Rheumatol Int (2013) 33:939–942
DOI 10.1007/s00296-012-2458-5

ORIGINAL ARTICLE

The frequency of antibodies against cyclic citrullinated peptides and rheumatoid factor in healthy population: a field study of rheumatoid arthritis from northern turkey

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Received: 13 January 2012 / Accepted: 7 July 2012 / Published online: 25 July 2012
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Abstract Rheumatoid arthritis (RA) is a chronic, systemic and an autoimmune disease characterized by inflammation of the synovial membrane that affects approximately 1 % of the total world population. Rheumatoid factor (RF) is a widely used auto antibody in diagnosis of the RA and found positive in 50–80 % of the patients but with a lower specificity. On the other hand, anti-cyclic citrullinated peptide (anti-CCP) is the latest serological marker with a specificity around 98 %. This field survey was conducted in different regions to investigate the frequency of RF and anti-CCP and also frequency of RA in a northern province of Turkey. This study was conducted in 70 local areas (12 urban and 58 rural) in the province of Tokat, which is located in northern Turkey. The population of Tokat was reported to be 828,000 at the last census and about 530,000 individuals aged > 18years old. The study population of 941 subjects (462 male and 479 female; urban 501 and rural 440) was selected by random sampling method among 530,000 individuals. Of the 941 healthy controls assigned to the study, 479 of them were female (51 %) and 462 of them were males (49 %), and median age of all participants was 41 ± 17. Twenty-six subjects were RF positive (2.8 %), and 9 patients were anti-CCP positive (1 %). The presence of both RF and anti-CCP antibodies has also been shown in two patients (0.2 %). In conclusion, we demonstrated that the frequency of RA was 0.53 %, RF presence was 2.8 %, and anti-CCP presence was 1 % in total 941 healthy subjects enrolled into study.

Keywords Rheumatoid arthritis · Field study · Rheumatoid factor · Anti-cyclic citrullinated peptid

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic and an autoimmune disease characterized by inflammation of the synovial membrane that affects approximately 1 % of the total world population [1, 2]. Early diagnosis and treatment is vitally important in RA to prevent possible joint deformity that may be seen in the further stages of the diseases [3]. Rheumatoid factor (RF) is a widely used auto antibody in diagnosis of the RA and found positive in 50–80 % of the patients but with a lower specificity. Higher levels of RF have been found in other rheumatologic and some infectious diseases, and it increases with age [4, 5].

On the other hand, anti-cyclic citrullinated peptide (anti-CCP) is the latest serological marker with a specificity around 98 %. Anti-CCP would be present in the early

stage, and its positivity can provide prognostic information on the severity of the disease [6]. Besides that, anti-CCP positivity is placed in the new RA diagnosis criteria of the American Collage of Rheumatology (ACR) in 2010 [7].

Up-to-date, there are several studies conducted to investigate the frequency of RF and anti-CCP among the healthy controls but the blood samples were collected from the people admitted to the hospitals and blood banks. This field survey was conducted in different regions to investigate the frequency of RF and anti-CCP and also frequency of rheumatoid arthritis in a northern province of Turkey.

Materials and method

This study was conducted in 70 local areas (12 urban and 58 rural) in the province of Tokat, which is located in northern Turkey. The population of Tokat was reported to be 828,000 at the last census and about 530,000 individuals aged $>$ 18 years old (http://www.die.gov.tr). All urban regions and some of the rural regions selected by the cluster sampling method were included in the study. The study population of 941 subjects (462 male and 479 female; urban 501 and rural 440) was selected by random sampling method among 530,000 individuals. The number of individuals to be screened in each district was determined through the proportion of the district to the total population of all districts of Tokat. Consecutive houses in randomly selected streets in each district were visited until the target number of individuals had been interviewed with a standard questionnaire. Informed consent was obtained from all patients by a questionnaire at the time of enrollment, and the study protocol was approved by the Ethics Committee of Gaziosmanpasa University.

All subjects assigned into the study were questioned for the presence of RA by an internist. After a physical examination performed for all participants, serum blood samples were obtained from all subjects and stored at -80 °C until analysis was performed. All serum samples were obtained during morning hours. RF was measured with the Nephelometer, which detects IgM-RF, and the normal range was between 0 and 20 IU/ml. Anti-CCP was measured using enzyme-linked immunosorbent (ELISA) that is including the second generation anti-CCP antibody assays (anti-CCP2), and manufacturer's cut off for positivity was $\leq$25 U/ml. The subjects with positive RF and anti-CCP were invited to hospital for further investigation. The patients were diagnosed in accordance with 1987 ACR diagnosis criteria for RA [8].

The Kolmogorov–Smirnov normality test was performed to measure the normal distribution of constant variables used in the study. Accordingly, in order to compare the constant variables of two groups, we have used independent two-sample t test. All variables demonstrated with $\pm$ standard deviation. Categorical variables of all groups were compared with chi-square test, and we have used (n) for numbers and (%) for the percentage. Possible relationships between the values were detected using the Pearson and Spearman correlation analyses, and p values below 0.05 were accepted as statistically significant.

Results

Of the 941 healthy controls assigned to the study, 479 of them were female (51 %), 462 of them were males (49 %) and median age of all participants was 41 $\pm$ 17. Demographic features of participants are demonstrated in Table 1. Twenty-six subjects were RF positive (2.8 %), and 9 patients were anti-CCP positive (1 %). The presence of both RF and anti-CCP antibodies has also been shown in two patients (0.2 %). When we evaluated those subjects with RF, after a detailed physical examination, of the 26 individuals, 3 participants were diagnosed with RA. Two of the three patients diagnosed with RA were also anti-CCP positive. Similarly, when we have evaluated the 9 anti-CCP-positive participants, 4 of them were diagnosed with RA following the physical examination. In total, 5 patients out of 941 participants were diagnosed with RA, and 3 of the patients were diagnosed before and having RA treatment during the detection period, while two patients were without diagnosis and treatment.

We identified 51 HBsAg-positive, and 20 anti-HCV-positive patients out of 941 participants. Of the 51 HBsAg-positive patients, 3 of them had concurrent RF positivity and none of the patients had anti-CCP positivity. Only patients from the group of 20 anti-HCV-positive patients had concurrent RF presence, and again none of the patients had anti-CCP positivity. Our analyses revealed that there is no a significant difference and correlation between serological markers including RF and anti-CCP and hepatitis markers like HBsAg and anti-HCV.

Median ages of the total subjects assigned to the study was 41 $\pm$ 17, and it was 52 $\pm$ 18 years in RF-positive group and 52 $\pm$ 14 years in anti-CCP-positive group. There was a positive and statistically significant relationship between age and RF and anti-CCP positivity (respectively, $r = 0.111$, $p = 0.001$; $r = 0.066$, $p = 0.044$). Gender-based serological analyses revealed that RF was present in 17 (3.5 %) and anti-CCP were present in 4 female participants (0.8 %). On the other hand, RF found positive in (1.9 %) and anti-CCP found positive in 5 male participants (0.8 %) among 462 total male participants. Concerning the smoking history, 559 never smoked, 96 quitted and 285 participants were already smoking. In other words, smoking did not have a significant

Table 1 Demographic characteristics of the 941 individuals

	n (%)
Sex	
Female	479 (51)
Male	462 (49)
Residence	
Rural	440 (46.7)
Urban	501 (53.3)
Age distributions	
18–29 years	318 (33.7)
30–39 years	212 (22.5)
40–49 years	151 (16)
50–59 years	121 (12.8)
60–69 years	96 (10.2)
≥70	39 (4.1)
RF positivity	26 (2.8)
Anti-CCP positivity	9 (1)
RF and anti-CCP positivity	2 (0.2)

effect on anti-CCP antibody or RF presence (respectively, $p = 0.058$ and $p = 0.435$).

Discussion

Although anti-CCP is a recent and widely accepted serological marker, there are limited epidemiological studies conducted. To the best of knowledge, this is the first field study that evaluates the frequency of the anti-CCP positivity among the population. We demonstrated that the frequency of RA was 0.53 %, RF presence was 2.8 % and anti-CCP presence was 1 % in total 941 healthy subjects enrolled into study.

Excluding the endemic differences, RA affects almost 1 % of the total world population. Prevalence studies held in Turkey demonstrated a range of percentage between 0.32 and 1 % [9–12]. Similarly, we have found the RA prevalence as 0.53 %, and it was in parallel with the findings of the previous prevalence studies held in our country.

Most of the recent studies revealed that early diagnosis and treatment would prevent the joint deformity and functional limitations. Because of the progressive nature of the diseases, early diagnosis is vitally important for the patients [13]. RF is relatively old, and for decades, it has been used in the diagnosis of RA. With a high sensitivity (50–80 %) and insufficient specificity, RF could be present in other inflammatory diseases [5].

Anti-CCP as a recent serological marker has been viewed as highly specific to RA (95–98 %) but with a similar sensitivity as RF (70–80 %). Although anti-CCP is specific for the patients with RA, it can be positive in other diseases including tuberculosis (37 %), systemic lupus erythematosus (17 %), Sjögren syndrome (7.5 %), scleroderma (10.6 %) and polymyositis or dermatomyositis (14 %) [14–18]. In our analyses, we have underestimated the RF and anti-CCP presence in other diseases like viral hepatitis and rheumatic diseases; we have found that RF presence was 2.8 % and anti-CCP presence was 1 % in total 941 healthy subjects.

There is limited number of field studies detecting the RF and anti-CCP presence among the healthy subjects. Most of the studies focused on the specificity and sensitivity of RF and anti-CCP for the RA and healthy controls were chosen from blood donors. In one of the recent study, Li et al. investigated RA and anti-CCP positivity in a total of 1,018 healthy donors, 212 patients with RA, and 435 patients with other connective tissue disease, and they found out that anti-CCP was present in 2.6 % and RF was present in 21.5 % of the healthy donors [19]. Goeldner et al. have shown that RF was positive in 6 % and anti-CCP was positive in 1 % of the total 100 healthy controls [20]. In a systematic analysis of the literature conducted by Avouac et al., it is found out that anti-CCP was positive in 6 (0.4 %) out of 1,561 healthy controls [21]. Our findings concerning the RF presence were relatively low (2.8 %) in contrast to the other studies conducted in other countries. It is thought that the differences could be resulted due to geographic differences, median age of the participants and endemic differences of other inflammatory diseases that effects RF formation. However, our findings concerning the anti-CCP positivity were in parallel with the findings of those studies (1 %). The frequencies of RF and anti-cyclic citrullinated peptide in previous studies are shown in Table 2.

According to the gender-based analyses of RA, women are experiencing two or three times more cases of the disease than men [1]. In our study, RF positivity of females was 3.5 % and RF positivity of males was 1.9 %, but the difference was not statistically significant. While RF

Table 2 Frequency of rheumatoid factor and anti-cyclic citrullinated peptide in previous studies

Author	Population	RF positivity	Anti-CCP positivity
Li et al. [19]	Healthy donors ($n = 1,018$)	21.5 %	2.6 %
Goeldner et al. [20]	Healthy controls ($n = 100$)	6 %	1 %
Avouac et al. [21]	Literature review ($n = 1,561$)	NA	0.4 %
Our study	Field study ($n = 941$)	2.8 %	1 %

presence was 2–10 % among the healthy controls, it is found 20 % among the individuals with 65 years and older [22]. Studies have shown a link between cigarette smoking and RA and smoking accepted to be a risk factor for RA [1]. Also there are studies demonstrating the association between smoking and RF positivity [23]. However, in our study, there was not a statistically significant difference concerning the RF and anti-CCP presence between the participants with or without smoking history.

In conclusion, we demonstrated that the frequency of RA was 0.53 %, RF presence was 2.8 % and anti-CCP presence was 1 % in total 941 healthy subjects enrolled into study. There was a positive correlation between age and RF positivity but not anti-CCP. Considering the endemic differences, there is a need for the further field studies covering different geographic locations with larger subjects to verify our findings.

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