ORIGINAL ARTICLE

The association of immunoglobulin A, immunoglobulin G and anti-cyclic citrullinated peptide antibodies with disease activity in seronegative rheumatoid arthritis patients

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Background: Rheumatoid arthritis (RA) is a common autoimmune disease that is associated with progressive disability, systemic complications, and early death. The present study was aimed to investigate the level of immunoglobulin G (IgG) and IgA isotypes and anti-cyclic citrullinated peptide (CCP) antibody and to assess their association with disease severity based on disease activity score (DAS-28) in patients with IgM rheumatoid factor (IgM-RF) negative RA. **Materials and Methods:** In this cross-sectional study, 62 RA patients with IgM-RF negative were assessed. Radiographs were obtained for all RA patients. The RF (IgG, and IgA) and anti-CCP were measured by using the enzyme-linked immunosorbent assay. Values of cut-off points over 15 UI/mL for IgA IgA-RF, 20 UI/mL for IgG-RF and over 20 units for anti-CCP were considered positive. DAS-28 score was compared in regard to the IgA-RF and IgG-RF and anti-CCP positivity using Mann–Whitney test. **Results:** DAS-28 score in IgA-RF positive was significantly higher than IgA-RF negative (mean score, 6.03 ± 0.33 vs. 5.44 ± 0.76 respectively, $P = 0.035$). In IgG-RF positive patients, DAS-28 score was similar to patients with IgG-RF negative (5.64 ± 0.59 vs. 5.46 ± 0.78 respectively, $P = 0.396$). Furthermore, in patients with anti-CCP positive DAS-28 score was significantly higher than patients with anti-CCP negative (5.72 ± 0.61 vs. 5.38 ± 0.79 respectively, $P = 0.049$). **Conclusion:** Findings indicated that there was a significant association between the amounts of IgA and anti-CCP with severity of disease in RF negative RA patients while there was no significant association between the amounts of IgG and severity of RA disease.

Key words: Disease activity score-28, immunoglobulin A, immunoglobulin G, rheumatoid factor, seronegative rheumatoid arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic, systemic and inflammatory autoimmune malady that causes symmetrical polyarthritis of joints.[1-3] RA in 2010 resulted in about 49,000 deaths worldwide.[4] It has long been recognized that women are more commonly in risk of RA[5] and are affected 2-4 times more than men.[6] The disease is generally starts in women between 40 and 50 years of age, while in men is rather later.[7] The incidence of RA increases with age, but the peak incidence occurs at 60-70 years.[6] Immunological studies such as testing for the presence of serum rheumatoid factor (RF) are needed for diagnosis of RA.[8] Several studies have investigated the immunoglobulin (Ig) class of RF in seronegative and seropositive RA patients.[9,10] Naka *et al*. have indicated the efficacy of consecutive test of serum IgG-RF level in RA diagnosis and suggested that this test could be effectively used in daily handling of RA patients.[11] In seronegative cases that usually include 15% of patients, the negative RF does not rule out RA.[12] Being negative during the early stages of the disease, observability in a wide range of rheumatic and non-rheumatic cases and being positive in almost 10% of the healthy individuals have indicated that RF test is not exact enough.[13] One of the antibodies mostly elevated in patients with RA, with a specificity of 95-98% among patients meeting the criteria for RA is anti-cyclic citrullinated peptide (anti-CCP).[14-16] High-specificity and early-phase presence of anti-CCP antibodies have changed this test as of the most important clinical assays. Anti-CCP antibody also in cases with chronic hepatitis C that have RF and some rheumatic symptoms is scarcely seen.[17-19] Although autoantibodies in RA are helpful both for prognosis and diagnosis, but their role in RF negative individuals and its association with disease severity is not clearly understood. So in the present study we aimed to investigate the level of IgG and IgA isotypes and anti-CCP antibody and to assess their association with disease severity based on disease activity score (DAS-28) in patients with IgM-RF negative RA, who referred to

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MATERIALS AND METHODS

The present study was a cross-sectional study which was conducted between January, and May, 2013, on 62 RA patients with RF negative who were being evaluated at the Rheumatology Arthritis Clinic of Noor and Aliasghar Hospital in Isfahan, Iran. All studied patients were early diagnosed active RA, not have been under treatment of disease-modifying anti-rheumatic drugs or corticosteroids or anti-inflammatory dose of non-steroidal anti-inflammatory drugs. Eligibility was define as age older than 18 years old in both gender, with RA based on the American College of Rheumatology criteria (2010) and no history of chronic disease. Furthermore, pregnant women's and patients who were unable to give valid consent were excluded from the study. The ethics committee of Isfahan University of Medical Sciences investigates and approves this study, and written informed consent was obtained from all studied patients.

Collected data included age, sex combination, duration of disease, DAS-28 score, extra-articular manifestations, IgA-RF, IgG-RF and anti-CCP positive which were assessed in all patients. DAS-28 was used to assess the activity of RA (tender and swollen joint count [0-28], patients' assessment of disease activity [0-100 mm] and erythrocyte sedimentation rates). Also, based on information in serological tests for RF all studied patients were negative for IgM-RF.

The RF (IgG, and IgA) and anti-CCP were measured by using an enzyme-linked immunosorbent assay (ELISA), according to the producer protocol. Values of cut-off points over 15 UI/mL for IgA-RF and 20 UI/mL for IgG-RF were considered positive. Also, value of cut-off points over 20 20 for anti-CCP units considered positive.

The sample size was calculated using an estimate of proportion formula which prevalence of negative RF in RA patients previously reported around 20%, with two-sided log-rank test, α =0.05, and 80% power. All statistical analyses were done using statistical package for the social sciences software for windows, version 20 (SPSS,. Chicago, IL, USA). Descriptive data are reported as mean ± standard deviation, median interquartile range or number (%) as appropriate. Mann–Whitney test was used to comparing all studied variables between patients in regard to the IgA-RF and IgG-RF and anti-CCP positivity. The level of significance is considered to be <0.05.

RESULTS

Sixty-two IgM-RF negative patients with RA (59 women and 3 men) entered the study. The mean age of patients was 53.4 ± 11.8 years, with an age range of 23-78 years old. Table 1 shows the characteristics of the 62 patients with RF at baseline. Median disease duration in patients was 24 months. Mean of DAS-28 score in patients was 5.49 ± 0.75. Of the patients with RF, 10 patients (16.1%) had extra-articular manifestations. As to the serology, five of patients (8.1%) were positive for IgA RF, nine (14.5%) were positive for IgG and 20 of patients (32.3%) were positive for anti-CCP.

Disease activity score-28 score was compared in regard to the IgA-RF and IgG-RF and anti-CCP positivity using Mann–Whitney test and findings are shown in Table 2. DAS-28 score in IgA-RF positive was significantly higher

Table 1: Characteristics of the 62 patients with RF at baseline

Characteristics	Statistics
Age (year)	53.4±11.8
Gender (female/male)	59/3
Disease duration (month)	24 (6.75-24)
DAS-28 score	5.49±0.75
Swollen joint count (0-28)	7 (6-9)
Tender joint count (0-28)	10 (8-12)
ESR (mm/h)	28.5 (13.75-39.25)
VAS disease activity (0-100 mm)	50 (50-60)
Extra-articular manifestations	
Vasculitis	0
Subcutaneous nodules	1 (1.6)
Lung involvement	0
Anemia	8 (12.9)
Sjogren's syndrome	1 (1.6)
IgA level	12.4 (8.3-16.5)
IgA positive	5 (8.1)
IgG level	12.3 (8.7-16.8)
IgG positive	9 (14.5)
Anti-CCP level	8 (5.1-67.5)
Anti-CCP positivity	20 (32.3)

Data are mean±SD, median (IQR), *n* (%). Anti-CCP=Anti-cyclic citrullinated peptide antibodies; ESR=Erythrocyte sedimentation rate; SD=Standard deviation; RF=Rheumatoid factor; DAS=Disease activity score; VAS=Visual analogue scale; IQR=Interquartile range

Table 2: Comparison of DAS-28 of 62 RF negative patients with rheumatoid arthritis

Characteristics	DAS-28		*P*
	Positive (*n*=5)	Negative (*n*=57)	
IgA	6.03±0.33	5.44±0.76	0.035
	5.99 (5.77-6.31)	5.45 (5.00-5.89)	
	Positive (*n*=9)	Negative (*n*=53)	
IgG	5.64±0.59	5.46±0.78	0.396
	5.63 (5.35-5.93)	5.50 (5.00-5.94)	
	Positive (*n*=20)	Negative (*n*=42)	
Anti-CCP	5.72±0.61	5.38±0.79	0.049
	5.77 (5.41-6.04)	5.34 (4.89-5.83)	

Data are mean±SD and median (IQR). *P* values derived from Mann-Whitney. DAS=Disease activity score; VAS=Visual analogue scale; IQR=Interquartile range; SD=Standard deviation; RF=Rheumatoid factor; Anti-CCP=Anti-cyclic citrullinated peptide antibodies

than IgA-RF negative (mean score, 6.03 vs. 5.44 respectively, $P = 0.035$). In IgG-RF positive patients, DAS-28 score was similar to patients with IgG-RF negative (mean score, 5.64 vs. 5.46 respectively, $P = 0.396$). Also, in patients with anti-CCP positive DAS-28 score was significantly higher than patients with anti-CCP negative (mean score, 5.72 vs. 5.38 respectively, $P = 0.049$).

DISCUSSION

Autoantibodies and their role as effective agents in prognosis and diagnosis of RA are well described. However, our knowledge in IgM-RF negative patients and its association with disease severity is not fully understood. This study sought to examine the level of IgG and IgA isotypes and anti-CCP antibody and to assess their association with disease severity based on DAS-28 in individuals with RF negative RA.

In our study we have found that there is a significant association between the amounts of IgA with severity of disease in RF negative RA individuals, while we could not find any significant association between the amounts of IgG and severity of disease. In accordance to our finding Teitsson indicated that IgA-RF can be a more specific prognosticator of disease severity than IgM-RF or IgG-RF.[20]

Rantapaa-Dahlqvist *et al*.[21] in a case control study 67 patients with RA were assessed and the prevalence of autoantibodies in studied patients was 33.7% for anti-CCP, 16.9% for IgG-RF, and 33.7% for IgA-RF which all were highly significant compared with controls. Also, authors in this study revealed that the presence of anti-CCP and IgA-RF predicted the development of RA, with anti-CCP antibody having the highest predictive value. In the present study the prevalence of autoantibodies in RF negative RA patients was 32.3% for anti-CCP, 14.5% for IgG-RF, and 8.1% for IgA-RF. Although studied patients in these two studies were different, (patients with RA in Rantapaa-Dahlqvist study and RF negative RA patients in the present study) the prevalence of anti-CCP and IgG-RF were similar, but the prevalence of IgA-RF in our study was lower than Rantapaa-Dahlqvist study.

In a study that assayed the IgA-RF, using ELISA in 114 sera of patients with active RA, it has been indicated that determining of IgA-RF could be practically applied in the formation of the harsh course of RA with systemic damages.[22] Our results is also indicated a significant association between IgA-RF and severity of disease. Moreover, some studies have explained that the combined increase in IgM and IgA-RF, with or without IgG-RF, is the most frequent RF pattern found in RA individuals.[23-25] This shows that IgG-RF has little effect on the severity of disease that is in accordance with our results.

We found that there is a statistically significant association between the amounts of serum anti-CCP antibodies and severity of disease in RF negative RA patients. Similar to our result in a study that assessed the predictive value of anti-CCP antibody in RA patients it have been suggested that in anti-CCP–positive individuals severe radiologic damage is significantly more than cases with anti-CCP negative antibody.[26] Also in a study conducted upon 150 long-standing RA patients there was intense correlation founded between more disease activity and anti-CCP positivity that is somewhat keeping with our finding.[7] In a cross-sectional study by Karimifar *et al.*,[27] 90 patients with RA were assessed for evaluating the relation between anti-CCP1 antibody titer and DAS-28. Authors reported meaningful difference in DAS-28 between two groups of anti-CCP1 positive and negative patients 5.07 ± 1.1 and 3.5 ± 1.5 respectively ($P < 0.001$). Also, in another study reported that a significant difference between anti-CCP positive and anti-CCP negative RA patients was found for RF.[28] Similarly, in the present study in patients with anti-CCP positive DAS-28 score was significantly higher than patients with anti-CCP negative (5.72 ± 0.61 vs. 5.38 ± 0.79 respectively, $P = 0.049$).

One of the limitations toward this study was the lack of literatures regarding association of autoantibodies with severity of disease especially in RF negative RA patients that could hinder useful intervention in practice. Also, designed of this study (cross-sectional design) and a small number of patients (which makes it difficult to stratify into subgroups) are the other limitations of our study, so the results need to be confirmed in other studies. Therefore, future studies with appropriate sample size are necessary to specifically assess association between the amounts of IgA, IgG and anti-CCP with severity of disease in RF negative RA patients.

CONCLUSION

Our findings indicated that there was a significant association between the amounts of IgA and anti-CCP with severity of disease in RF negative RA patients while there was no significant association between the amounts of IgG and severity of RA disease.

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AUTHORS' CONTRIBUTION

MK and HM carried out the design and coordinated the study, participated in most of the experiments and prepared the manuscript. MB provided assistance in the design of

the study, coordinated and carried out all the experiments and participated in manuscript preparation. MA provided assistance in the design of the study,analysis and manuscript preparation. All authors have read and approved the content of the manuscript.

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