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Interleukin -21 gene RS 2221903 polymorphisms in rheumatoid arthritis patients

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Abstract
Background: Rheusmatoid arthritis (RA) is a chronic systemic disease characterized by articular involvement and extra-articular manifestation. A cytokine with immunomodulatory properties, interleukin-21 (IL-21) influences both the innate and acquired immunity. The aim of this work was to detect IL-21 polymorphism (rs2221903) in cases with RA and determine its role in the pathogenesis and activity of the disease.
Methods: This research was carried out on 60 cases with RA, cases were divided into three groups Group I: RA cases with an active form, group II: RA cases in inactive form, group III: apparently healthy individual who will be selected with matched age and sex.
Results: There was statistically significant relationship between activity of RA and genotypes (P=0.04), three groups in the genotype distribution (p=0.016), reference (C) and variant allele (T) distribution (p=0.003) between the three groups and in genotype distribution between RA cases and control (P=0.003), this meaning that IL-21 is susceptibility locus for RA.
Conclusions: Results indicate that IL-21(rs2221903) has a role in the pathogenesis and activity of RA. It is also susceptibility locus for RA.

Keywords: Interleukin-21 gene rs2221903 polymorphisms, rheumatoid arthritis

Introduction
Chronic inflammation and progressive joint degeneration are hallmarks of rheumatoid arthritis (RA), an inflammatory illness. It's a long-term condition that has devastating effects on people's health and quality of life. Unfortunately, conventional medications are the only option for managing RA's symptoms. Factors in both the genetic and environment hold major responsibility for this disease [1].
Different immune system components are involved in the development of rheumatoid arthritis. Tight interplay between cells and mediators of the innate and adaptive immune systems amplifies and sustains inflammation and tissue remodelling in people who are genetically predisposed to develop RA [2].
Pro-inflammatory cytokines play an important role in RA pathogenesis by triggering an inflammatory response and the production of additional inflammatory mediators. Cytokines and chemokines attract and accumulate immune cells. By its late stages, RA may be understood to be a cell-autonomus genetic and epigenetic illness, in contrast to its early stages, when the involvement of anticitrullinated protein antibodies in RA induction predominates [3].
CD4+ T cells, such as T follicular helper cells, Th17 cells, and natural killer (NK) T cells, release interleukin 21, an immunomodulatry cytokine with pleiotropic effects on innate and adaptive immune responses [4].
Activated CD4+ and CD8+ T cell proliferation is stimulated, but inducible regulatory T cell differentiation is blocked. It has the ability to directly affect B cells, which can then set off an immunological response. Thus, IL-21's influence on B cells may contribute to the onset of autoimmune illnesses. IL-21 is a cytokine that plays a crucial role in the onset and progression of RA, as well as in the ongoing inflammation that plagues the disease's affected joints and tissues [5].
Production of IL-21 is under genetic control. IL- 21 is encoded by IL-21 gene on chromosome 4, its location 4q27 and it has 5 exons. Single nucleotide polymorphisms (SNP)

have been described in IL-21 gene. These polymorphisms are found to be related to pathogenesis and the activity of RA. One SNP (rs2221903) T>C give rise to amino acid substitution [6].

The aim of this work was to detect IL-21 polymorphism in cases with RA and determine its role in the pathogenesis and activity of the disease.

Patients and Methods

This research was carried out on 60 cases with RA. The Ethical Committee of Tanta University Hospitals gave their approval to the research. The patient gave their informed, signed consent.

Exclusion criteria were Cases with liver and renal diseases and other autoimmune diseases.

Cases were divided in to three equal groups: Group I: RA cases with an active form, group II: RA cases in inactive form, group III: apparently healthy individual who will be selected with matched age and sex.

All cases were subjected to: full history taking, Full clinical examination (Assessment of disease activity by DAS28 (Disease Activity Score of 28 joints, extra-articular manifestation such as, subcutaneous nodules, interstitial lung disease, pleural effusion, pericarditis, splenomegaly and leukopenia), laboratory investigation (rheumatoid factor, ESR, CRP, Ant citrullinted cyclic protein antibody (ACCP), specific tests to exclude other autoimmune diseases such as ANA (antinuclear antibody, Anti-dsDNA antibodies, Antiphospholipid antibodies, specific laboratory investigation, molecular analysis: IL21 rs 2221903 was performed. Genomic DNA was isolated, purified and was polymerase chain reaction amplified by TaqMan real time PCR).

Venous blood sample were withdrawn from all subjects and divided into three parts 1: collected on sterile ethylene diamine tetra-acetate 'EDTA' (vacutainer) tubes and was used for DNA extraction and stored at -20, 2: (4ml) collected in plain tubes. Clotted samples were centrifuged within one hour of sampling, at 3000 rpm for 10 minutes. The serum was then separated and kept in an eppendorf tube and was used for RF, CRP, and ACCP 3: anticoagulated blood is allowed to stand in narrow vertical glass tube for ESR.

Principle of RF test

The latex agglutination test was the basis for the serological technique used. Since RF is an IgM class antibody that targets the Fc region of the IgG molecule, it can be detected through agglutination of latex particles that have been coated with IgG. A positive reaction, marked by the agglutination of latex particles, indicates the existence of rheumatoid factor at a level that can be measured. If the RF concentration is more than 18 IU/ml, the latex particles in the suspension will agglutinate after two minutes. The suspension of latex particles did not agglutinate within two minutes, yielding a negative result [7].

Methods to measure RF

Isotonic saline used for a rough quantitative estimate In the qualitative approach, dilute the positive test sample by the following factors: 1: 2, 1: 4, 1: 8, 1: 16, 1: 32, 1: 64, 1: 128, etc. Use a sample from each dilution in the qualitative testing technique.

A positive test result is indicated by a titer that is greater than or equal to the reciprocal of the maximum dilution. By reading the titer at the final dilution step where agglutination was still apparent, we may estimate the rheumatoid factor concentration, which we write as RF in IU/mL= IU/mL Titre sensitivity of latex Gamma globulin reagent [8].

Erythrocyte sedimentation rate (ESR)

Red blood cells (RBCs) settle out from the plasma when anticoagulated blood is left standing in a narrow vertical glass tube for an extended period of time. The rate at which they are settling is expressed in millimeters per hour (mm/hr) of clear plasma at the top of the column.

Wintrobe's method and Westergren's method are the two most common approaches to calculating ESR.

The outcomes from each technique are only marginally varied. When the ESR is low, Wintrobe's method is better, but when it's high, Westergren's method is the chosen one.

Procedure: Thoroughly combine the anticoagulated blood, fill the tube with blood using the rubber bulb until the 0 mark is reached, and then remove any remaining blood from the bottom of the tube using cotton. Pipette should fit tightly to prevent leakage; pipette should be held vertically; tube should be left undisturbed for 1 hour before result is read [9].

Normal values for males: 0-10 mm/hr, females: 0-15 mm/hr

As an acute phase reactant, C-reactive protein (CRP) levels increase rapidly in response to tissue injury, the onset of infection, or other inflammatory stimuli. The method of latex agglutination is used in the CRP test. A positive reaction, characterized by the agglutination of latex particles, indicates the presence of CRP at a level that can be measured. Normal: High: 10 mg/L or higher, low: 10 mg/L or higher

When comparing the first and second generation ACCP enzyme-linked immunosorbent assays (ELISAs), the third generation ELISA offers higher sensitivity for detecting of RA [10].

Reference Range: Negative less than 20u/ml, Low/weak positive20-39 u/ml, moderate positive more than 40-59u/ml, strong positive more than 60 u/ml. Analysis of IL21 rs 2221903 gene polymorphism by Real Time PCR using TaqMan probes and primers on Applied biosystems Step One Real-Time PCR system: Manual extraction of genomic DNA by GeneJET Genomic DNA Purification Kit supplied by Thermo Fisher Scientific from peripheral blood leucocytes of EDTA anticoagulated blood, amplification of the extracted DNA and genotypes were determined by Real-Time PCR.

Following PCR amplification, an endpoint plate read was done using an Applied Biosystems Real-Time PCR System. Fluorescence measurements were taken at this time, and the Sequence Detection System (SDS) Software plotted values based on signals from individual wells. Allele distribution was displayed as a scatter plot of fluorescence signals. Plate Layout was created, analyzing the plate read document, doing automatic allele calls, and converting allele calls to genotypes. Figure 1.

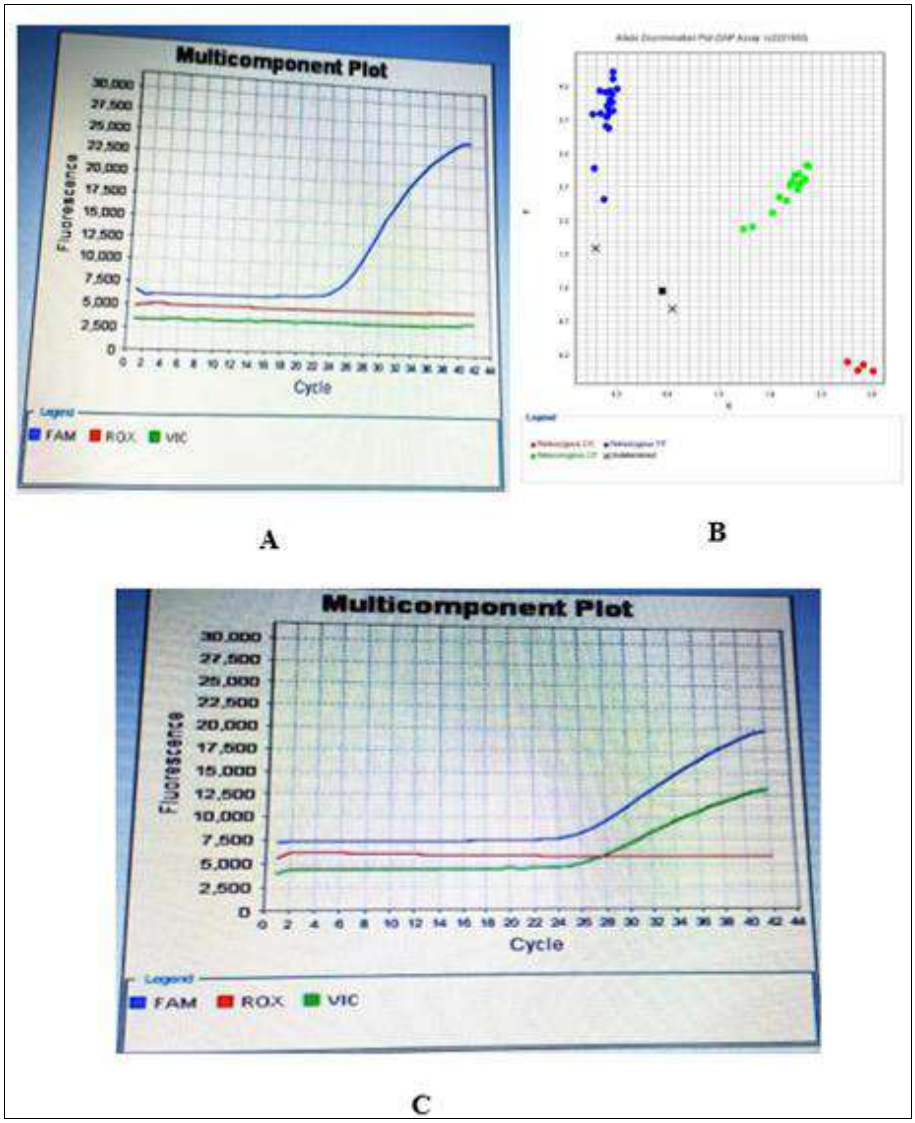

Fig 1: A Multicomponent plot showing homozygosity (A) and heterozygosity(C) for allele, B: Allelic discriminative plot showing dyes of the PCR probe

Statistical analysis

IBM's statistical software, SPSS, version 20.0, was used to examine the data provided into the computer. IBM Corp., Armonk, New York. Quantitative and percentage descriptions of qualitative data were provided. The normality of distribution was checked using the Shapiro-Wilk test. The minimum and maximum values, as well as the mean, standard deviation, median, and interquartile range (IQR), were used to characterize the quantitative data. Results were considered statistically significant at the 5% level.

Different groups were compared using the following statistical methods: Student t-test (for continuous variables with normal distributions),Chi-square test (for categorical variables), Monte Carlo adjustment (for chi-square when greater than 20% of cells have expected count less than 5), the Mann Whitney test (for not normally distributed quantitative variables), and the Hardy-Weinberg equilibrium. (The Hardy-Weinberg was used to determine the equilibrium of the investigated sample).

Results

There was statistically significant between three groups (p=0.017) and between groups I and group II (p=0.013). Table 1

Table 1: Descriptive analysis of the investigated cases according to DAS28 and age

		Mean ± SD.	Median (IQR)	p
DAS28	Active (>5) n= (20)	5.98 ± 0.56	5.88 (5.54 – 6.26)	-
	Inactive (<5) n= (20)	2.99 ± 0.13	3.0 (2.92 – 3.05)	
	Total n= (40)	4.49 ± 1.56	4.30 (3.0 – 5.88)	
Age (years)	Group I n= (20)	48.95 ± 12.58	49.50 (36.0 – 57.0)	0.017*
	Group II n= (20)	41.0 ± 7.59	39.0 (35.0 – 49.5)	
	Group III n= (20)	44.05 ± 2.24	45.0 (44.0 – 45.0)	
Sig. bet. Grps		p_1=0.013*,p_2=0.177,p_3=0.503		

Data are presented as Mean ± SD, IQR: Inter quartile range *: Statistically significant at p ≤ 0.05.

There was statistically significant variattion in onset, ESR, anticitrullinated cyclic peptide antibody (ACCP), swollen and tender joints. No statistically significant variattion in duration, rheumatoid factor and disability between the two investigated groups. (Table 2).

Table 2: Comparison between the two investigated groups according to onset, duration, RF, inflammatory markers, ACCP, tender, swollen and disability

		Group I (n = 20)	Group II (n = 20)	Test of Sig.	P value
Age of onset (years)		40.95 ± 11.54	31.60 ± 11.13	t= 2.607*	0.013*
		37.0 (31.5-48.5)	31.0 (25.50-42.0)		
Duration (years)		8.10 ± 6.13	7.77 ± 6.31	U= 196.50	0.925
		5.0 (3.50-12.0)	6.50 (1.50-12.50)		
RF	Negative (≤14)	8 (40.0%)	4 (20.0%)	χ^2= 1.905	0.168
	Positive (>14)	12 (60.0%)	16 (80.0%)		
RF		34.80 ± 36.77	63.85 ± 100.2	U= 158.0	0.265
		19.0 (12.0-48.0)	32.0 (18.0-48.0)		
ESR (2nd hour)		77.05 ± 26.10	18.40 ± 4.30	t= 9.917*	<0.001*
		77.0 (57.50-95.0)	20.0 (15.0-22.0)		
CRP		19.74 ± 21.87	7.43 ± 4.66	U= 89.50*	0.002*
		14.15 (7.55-19.0)	6.0 (5.0-7.0)		
ACCP	Negative ≤20)	6 (30.0%)	2 (10.0%)	χ^2= 2.500	FEp= 0.235
	Positive (>20)	14 (70.0%)	18 (90.0%)		
ACCP		24.43 ± 9.13	159.1 ± 99.09	t= 6.053*	<0.001*
		23.50 (19.0-30.0)	160.0 (120.0-195.0)		
Tender joints		10.10 ± 6.60	0.20 ± 0.62	1.000*	<0.001*
		8.0 (4.50-15.50)	0.0 (0.0-0.0)		
Swollen joints		2.75 ± 3.21	0.20 ± 0.62	85.00*	0.001*
		2.0 (0.0-4.50)	0.0 (0.0-0.0)		
Disability		6.45 ± 1.36	7.0 ± 0.86	154.0	0.221
		6.50 (6.0-7.0)	7.0 (6.0-8.0)		

Data are presented as Mean ± SD, median (IQR): Inter quartile range χ^2: Chi square test U: Mann Whitney test t: Student t-test FE: Fisher Exact *: Statistically significant at $p \leq 0.05$.

There was statistically significant variation between the three groups in the genotype distribution, reference (C) and variant allele (T) distribution. Table 3

Table 3: Comparison between the three investigated groups according to IL-21 gene (rs2221903)

	Group I (n = 20)	Group II (n = 20)	Group III (n = 20)	χ^2	p
IL-21 gene					
C/C	1 (5.0%)	3 (15.0%)	10 (50.0%)	11.893*	MCp= 0.016*
C/T	10 (50.0%)	8 (40.0%)	6 (30.0%)		
T/T	9 (45.0%)	9 (45.0%)	4 (20.0%)		
HWχ^2 (p)	0.726 (0.394)	0.292 (0.589)	2.321 (0.128)		
Allele					
C	12 (30.0%)	14 (35.0%	26 (65.0%)	11.674*	0.003*
T	28 (70.0%)	26 (65.0%	14 (35.0%)		

χ2: Chi square test, MC: Monte Carlo *: Statistically significant at $p \leq 0.05$

There was statistically significant variation in the distribution of genotypes and alleles between group I and group II, between group I and group III, group II and group III, r RA cases (group I + II) and control group (group III). Table 4 Figure 2

Table 4: Comparison between Group I + II and Group III according to IL-21 gene (rs2221903)

	Group I + II (n = 40)	Group III® (n = 20)	χ^2 (p)	p_1	OR(LL – UL 95%C.I)
IL-21 gene					
C/C®	4(10.0%)	10(50.0%)	χ^2=12.166* (p=0.002*)		1.000
C/T	18(45.0%)	6(30.0%)		0.008*	7.500 (1.703 – 33.034)
T/T	18(45.0%)	4(20.0%)		0.003*	11.250(2.301 – 54.997)
Allele					
C®	26(32.5%)	26(65.0%)	χ^2=11.41* (p=0.001*)		1.000
T	54(67.5%)	14(35.0%)		0.001*	3.857 (1.732 – 8.590)

Data are presented as frequency, Chi square test, OR: Odd's ratio, ®: Reference group, CI: Confidence interval LL: Lower limit, UL: Upper Limit

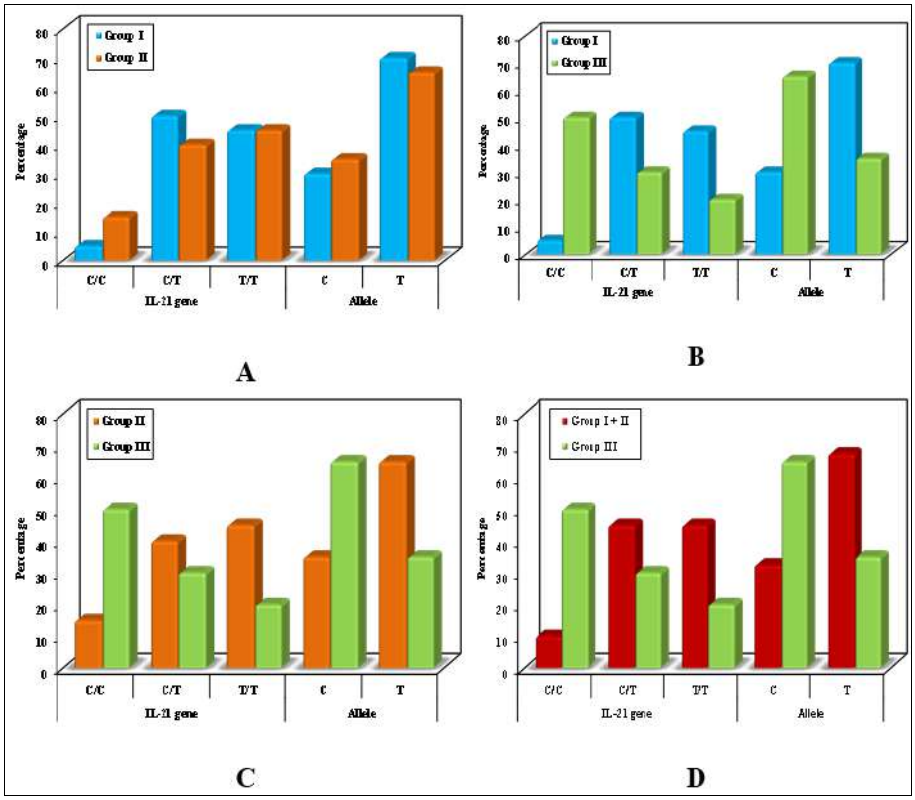

Fig 2: Comparison according to IL-21 gene (rs2221903) A:between Group I and Group II according to IL-21 gene (rs2221903), B: between Group I and Group III C: between Group II and Group III, D: between Group I + II and Group III

There was statistically significant relation between IL21 genotypes and ESR (P=0.01). There was no statistically significant relation between IL21 genotypes (C/C, C/T and T/T) with different parameters (onset, tender, swollen joints, RF, ACCP, CRP) in group I. Table 4

Table 5: Relation between IL-21 gene (rs2221903) with different parameters in group I (rheumatoid arthritis cases with an active form) (n=20)

	IL–21 gene			Test of Sig.	P
	C/C (n = 1) #	C/T (n = 10)	T/T (n = 9)		
Onset (years)					
Mean ± SD.	34.0	41.10 ± 11.16	41.56 ± 13.03	t= 0.082	0.936
Median (Min. - Max.)		42.50(25.0–56.0)	36.0 (29.0 – 68.0)		
Tender					
Mean ± SD.	9.0	9.20 ± 6.75	11.22 ± 7.05	U= 36.50	0.497
Median (Min. - Max.)		7.0 (3.0 – 22.0)	8.0 (2.0 – 21.0)		
Swollen					
Mean ± SD.	9.0	2.60 ± 2.67	2.22 ± 3.35	U= 37.50	0.549
Median (Min. - Max.)		2.0 (0.0 – 7.0)	1.0 (0.0 – 10.0)		
RF					
Mean ± SD.	10.0	47.80 ± 47.06	23.11 ± 16.68	U= 38.50	0.604
Median (Min. - Max.)		24.0(10.0–128.0)	20.0 (10.0 – 64.0)		
ACCP					
Mean ± SD.	22.0	23.80 ± 8.04	25.40 ± 11.10	t= 0.363	0.721
Median (Min. - Max.)		23.0 (14.0 – 40.0)	24.0 (3.60 – 40.0)		
ESR (2nd hour)					
Mean ± SD	100.0	62.70 ± 25.37	90.44 ± 19.20	t= 2.663*	0.016*
Median (Min. - Max.)		59.0(29.0–120.0)	90.0(60.0–120.0)		
CRP					
Mean ± SD.	96.0	14.19 ± 13.19	17.44 ± 13.0	U= 32.50	0.315
Median (Min. – Max.)		13.0 (2.20 – 48.0)	15.0 (2.60 – 48.0)		

Data are presented as Mean ± SD, Median (Min. - Max.) test U: Mann Whitney test t: Student t-test *: Statistically significant at $p \leq 0.05$.

There was no statistically significant relation between IL21 genotypes (C/C, C/T and T/T) with different parameters (onset, tender, swollen joints, RF, ACCP, ESR, CRP) in group II.

Table 6: Relation between IL-21 gene (rs2221903) with different parameters in group II (rheumatoid arthritis cases with an inactive form) (n = 20)

	IL-21 gene			Test of Sig.	P
	C/C (n = 3)	C/T (n = 8)	T/T (n = 9)		
Onset (years)					
Mean ± SD.	31.33 ± 0.58	35.50 ± 11.12	28.23 ± 12.56	F= 0.894	0.427
Median (Min. – Max.)	31.0 (31.0-32.0)	36.50 (22.0–49.0)	29.0 (0.08-42.0)		
Tender					
Mean ± SD.	0.0 ± 0.0	0.50 ± 0.93	0.0 ± 0.0	H= 3.167	0.205
Median (Min. – Max.)	0.0 (0.0-0.0)	0.0 (0.0-2.0)	0.0 (0.0-0.0)		
Swollen					
Mean ± SD.	0.0 ± 0.0	0.50 ± 0.93	0.0 ± 0.0	H= 3.167	0.205
Median (Min. – Max.)	0.0 (0.0-0.0)	0.0 (0.0-2.0)	0.0 (0.0-0.0)		
RF					
Mean ± SD.	32.0 ± 0.0	111.50 ± 149.86	32.11 ± 20.25	H= 0.442	0.802
Median (Min. – Max.)	32.0 (32.0-32.0)	27.0(10.0–350.0)	32.0 (10.0-64.0)		
ACCP					
Mean ± SD.	132.33 ± 99.88	180.13 ± 78.87	149.39 ± 120.70	F= 0.309	0.739
Median (Min. – Max.)	190.0 (17.0-190.0)	179.5 (22.0-300.0)	120.0 (12.0-433.0)		
ESR (2nd hour)					
Mean ± SD.	19.33 ±1.15	18.87 ±5.41	17.67 ±4.09	F= 0.230	0.797
Median (Min. - Max.)	20.0 (18.0-20.0)	20.0 (12.0-25.0)	20.0 (10.0-22.0)		
CRP					
Mean ± SD.	10.33 ±2.89	6.29 ±2.08	7.48 ±6.41	H= 4.808	0.090
Median (Min. – Max.)	12.0 (7.0-12.0)	6.0 (5.0-11.30)	6.0 (2.30-24.0)		

Data are presented as Mean ± SD, Median (Min. – Max.) test U: Mann Whitney test t: Student t-test *: Statistically significant at $p \leq 0.05$.

There was no statistically significant relation between IL21 genotypes (C/C, C/T and T/T) with different parameters (onset, tender, swollen joints, RF, ACCP, ESR, CRP) in RA cases (group I + II). Figure 3.

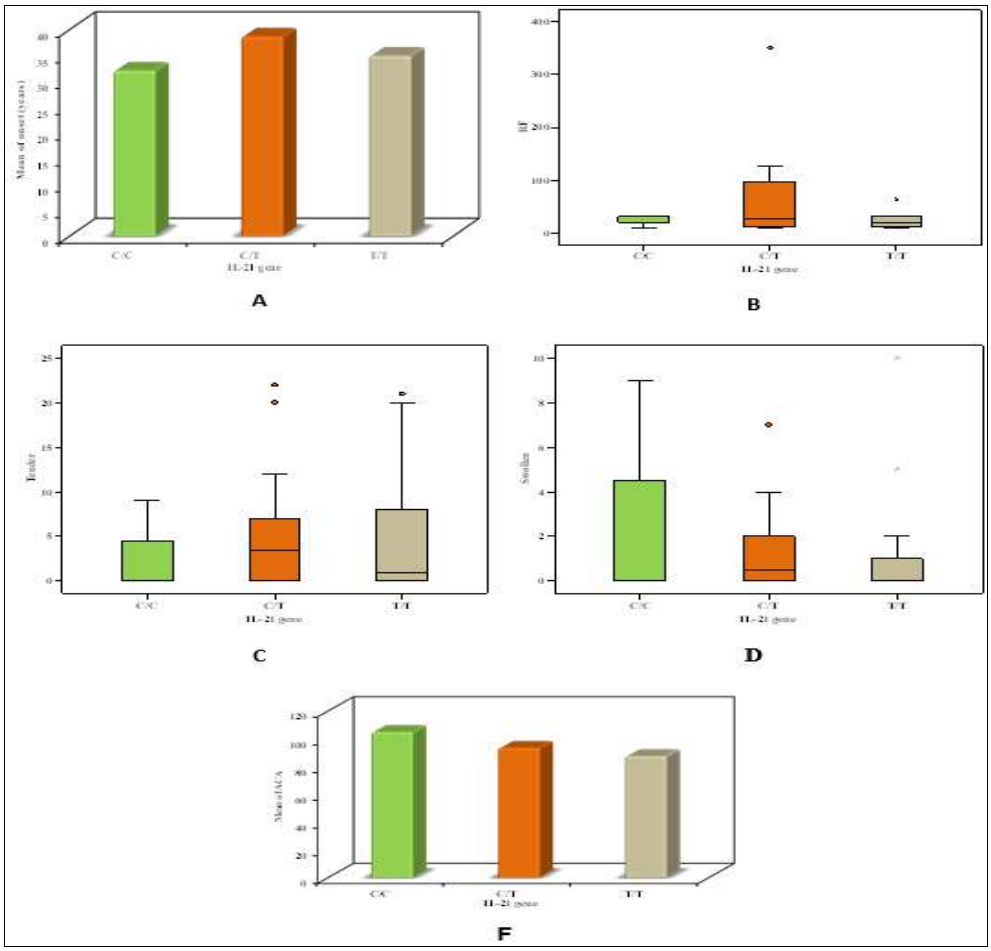

Fig 3: Relation between IL-21 gene (rs2221903) with A:onset, B:RF, C:tender, D:swollen and F: ACCP in group I + II (rheumatoid arthritis cases) (n = 40)

Discussion

Rheumatoid arthritis is a long term multifactorial autoimmune disease that primarily affects joints leading to joint destruction and numerous extra-articular manifestations. Onset is most frequently during middle age, affects approximately 0.8 percent of adults worldwide and women are affected 2.5 times as frequently as men and leads to substantial disability [11].

Interleukin 21 is an immunomodulatry cytokine, CD4+ T cells produce this cytokine, which has a pleiotropic impact on both innate and acquired immunity via the production of IL-17A and IL-17F [4].

IL-21 is a cytokine that has been linked to RA pathogenesis and the formation and maintenance of an inflammatory condition in joints and tissues in RA cases, according to a number of studies. According to Tang *et al*. Previous research has linked elevated IL-21 levels in RA cases can be used as measures of disease activity [12].

Genotype seems to be an important determinant of both incidence and severity of RA. IL- 21 is encoded by IL-21 gene on chromosome 4, its location 4q27 and it has 5 exons. SNP rs 2221903 T>C located at position 122617 of translated sequence of IL-21 give rise to amino acid substitution (A/G) in signal peptide of IL-21 [6].

As regard age distribution, the distribution is statistically significant between three groups (p=0.017) and also statistically significant between group I and group II (p=0.013), this result agrees with research performed by Eltahir *et al*. [13] who reported that, frequency of RA was higher among adult ages more than 31 years. This research disagreed with the research performed by Malinowski *et al*. [14]who reported that there is no statistically significant variattion between investigated groups (P=0.8).

This research showing that, age at onset was 40.95±11.54 in group I and 31.60±11.13 in group II, and this result agreed with a research performed by Malinowski *et al*. [14] who reported that age at onset According to age of onset RA classified into early onset EORA and late onset LORA (more than 60 years). Ke *et al*. [15] reported that early onset associated with worse prognosis, upgraded systemic inflammatory status, declined physical and functional assessment, large joints were more affected, (DAS) higher, misdiagnosed as osteoarthritis, greater decline in life quality. Cases with early onset disease before age of 40 years (41.8±12.8) and those with late onset after age of 60 years had a high risk of death from CVD Lee *et al*. [16].

Duration in group I was 8.10 ± 6.13 and 7.77 ± 6.31 in group II, agree with research performed by Gao *et al*. [17] disagree with research performed by Malinowski *et al*. [14] who reported that disease duration was 10.07±8.32.

The value of DAS28 in RA was 5.98 ± 0.56 in group I and was 2.99 ± 0.13 in group II. Agree with research performed by Paradowska-Gorycka *et al*. [18] DAS28 was 5.16±1.38 in group I, disagree with research performed by Malinowski *et al*. [14] disease activity was 3.45 ±2.39.

DAS is a tool for staging RA, assessing activity, and can be used as additional tool for venous thromboembolism risk stratification in cases with RA Molander *et al*. [19].

There was a statistically significant relation between ESR and activity of disease (P=0.001), our result was in agreement with research performed by Cao *et al*. [20] (P=0.05). It is used for monitoring response to treatment, and it is the most impotant predictor of flare in sustained remission cases Vodencarevic *et al*. [21].

Regarding CRP, there were statistically significant relation between CRP and activity of disease (P=0.002), this result agreed with research performed by Cao *et al*. [22] (P=0.001).

Regarding RF in the two investigated groups, they were positive 60% in group I and 80% in group II, this result was in agreement with research performed by Malinowski *et al*. [14] who reported that they were positive in 75.36%. Menglei [23] RF autoantibody has been suggested as a potential biomarker for RA susceptibility screening. It has long been understood that the absence of RF in blood constitutes the seronegative RA phenotype. Seropositive and seronegative RA appear to act differently during the course of illness and in a number of ways, as observed by Paalanen [24], seronegative cases may present with more severe clinical manifestation. Nnotwithstanding higher disease activity at disease beginning, they appear to have a better prognosis.

The presence of anti-CCP antibodies is highly predictive of the development of RA more than 20 years before the onset of symptoms and of the disease's severity among individuals with already existing RA, they were positive 70% and 90% in group I and II respectively, agree with research performed by Paradowska-Gorycka *et al*. [18] they were positive in 80%. disagree with research performed by Hao *et al*. [25] they were positive in 62%.

ACCP status is delineator of RA disease endotypes (ACCP+ and ACCP-) with similar clinical manifestation but different pathophysiology. ACCP+ antibodies are associated with more aggressive disease (more erosive) with variattion in the pattern of early joint involvement and bad prognosis. Two endotypes could be evidence of disparate development with distinct underlying immunological mechanisms Floudas *et al*. [26].

On comparison between two investigated groups according to tender and swollen joints. There was statistically significant variattion in swollen and tender joint between two groups (P=0.001), this result agreed with research performed by Hao *et al*. [25]. (P=0.001). Disagree with research performed by Zhang *et al*. [27] who reported that there was no statistically significant variattion between two investigated groups (P=0.1).

There is statistically significant variattion in genotype (p=0.002) and allele (p=0.001) distribution between RA cases (group I + II) and control group (group III). This means that IL-21 rs2221903 is susceptibility locus for RA.

Our results disagree with research performed by Malinowski *et al*. [14] who reported that there were insignificant variattion in the distribution of the investigated genotypes between RA cases and controls (P=0.1).

A comparison between two investigated groups according to genotype distribution shows that there is statistically significant variattion in the distribution of genotypes and alleles between group I and group II (P=0.04). There were 5.0% and 15.0% in CC (homozygous reference) genotype, 50.0% and 40.0% in TC (heterozygous) and 45.0% in TT (homozygous variant) in the two groups respectively, disagree with genotype distribution in research performed by Malinowski *et al*. [14] who found that they were 14.1% and 11.6% in CC (homozygous reference) genotype, 49.0% and 41.0% in CT (heterozygous) and3 6.9% and 47.4% in TT (homozygous variant).

According to allele frequency between two groups, they were 30% and 35% for C allele (wild allele), 70% and 65% for T allele (variant allele), which agree with allele frequency in research performed by Malinowski *et al*. [14]

who found that they were 35.90% and 33.43% for C allele and 64.10%, 66.57% for T allele

On comparing genotype distribution between group I (active RA) and group III (control) (table10) there is statistically significant variattion in genotype (p=0.006) and allele (p=0.002) distribution between group I and group III, there were 5% for CC, 50% for CT, 45% for TT in group I and 50% for CC, 30% for CT, 20% for TT in group III, agree with research performed by Hao *et al*. [25]. there were more frequency of TT genotype (50%) in the active group, disagreed with research performed by Malinowski *et al*. [14]there were increased frequency of rs2221903 CT and CC genotypes in cases with active form of RA.

There is no statistically significant relation between IL21 genotypes (C/C, C/T and T/T) with different parameters (onset, tender, swollen joints, RF, ACCP) in group I, II, III, agree with research performed by Malinowski *et al*. [14] there were no statistically significant relationship.

There is statistically significant relation between IL21 genotypes (C/C, C/T and T/T) and activity of RA (P=0.04), agree with research performed by Malinowski *et al*. [14] there were statistically significant relation between IL21 genotypes (C/C, C/T and T/T) and activity of RA (P=0.03). Agree with research performed by Hao *et al*. [25] there were statistically significant relation between IL21 genotypes (C/C, C/T and T/T) and activity of RA (P=0.02), agree with research performed by Cao *et al*. [22] there were statistically significant relationship (P=0.001).

Hao *et al*. [25]. Plasma IL-21 levels in patients with RA were shown to be considerably higher than in healthy controls (P=0.001). There was a significant positive correlation between IL-21 and DAS28, suggesting that this cytokine is linked to illness progression.

As a result of this research, it is recommended that it be performed on a large number of cases and healthy subjects. Therapeutic approaches aimed at IL-21 may prove useful in the management of RA. IL-21R.Fc-mediated inhibition of the interleukin-21 receptor Reduced IL-21-induced proliferation and IL-6/TNF release to improve inflammation or joint destruction induced by matrix metalloproteinase [25].

Conclusions

Results indicate that IL-21 (rs2221903) has a role in the pathogenesis and activity of r RA. It is also susceptibility locus for RA.

Recommendation: large number of cases and healthy subjects are recommended, therapeutic strategies targeting IL-21 might be effective for the treatment of RA. Blockade of IL-21/IL-21 R pathway with IL-21R.Fc Attenuated IL-21 induced proliferation and secretion of TNF and IL-6 to improve inflammation or joint destruction induced by matrix metalloproteinase

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Conflict of Interest

Nil

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