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ASSOCIATION BETWEEN INTERLEUKIN-21 RS2055979 C/A GENE POLYMORPHISM AND RHEUMATOID ARTHRITIS SUSCEPTIBILITY IN A SAMPLE OF IRAQI PATIENTS.

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Abstract: Rheumatoid arthritis (RA) is a systemic inflammatory disease that mostly affects the joints. It is the most common inflammatory joint condition, characterized by cartilage and bone erosion, resulting to functional decline and disability if left untreated. As a result, cartilage and joint degradation, as well as disability. This study aims to find a relationship between interleukin -21 rs2055979 C/A polymorphism and predisposition to rheumatoid arthritis development in a sample of Iraqi patients. The study includes one hundred subject of Iraqi patients were diagnosis in the Rheumatology Unit of AL-Hindyia General Hospital in Karbala province. Samples were divided to two groups. The first group was including patients while second group was including apparently healthy. DNA was extracted, then the Genotyping polymorphism (rs2055979) of the gene Interleukin-21 was done by RT-PCR. The results of rheumatoid factors-antibody showed highly significant association in patients with rheumatoid arthritis than healthy control with ($P<0.01$). The results of C-reactive protein showed that were significant association between the two study groups in C-reactive protein with ($P<0.01$). Also, the results of rheumatoid factor showed that were significant association between the two study groups in RF with ($P<0.01$) The genotyping and allele frequencies of IL-21 rs2055979 C/A for the two groups appeared that there were significant differences in genotype between patients and controls. Compared CA genotype between control and patients, heterozygous CA genotype was significant different from controls ($X^2=2.55$, OR=0.615); and the CC genotype was significantly increased risk for RA ($X^2=8.59$, OR=1.86).

Keywords: Rheumatoid arthritis (RA), Genetic polymorphism, CRP and RF

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that mostly affects the joints. It is the most common inflammatory joint condition, characterized by cartilage and bone erosion, resulting to functional decline and disability if left untreated. As a result, cartilage and joint degradation, as well as disability, ensue [1]. Rheumatoid arthritis affects roughly 5 people out of every 1000, and it can cause serious joint damage and disability. Arthralgia, edema, redness, and even a reduction in range of motion are all symptoms of symmetrical joint involvement [2]. It affects at least twice as many women as it does males, and while it can strike at any age, moreover, it is most common in people over 50 [3]. According to that, cytokines play an important role in the pathophysiology of RA, they play a role in the initiation and maintenance of inflammation, making them therapeutic targets [4]. The control of cytokines is imbalanced, resulting in low levels of

inhibitory cytokines and increased levels of pro-inflammatory cytokines, both of which contribute to the chronic inflammatory state. Cell-cell interaction or soluble mediators - cytokines - mediate and determine the systemic response to inflammation and cellular activation. Cytokines form large networks with both synergistic and antagonistic interactions, resulting in both negative and positive effects on target cells [5]. The prognosis of RA is also dependent on early detection and management. The medical history, clinical findings (including imaging modalities), and serological laboratory tests are the three pillars of rheumatological disease diagnosis [6].

Materials and Methods

One hundred volunteers were taken in this study. Fifty with RA patients and fifty apparently healthy, who randomly selected between November 2021-February 2022 at the Rheumatology Unit of AL-Hindyia General Hospital in Karbala province. A questionnaire has been taken from the patients, and the case sheet included age, gender, residence, height, weight, and previous history of the disease. In this study,100 volunteers were use and divided to two groups, the first group was include patients while second group was include apparently healthy. Two ml of peripheral blood from all select subjects were collected and placed into sterile plain tube that contained EDTA and three ml of serum were collected and placed into sterile plain tube. The blood and serum were placed in a cool - box under aseptic conditions and transfer to the laboratory. Serum CRP and RF measured by latex method. Genotyping of polymorphism (rs2055979) of the *IL-21* gene was done, by using Taq man SNP Genotyping Assays. A set of primers was used to amplify specific region within the IL-21 gene. The forward primer 5'-GGAAACTCTGGAAAGAACTCTAACC-3' and the Reverse primer 5'-GCACATTCAGCTTATTGGAAAG-3'. Fam-Probe 5'-AGCATCTCATGCACCTT-3' and Hex-Probe 5'-AGCATCTCATTCACCTTG-3'. The thermal cycling program was as follows: Carryover prevention in 50 C° for 2 min, followed enzyme activation in 95 C° for 10 min, followed by 40 cycles of two steps (first one was denaturation 95 C° for 30 second and second step of annealing for 1 min sec (60 C°).

Statistical Analysis

The statistical analysis system- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test and LSD test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio in this study. SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

Results and Discussion

Distribution of Rheumatoid Arthritis Patients and Control Group According to Age.

Results of rheumatoid arthritis patients as well as apparently healthy control group were studied according to age (Table 1).

The results showed that were a significant differences association between the two study groups in age. The mean age of patients group mean± SD (52.60±12.307) years old, and control groups were mean± SD (39.42±9.498) years old. The age of subjects more than 50 years old have great incidence for getting Rheumatoid arthritis and this results is agreed with [7].Who showed that the mean of ages of rheumatoid arthritis onset increased significantly from 55.8 years in 2002–2003 and 57.0 years in 2007–2008 to 59.9 years in 2012–2013. The peak age shifted from the 50-59 years age group in 2002-2003 to the 60-69 years age group in 2012-2013. In [8] showed that disease onset could occur at any age, but peak incidence occurs within the fourth and fifth decades of life. Moreover, the previous result agreed with[9]. They found that RA can affect people of any age, but peak onset is from age 50 to 59 years.

Table (1): Distribution of rheumatoid arthritis patients and control group according to age

Variable	Study Groups		P values
	Mean ± SD		
	Patients	Control	
Age	52.60 ± 12.307	39.42± 9.498	**0.001** **

SD: Standard Deviation, P: Probability, Significantly* ($p \leq 0.05$), ** ($p \leq 0.01$).

Distribution of Rheumatoid Arthritis Patients and Control Group According to Residence.

Distribution of rheumatoid arthritis patients and as well as apparently healthy control group was studied according to residence (Table 2). The results showed that were a no significant association between the two study groups in residence, the study revealed that the incidence of rheumatoid arthritis among patients group who lived in urban areas was (60%) and (66%) in healthy control while the incidence of rheumatoid arthritis who lived in rural areas was (40%) in patients group and (34%) in healthy control group. Present results show that in both groups there is no significant incidence of rheumatoid arthritis among people who lived in urban and rural areas. This result agreed with [10]. There is no evidence that social class influences the onset of rheumatoid arthritis.

Table (2): Distribution of rheumatoid arthritis patients and control group according to residence

Geographical distribution		Patients (50)		Control (50)		P-value
		Freq.	%	Freq.	%	
Valid	Urban	30	60.0	33	66.0	0.41 NS
	Rural	20	40.0	17	34.0	
	Total	50	100.0	50	100.0	

P: Probability, NS: Non significant

Distribution of Rheumatoid Arthritis Patients and Control Group According to Rheumatoid Factor.

Distribution of rheumatoid arthritis patients and as well as apparently healthy control group was studied according to rheumatoid factor (Table 3). The results of rheumatoid factors-antibody in the current study showed highly significant association in patients with rheumatoid arthritis than healthy control with p-value 0.001. The current study observed that RF in patients with RA was positive in (76%) and negative in (24%); additionally, in a control group, RF was negative in (88%) and positive (12%).The presence of elevated RF in the blood plasma and synovial fluids, which accumulate over time, was used to diagnose rheumatoid arthritis. Rheumatoid factor is an antibody that recognizes the Fc or conserved component of human antibodies. It is present in 60% to 90% of RA patients with developed RA but less than 50% of patients with early RA. Erik Waaler discovered RA by detecting rheumatoid factors (RF) such as anti-immunoglobulin G (IgG), anti-IgM, and anti-IgA. The American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification guidelines include positive RF in 80 percent of RA patients as an important method in the diagnosis of rheumatoid arthritis. However, RF has a low sensitivity and specificity since it can be seen in other autoimmune disorders and pathogens, and not all RA patients had positive RF [11].the result of the current study is agreed with a previous study done in Iraq for RA patients [12]. which found that RF has a low specificity in RA patients and that RF is positive in about 4% of the control group and agreed with another previous study [13].

Table (3): Distribution of rheumatoid arthritis patients and control group according to rheumatoid Factor.

RF		Patients (50)		Control (50)		OR	X^2	p-value
		Freq.	%	Freq.	%			
Valid	Positive	38	76.0	6	12.0	1.700	2.135	0.001**
	Negative	12	24.0	44	88.0	1.074		
	Total	50	100.0	50	100.0			

OR: Odds Ratio, X^2: Pearson's Chi Square, P: Probability, the difference is highly significant at (< 0.01).

Distribution of Rheumatoid Arthritis Patients and Control Group According to C-reactive protein.

Distribution of rheumatoid arthritis patients and as well as apparently healthy control group was studied according to C-reactive protein (Table 4). The results showed that were significant association between the two study groups in C-reactive protein with (P<0.01). The current study observed that CRP in patients with RA is positive in (80%) and negative in (20 %); furthermore, in the control group, CRP is positive in (14 %) and negative in (86 %).C-reactive protein sensitivity was found to be around 80 % in the current study, indicating that it has little specificity in RA patients. This result is agreed with [14]. They found that C-reactive Protein is normal at presentation in 35–45 % of rheumatoid arthritis patients. CRP has low specificity, and other studies also recorded similar results [15]. CRP has involved in the host's defense mechanisms against

infectious agents as well as the inflammatory response [16]. Following an inflammatory event, IL-6 stimulates hepatocytes to produce the acute-phase reactant C-reactive protein [17]. Smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes have also been shown to express CRP. CRP binds to immunoglobulin Fc gamma receptors (FcgR), causing proinflammatory cytokines to be generated, resulting in an inflammatory amplification loop [18]. CRP can play a direct role in bone degradation in RA, according to may preclinical evidence.

Table (4): Distribution of rheumatoid arthritis patients and control groups according to C-reactive protein.

CRP		Patients (50)		Control (50)		OR	X^2	p-value
		Freq.	%	Freq.	%			
Valid	**Positive**	40	80.0	7	14.0	1.750	2.035	0.001**
	Negative	10	20.0	43	86.0	1.094		
	Total	50	100.0	50	100.0			

OR: Odds Ratio, X^2: Pearson's Chi Square, P: Probability, the difference is highly significant at (< 0.01).

Genotype Distribution and Allele Frequency of IL-21 rs2055979 C/A in Patients and Control groups

The genotype and allele frequencies of the IL-21 rs2055979 C/A for the two study groups (controls and patients) are shown in table (5). All genotype frequencies of the control group and patients group confirmed to the Hardy-Weinberg equilibrium (HWE).

Table (5): Genotype of IL-21 rs2055979 C/A gene polymorphism with allele frequency.

Genotype rs2055979 C/A	Patients No. (%)	Control No. (%)	Chi-Square (χ^2)	O.R	p-value
CC	5 (10.00%)	35 (70.00%)	9.82 **	Ref. =1	0.0001
CA	13 (26.00%)	5 (10.00%)	2.55*	0.615	0.05
AA	32 (64.00%)	10 (20.00%)	8.59 **	1.86	0.0071
Total	50 (100%)	50 (100%)			
Allele	**Frequency**				
C	0.23	0.75			
A	0.77	0.25			

* (P≤0.05), ** (P≤0.01)

OR: odds ratio; X^2: Person's Chi Square

Results from table (5) show that the genotype and allele frequencies of IL-21 rs2055979C/A for the two study groups appeared that there were significant differences between Rheumatoid arthritis patients and control group. Compared CA genotype CA genotype between control and patients, heterozygous CA genotype was associated with increased risk for rheumatoid arthritis (X^2= 2.55, P-value =0.05). Compared AA genotype between control and patients, homozygous AA genotype was associated with significantly increased risk for rheumatoid arthritis (X^2=8.59, P-value =0.0071). In addition, allele frequency for the A allele is associated with significantly increased risk for rheumatoid arthritis. This result is agreed with [19],they investigate the prevalence of homozygous mutant (AA) of the rs2055979 polymorphism was considerably greater (p 0.0001, X^2 = 34.73, OR = 4.342). Additionally, there were more mutations (CA + AA) in RA than in controls (p = 0.001, 2 = 10.71, OR = 1.901). Additionally, the frequency of the mutant allele (A) was even higher in patients compared to healthy controls (P 0.0001, 2 = 35.53, OR = 2.149), showing a crucial genetic susceptibility factor on predisposition to the development of RA. The SNPs IL-21 rs2055979C/A are located in intron 3region, and their single-nucleotide changes. The IL-21 gene, also known as Za11 or CVID11, is located on human chromosome 4q27, and encodes a member of the common-γ chain family of cytokines with immune regulatory activity [20]. The protein encoded by IL-21 is known to be involved in both innate and adaptive immune responses by inducing the differentiation, proliferation and activity of multiple target cells, including macrophages, natural killer cells, B cells and cytotoxic T cells [21]. Dysregulation of this gene may result in multiple immune-mediated diseases including RA, systemic lupus erythematosus, psoriasis and chronic inflammatory diseases [22]. In [23] they found serum IL-21 levels were increased in patients with SLE compared with controls (P<0.01). Moreover, genotypes carrying the IL-21 rs2055979 A variant allele were associated with increased IL-21 levels compared to the homozygous wild-type genotype in patients with SLE. The positive association was also observed in hepatocellular carcinoma [24].

Conclusion

The current study observed the mean age of patients group mean± SD (52.60±12.307) years old, and control groups were mean± SD (39.42±9.498) years old. The current study observed that RF in patients with RA was positive in (76%) and negative in (24%); additionally, in a control group, RF was negative in (88%) and positive (12%). The current study observed that CRP in patients with RA is positive in (80%) and negative in (20 %); furthermore, in the control group, CRP is positive in (14 %) and negative in (86 %). The genotyping and allele frequencies of IL-21 rs2055979 C/A for the two groups appeared that there were significant differences in genotype between patients and controls. Compared CA genotype between control and patients, heterozygous CA genotype was significant different from controls (X^2=2.55, OR=0.615); and the CC genotype was significantly increased risk for RA (X^2=8.59, OR=1.86). In addition, allele frequency for C

allele is associated with significantly increased risk for RA. For the patients group the allele frequency of (C) 0.23 %, but (A) allele frequency 0.77 %, while for control groups the allele frequency of (C) was 0.75 %, but (A) allele 0.25 %. Moreover, the IL-21 rs2055979 C/A genotype was associated with increased risk for development of RA in Iraqi patients.

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