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Association of Interleukin-10 Genotypes and Anticyclic Citrullinated Peptide Antibodies with Rheumatoid Arthritis

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Abstract

Background: According to recent evidence, there is an association between some genetic factors and rheumatoid arthritis (RA). The aim of this study was to determine whether genetic variations in the interleukin 10 (IL10) and anti-cyclic citrullinated peptide (Anti-CCP) antibody loci were linked to RA.

Methods: In this hospital-based case-control study with 224 cases and 194 healthy individuals, we investigated the association of IL-10 genotypes and anti-CCP antibodies with RA. Independent sample t, chi-square, and Fisher exact tests were used to assess the association between study variables.

Results: Frequency of IL-10 -1082 A/G genotype in RA patients is significantly higher than the control group (odds ratio [OR], 1.67 [95% CI, 1.11-2.51]) (p=0.009), while the frequency of IL-10-1082 A/A and G/G polymorphisms in RA patients was lower than controls and this finding for G/G polymorphism was statistically significant (p=0.01). No significant difference was observed between the 2 studied groups regarding IL-10-592 C/C, C/A, and A/A polymorphisms (p>0.05). The chance of RA occurrence among persons with positive anti-CCP was significantly (63.3 times [22.7-176.5]) higher than individuals with negative anti-CCP (p<0.001).

Conclusion: According to our data, the chance of anti-CCP positivity in persons who have IL-10 genotype 1082 A/G is higher. Further studies are recommended to determine the relationship between IL-10 genotype 1082 G/A and RA. If such a relationship is proven, this finding as a diagnostic clue can help rheumatologists in the early detection of RA.

Keywords: Interleukin-10, Anti-Cyclic Citrullinated Peptide Antibodies, Rheumatoid Arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is characterized by inflammation and destruction of the joint (1). This condition as the most common chronic inflammatory disease of the joints is highly important, as it is associated with the involvem ent of other organs (2). A wide range of preinflammatory cytokines and secreting inflammatory cells, such as monocytes, lymphocytes, and macrophages, are involved in the pathogenesis of RA (3). Based on the existing evidence, cytokine levels, such as IL-1, IL-6, and TNF-α, are increased in the joints and blood and will cause disease and destruction of joints' cartilage (4,

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↑What is "already known" in this topic:
Interleukin 6 (IL-6) as a pleiotropic cytokine has a central role in the pathophysiology of rheumatoid arthritis (RA). In patients with RA, a wide range of autoantibodies, such as anti-IgG (RF) and cyclic citrullinated peptide antibodies (Anti-CCP), are produced. In comparison with other autoantibodies, anti-CCP is a more sensitive and specific marker and can be associated with the severity of disease and cartilage degradation.

→What this article adds:
Our study revealed that the chance of anti-CCP positivity in persons who have IL-10 genotype 1082 A/G is higher. If this finding is approved by further studies, anti-CCP positivity can be used as a predictor factor for early detection of RA.

5). Interleukin 6 (IL-6) as a pleiotropic cytokine has a central role in the pathophysiology of RA. Interleukin 10 (IL-10) is an immunomodulatory cytokine that plays a pivotal role in the pathogenesis of autoimmune diseases. According to the existing evidence, IL-10 genotypes are immunoregulator cytokines, which can increase in patients with RA and it appears to be caused by genetic variants (6). It seems that the increase in IL-10 is a response to inflammatory and destructive cytokines. In fact, IL-10 decreases the effects of inflammatory cytokines and can prevent cartilage degradation (7). In addition, it stimulates B lymphocytes and is involved in promoting the production of autoantibodies (6).

In patients with RA, a wide range of autoantibodies, such as anti-IgG (RF) and cyclic citrullinated peptide antibodies (Anti-CCP), are produced. In comparison with other autoantibodies, anti-CCP is a more sensitive and specific marker and can be associated with the severity of disease and cartilage degradation (8).

IL-10 gene is a highly polymorph gene and previous studies have shown that the promoter of this gene has 11 positions of polymorphs (9). Three main polymorphisms situated at positions −1082, −819, and −592 of IL-10 gene and its major haplotypes can be associated with regulating IL10 promoter activity. Variants (-2849 A/G, -1082 A/G, -819 T/C, and -592 C/A) and the relationship between the positions of polymorphs, production of IL-10, and potential risk of RA have been indicated in several studies, although there have been controversial results (10, 11). To our knowledge, there has been no detailed investigation on the role of IL-10 and anti-CCP in RA. According to the high prevalence of RA in Iran and our region, Kurdistan, and also the genetic differences between people in this region and other parts of the world, this study was conducted to assess the possible association between RA and different genotypes of IL-10 as well as its relationship with anti-CCP.

Methods

This was a hospital-based case-control study conducted in Kurdistan province, northwest of Iran. Cases were RA patients (n=224) who were diagnosed based on the American College of Rheumatology criteria and recruited at a referral teaching hospital, Tohid hospital, in Sanandaj, Iran. The control group (n=194) were healthy individuals confirmed to be free of RA and without risk factors for RA who were matched for age, gender, and race with cases. Controls were also randomly selected at the same time and hospital in a different setting (hospital wards) from the setting of cases (rheumatology ward). The number of cases and controls at the beginning of the study was same but we did not find more controls because of the strict inclusion criteria. The matching methods that we used was frequency matching. Regarding selecting the control group, some inclusion criteria were considered as follows: no autoimmune disease, no history of arthritis in the past year, no RA patients in first degree relatives. Before beginning the data collection, written informed consent was obtained from all participants.

Required data for this study were collected in 2 consecutive steps. First, the demographic data of the 2 groups, including age and sex, were gathered through a checklist. Then, 10 mL blood sample was taken from each participant and sent to the selected laboratory. Blood samples were taken from patients in venoject tubes containing EDTA (100 micrograms EDTA per 5 mL of blood) and the plasma was separated and frozen at -20°. In the laboratory, the DNA was extracted via the phenol-chloroform method and determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using primers and specific limiting enzymes for variant genotypes of IL-10 (12).

For this process, a 2% agarose gel is used. The mixture is boiled for 3 to 5 minutes with buffer TBE. The gel temperature should reach about 60°. Then, the gel is placed in the tray gently. Gel coagulation takes 20 minutes. Then, the comb is removed and the PCR product that should be evaluated is inserted in the sink. Mix the samples with the loading buffer, which includes bromophenol blue 0.25%, Xylene cyanol 0.25%, and Ficoll 15%, to avoid leaving them before putting them in the electrophoresis tank. Eight microliter DNA and 2 microliter buffer were mixed and poured into the gel sinks.

The gel was stained with fluorescent ethidium bromide dye. For staining the gel, 10 mL ethidium bromide was dissolved in 100 mL of distilled water. The solution was kept in the dark in the laboratory. Gels were stained in the solution approximately 3 to 5 minutes. The gel was then examined using a transilluminator apparatus. Serum levels of anti-CCP antibodies were also measured in the 2 studied groups using the ELISA kit.

Data Analysis

All Analyses were performed in SPSS Version 20 by using the independent sample t, chi-square, and Fisher exact tests. OR and 95% CI and the corresponding P values were calculated to show the relationship between independent variables and RA quantitatively.

Results

The mean (SD) age of cases and controls was 49.6±12.5 and 48.9±7.8 years, respectively. A total of 24 (10.7%) of cases and 14 (7.2%) of controls were women. There was no significant difference between cases and controls with respect to age (p=0.741) and gender (p=0.213). Table 1 summarizes the difference in IL-10 polymorphism frequency between patients and controls.

As shown in Table 1, the frequency of IL-10-1082 A/G polymorphism in RA patients was significantly higher than the control group (p=0.009), while the frequency of IL-10-1082 A/A and G/G polymorphisms in RA patients was lower than controls and this finding for G/G polymorphism was statistically significant (p=0.010). No significant difference was found between the 2 studied groups regarding IL-10-592 C/C, C/A and A/A polymorphisms (p>0.05).

Table 2 shows that there was no significant difference between the two groups for each IL-10 allele independently (p>0.05). A total of 132 (31.58%) of the 418 studied participants were anti-CCP positive. The frequency of anti-CCP antibody or anti-CCP positive in cases (57.2%) was

significantly higher than in controls (2.1%) (p<0.001). On the other hand, the chance of RA occurrence among persons with positive anti-CCP was 63.3 times higher than those with negative anti-CCP (Table 3).

In terms of the association between each allele and the frequency of positive anti-CCP, the finding revealed that there was a significant relationship between allele A and antibody level, indicating that individuals who had this allele were more likely to have an increase in anti-CCP positivity (p<0.001). A significant relationship was also observed between allele G and the positive anti-CCP (p<0.001).

In addition to evaluating the association between RA and interlukin-10 genotypes and anti-anti-CCP, the potential association between anti-CCP and IL-10 was assessed. The results showed a significant difference between the positivity of anti-CCP and different alleles of IL-10-1082 so that the positive percent in AG was higher than that in AA and GG (Table 4).

Discussion

RA is a chronic autoimmune disease characterized by inflammation and destruction of the joints (13). A wide range of proinflammatory and inflammatory cytokines and their

Table 1. Association between Interleukin 10 genotypes and RA

IL-10 genotype		Case (N = 224)	Control (N =194)	OR (95%CI)	*P*
AG 1082					
A/A				0.74 (0.50-1.12)	0.142
	Yes	88 (39.3)	90 (46.4)		
	No	136 (60.7)	104 (53.6)		
A/G				1.67 (1.11 - 2.51)	0.009
	Yes	128 (57.1)	86 (44.3)		
	No	96 (42.9)	108 (55.7)		
G/G				0.34 (0.13 - 0.86)	0.012
	Yes	8 (3.6)	18 (9.3)		
	No	216 (96.4)	176 (90.7)		
CA 592					
C/C				1.14 (0.77 – 1.72)	0.410
	Yes	122 (54.5)	99 (51.1)		
	No	102 (45.5)	95 (48.9)		
C/A				0.91 (0.60 – 1.38)	0.621
	Yes	84 (38.0)	77(39.4)		
	No	140 (62.0)	117 (60.6)		
A/A				0.75 (0.35 – 1.61)	0.412
	Yes	16 (7.5)	18 (9.6)		
	No	208 (92.5)	176 (90.4)		

table 2. comparison of Frequency of Each Allele of Interleukin 10 between the 2 groups

IL-10 alleles		Case	Control	*P*
IL 10 – 1082 alleles				
A				0.801
	Yes	304 (67.9)	267 (68.8)	
	No	144 (32.1)	121 (31.2)	
G				0.801
	Yes	144 (32.1)	121 (35.5)	
	No	304 (67.9)	267 (68.8)	
IL 10 – 592 alleles				
C				0.211
	Yes	324 (72.3)	266 (68.6)	
	No	124 (27.7)	122 (31.4)	
A				0.412
	Yes	116 (25.9)	110 (29.3)	
	No	332 (74.1)	278 (71.7)	

Table 3. Comparison of positive/negative anti- CCP between the 2 groups

Anti CCP	Case, No (%)	Control, No (%)	OR (95%CI)	*P*
Positive	128 (57.2)	4 (2.3)	63.3 (22.7 – 176.5)	<0.001
Negative	96 (42.8)	190 (97.9)		

Table 4. Association between anti-CCP and IL-10

	Anti CCP		
IL-10 alleles	Positive, No (%)	Negative, No (%)	*P*
IL-10-1082			0.004
AA	46 (25.8)	132 (74.2)	
AG	76 (36.9)	130 (63.1)	
GG	10 (29.4)	24 (70.6)	
IL-10-592			0.921
AA	11 (31.4)	24 (68.6)	
AC	53 (32.7)	109 (67.3)	
CC	68 (30.8)	153 (69.2)	

secreting cells, such as monocytes, lymphocytes, and macrophages, are involved in the pathogenesis of this disease (14-16). IL-10, as an immunoregulatory cytokine that is elevated in patients with RA appears to be a response to increased inflammation and destructive cytokines (3). IL-10 reduces the effects of inflammatory cytokines and prevents them from destroying the cartilage. In addition, the effects of these cytokines stimulate B lymphocytes and interfere with the production of autoantibodies (17, 18). IL-10 is a highly polymorphic gene (8). Recent research has discovered that its promoter contains 11 polymorphic sites, which may be useful in preventing or contributing to autoimmune disorders like RA (19, 20).

In this study the association between IL10 genotypes and RA and also anti-CCP was investigated. The main aim of the study was to determine the IL10 genotypes and alleles that can be related to RA. According to the results, IL-10 genotype A/G was significantly associated with RA. Our results are compatible with some previous studies. In one study conducted in Turkey, there was a significant difference in the distribution of haplotypes of IL-10 between RA patients and healthy individuals so that polymorphism 1082A/G (P=0.008; OR, 1.44) and haplotypes GCC and ACC were associated with RA in Turkey (9). In another study in Poland IL-10 genotype A/G polymorphism at position 1082 was higher in RA patients with positive or negative rheumatoid factor (21).

In the genotypes of position 1082 IL 10, only the A/G genotype was significantly associated with RA. Two other genotypes of position 1082 interleukin 10, A/A and A/G, although were not statistically associated with RA, they were clinically important. Considering the higher frequency of A/A and A/G genotypes in the control group, they may have a protective role in RA and need to be studied further.

Unlike 1082, at 592 IL 10 position, none of the genotypes were associated with RA. The results were consistent with the study of Marinou et al in Royal Hallamshire Hospital, Sheffield (22). Furthermore, there was no further data or research in the literature to compare with our findings.

In the case of anti-CCP, there was a significant difference between healthy individuals and RA patients. Anti-CCP had a positive to negative ratio of 75% in individuals with RA and a ratio of 2.2% in healthy people. In general, we observed statistically significant associations between positive anti-CCP and RA. The results showed that anti-CCP was positive in some healthy individuals (2.2%). We can infer from this finding that healthy patients who test positive for anti-CCP may acquire RA diseases in the future. Our findings revealed that anti-CCP is highly correlated with the IL-10-1082 locus in RA disease and that anti-CCP positivity in healthy individuals may be an indicator of future RA development. Based on the results, none of the alleles at position 1082 or 592 showed a significant association with RA. The IL-10 1082 A/G genotype can be a risk factor for RA in general. Although anti-CCP positivity was linked to alleles 1082 (A, G) and 592 (A, C) of IL-10, allele A in the 1082 position was more effective than the other 2. Obtaining fewer controls than cases due to strict inclusion and exclusion criteria was a major weakness of this study, despite the sample size and number of cases and controls being rather large. It was impossible to recruit more controls in our study setting. Another limitation was the lack of data modeling and the extraction of OR values using a logistic regression, which did not offer more information to the study findings due to the small number of factors evaluated.

Conclusion

In this study, we assessed the relationship between RA and IL-10 genotypes/alleles and also the association between RA and anti-CCP antibodies. The results showed that IL-10 genotype A/G was significantly associated with RA. Although IL-10 alleles, 1082 A/G and 592 C/A, were associated with anti-CCP, IL-10 allele 1082 A was more associated with RA rather than other alleles. In summary, the chance of anti-CCP positivity in persons who have IL-10 genotype 1082 A/G is higher. Further studies are recommended to determine the relationship between IL-10 genotype 1082 G/A and RA. If such a relationship is proven, this finding as a diagnostic clue can help rheumatologists in early detection of RA.

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Conflict of Interests

The authors declare that they have no competing interests.

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