensitivity and lower specificity than anti-CCP. RF-T was present in 86% of patients with positive anti-CCP and 34.18% of patients with negative anti-CCP. These data showed that most of the anti-CCP positive patients were also positive for RF-T. The prevalence of anti-CCP antibody among

different populations is variable. Sockalingam et al. reported that 80.4% of Malaysian RA patients were positive for anti-CCP.[16] Sihvonen et al. found anti-CCP antibody in 66% of RA patients.[17] Jaskowski et al. reported the presence of anti-CCP in 65% of RA patients and 1% in healthy people.[18] This discrepancy may be due to different assays and cut off levels and also racial and genetic backgrounds.[19] The information about RF-T prevalence in RA patients is rare and as mentioned before, most of the studies assayed RF-IgM or other RF classes.

In our study, the frequency of both anti-CCP and RF-T was significantly higher in RA patients with high active disease compared to patients with remission and low active disease. Anti-CCP titer was not different in patients with different disease activity but RF-T titer was significantly higher in patients with active disease versus patients with remission and low active disease. Anti-CCP titer was correlated with RF-T titer (r=0.5, *p* *p*=0.000). The titer of both anti-CCP antibody and RF-T was correlated with CRP level and number of swollen joints in RA patients. This observation is very interesting, because joint swelling is a major sign of disease activity in RA patients. DAS28-CRP scores calculated using multiple parameters and the lower score of other parameters than the number of swollen joints can change the score. The RF-T positive patients had longer disease duration and lower age of disease onset. These findings suggested that the presence of RF may decrease the age of RA manifestations. Moreover, RF-T positive patients showed a higher level of ESR, CRP, number of swollen joints and DAS28-CRP score. It seems that the presence of RF is not just a marker but is associated with inflammatory markers even more than anti-CCP. We also analyzed the titer of anti-CCP and RF-T in RA patients with different disease activity. There was no significant change of anti-CCP antibody titer between patients with different disease activity but patients with high active disease had higher titer of RF-T compared to patients with remission and low active disease. In previous studies, Greiner et al. reported significant correlation between anti-CCP antibody and RF-IgM titer but no correlation between anti-CCP antibody titer with CRP and ESR.[20] Del Val del Amo et al. showed that the frequency of anti-CCP antibody in patients with high active disease was higher than other groups but there was not the same result for RF. They also reported that patients in remission had lower anti-CCP antibody titer than those with low, moderate or high disease activity.[21] In another study from Turkish population, there was a significant difference between anti-CCP positive and anti-CCP negative RA patients for RF, and no significant correlation between anti-CCP antibody and ESR, CRP, VAS, DAS 28 and significant correlation between RF and anti-CCP antibody was reported.[22] Ates et al. found the frequency of RF-T in 80.6% of RA patients but RF-T titer was not different between patients with active and inactive disease.[23]

In our study, the frequency of RF isotypes in RA patients was 46.52% for RF-IgM, 23.47% for RF-IgA and 21.74% for RF-IgG. 21.9% of patients were positive for all 3 RF isotypes and none of controls had the 3 RF isotypes. The single positivity of RF-IgG was not detected in RA patients. Among all 3 RF isotypes, only the frequency of RF-IgA was higher in patients with active disease compared to low active disease group. We first analyzed the sensitivity and specificity of anti-CCP, RF-T and RF isotypes. Then we evaluated the combination of anti-CCP and RF isotypes sensitivity and specificity Among RF isotypes, RF-IgG had higher specificity (99.4%) but lower sensitivity (21.7%). We found that specificity was increased when the combination of anti-CCP antibodies with RF-T or RF isotypes was used. In previous studies, the results of prevalence, sensitivity and specificity of RF isotypes were controversial. In Turkish population, it has been shown that RF-IgA is associated with severe joint damage in RA patients and its elevated level could be a marker of erosive disease.[23] In American African population, the sensitivity of all 3 RF-isotypes was higher than our study. Mikuls et al. reported that sensitivities were 70% for RF-IgM, 65% for RF-IgA and 39% for RF-IgG in RA patients and RF-IgA showed the highest specificity (94%).[24] In Troy et al. study, most of the RA patients (44%), had all 3 RF isotypes. The frequency of RF-IgM+, RF-IgA-, RF-IgG- pattern was 12%.[18] The common observation among most of the studies is that the presence of RF isotypes increases diagnostic specificity and decreases sensitivity. Based on this fact, we suggest that both anti-CCP and RF-T are suitable markers for screening purposes, and RF isotypes can be assayed as confirmatory tests in RA patients.

This study has demonstrated that in addition to anti-CCP antibody, RF-T was a suitable marker for RA diagnosis with moderate sensitivity and high specificity. We also determined the profile of major RF

isotypes in Iranian RA patients for the first time. The association of RF-T with lower age of onset and also the number of swollen joints in RA patients may be a predictive value for development of erosive joint lesions. We finally recommended using the combination of these different types of autoantibodies in clinical practice for better diagnostic or even therapeutic options.

ACKNOWLEDGEMENTS

We thank research deputy management of Tehran University of Medical Sciences for assistance and financial support.

REFERENCES

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011; 365(23):2205-19.
2. Song YW, Kang EH. Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies. QJM 2010; 103(3):139-46.
3. Lee AN, Beck CE, Hall M. Rheumatoid factor and anti-CCP autoantibodies in rheumatoid arthritis: a review. Clin Lab Sci 2008; 21(1):15-8.
4. Conrad K, Roggenbuck D, Reinhold D, Dörner T. Profiling of rheumatoid arthritis associated autoantibodies. Autoimmun Rev 2010; 9(6):431-5.
5. Vencovský J, Machácek S, Sedová L, Kafková J, Gatterová J, Pesáková V, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 2003; 62(5):427-30.
6. Berglin E, Johansson T, Sundin U, Jidell E, Wadell G, Hallmans G, et al. Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset. Ann Rheum Dis 2006; 65(4):453-8.
7. Lindqvist E, Eberhardt K, Bendtzen K, Heinegård D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis 2005; 64(2):196-201.
8. Zeng X, Ai M, Tian X, Gan X, Shi Y, Song Q, et al. Diagnostic value of anti-cyclic citrullinated Peptide antibody in patients with rheumatoid arthritis. J Rheumatol 2003; 30(7):1451-5.
9. Heidari B, Firouzjahi A, Heidari P, Hajian K. The prevalence and diagnostic performance of anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: the predictive and discriminative ability of serum antibody level in recognizing rheumatoid arthritis. Ann Saudi Med 2009, 29(6):467-70.
10. Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003; 48(10):2741-9.
11. Singwe-Ngandeu M, Finckh A, Bas S, Tiercy JM, Gabay C. Diagnostic value of anti-cyclic citrullinated peptides and association with HLA-DRB1 shared epitope alleles in African rheumatoid arthritis patients. Arthritis Res Ther 2010; 12(2):R36.
12. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;. 62(9):2569-81.
13. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315-24.
14. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39(1):34-40.
15. van Vollenhoven, RF. Sex differences in rheumatoid arthritis: more than meets the eye. BMC Med, 2009; 7:12.
16. Sockalingam S, Khuan CS, Sthaneshwar P. Prevalence of anti cyclic citrullinated peptide antibodies in Malaysian rheumatoid arthritis patients and its correlation with disease activity. Int J Rheum Dis 2009; 12(3):211-5.
17. Sihvonen S, Korpela M, Mustila A, Mustonen J. The predictive value of rheumatoid factor isotypes, anti-cyclic citrullinated peptide antibodies, and antineutrophil cytoplasmic antibodies for mortality in patients with rheumatoid arthritis. J Rheumatol 2005; 32(11):2089-94.
18. Jaskowski TD, Hill HR, Russo KL, Lakos G, Szekanecz Z, Teodorescu M. Relationship between rheumatoid factor isotypes and IgG anti-cyclic citrullinated peptide antibodies. J Rheumatol 2010; 37(8):1582-8.
19. Mimori T. Clinical significance of anti-CCP antibodies in rheumatoid arthritis. Intern Med 2005; 44(11):1122-6.
20. Greiner A, Plischke H, Kellner H, Gruber R. Association of anti-cyclic citrullinated peptide antibodies, anti-citrullin antibodies, and IgM and IgA rheumatoid factors with serological parameters of disease activity in rheumatoid arthritis. Ann N Y Acad Sci 2005; 1050:295-303.

21. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011; 365(23):2205-19.
22. del Val del Amo N, Ibanez Bosch R, Fito Manteca C, Gutierrez Polo R, Loza Cortina E. Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. Clin Exp Rheumatol 2006; 24(3):281-6.
23. Serdaroğlu M, Cakirbay H, Değer O, Cengiz S, Kul S. The association of anti-CCP antibodies with disease activity in rheumatoid arthritis. Rheumatol Int 2008; 28(10):965-70.
24. Ateş A, Kinikli G, Turgay M, Akay G, Tokgöz G. Effects of rheumatoid factor isotypes on disease activity and severity in patients with rheumatoid arthritis: a comparative study. Clin Rheumatol 2007; 26(4):538-45.
25. Mikuls TR, Holers VM, Parrish L, Kuhn KA, Conn DL, Gilkeson G, et al. Anti-cyclic citrullinated peptide antibody and rheumatoid factor isotypes in African Americans with early rheumatoid arthritis. Arthritis Rheum 2006; 54(9):3057-9.