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Association of IL-17A rs2275913, IL-23R rs11209026 polymorphisms and serum level of IL-17A with rheumatoid arthritis in Egyptian patients

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Abstract

Several studies have reported genetic polymorphisms at the IL-23/IL-17 axis linked to rheumatoid arthritis (RA) in many populations. We aimed to investigate the association of IL-17A rs2275913 and IL-23R rs11209026 polymorphisms with susceptibility to RA and, disease clinical features and the serum level of IL-17A in Egyptian patients. This case-control study included 94 RA cases and 74 controls. TaqMan genotyping assays were used for detection of gene polymorphism and the enzyme-linked immunosorbent assay was used to quantify IL-17A serum level. There was significant difference between RA patients and controls in genotypic distribution and allelic frequency of IL-17A rs 2275913 (p <0.0001). The GG genotype had 7 times higher risk for RA development (OR=7.04: 95% CI 2.11:23.46, p value= 0.001). Also, GG genotype was associated with higher level of serum IL-17 A compared to GA and AA genotypes (p<0.0001). Moreover, patients carrying the GG genotype had higher disease activity score 28 (DAS28) score (4.99±0.84) compared to patients with GA (2.73±0.52, p<0.0001) and patients with AA genotypes (2.67±0.41, p<0.0001). Genotypic distribution of IL-23R rs11209026 was significantly different between RA patients and controls (p <0.0001), but there was no difference between the allelic frequency in both groups (p=0.08). IL-23R rs11209026 SNP was not a risk for RA development. However, DAS28 was lower in AA genotype than AG and GG genotypes (p=0.002, p=0.009 respectively). The mean serum IL-17A level was higher among the RA patients (39.07±10.47 pg./ mL) compared to controls (15.23±1.88 pg/ mL; p <0.0001). Also, there was a positive correlation between IL-17A serum level and DAS28 score (Spearman r = 0.42; p value <0.0001). We concluded that the variant IL-17A (rs2275913) genotype could be a risk factor for RA in our population and IL-17A may play a crucial role in the development and pathogenesis of RA.

Keywords: Rheumatoid arthritis, IL-17A, IL-17A 197 GA rs2275913, IL-23R Arg381Gln rs11209026, SNP.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder with synovitis and varying degrees of damage to articular cartilage and bone. It could result in joint deformity and disabilities.[1] The prevalence of RA varies widely according to the geographical region. In 2019,

according to the Global Burden of Disease (GBD) study, the prevalent cases of RA globally were 18.6 million (95% UI: 17.0–20.4), and the age-standardized point prevalence of 224.2 per 100,000 population (95% UI: 204.9–246.0).[2] In 2019, the RA prevalence in Egypt was 84,338 (95% UI :70531- 100823) with an age-standardized point prevalence of 98.1 per 100,000 population (95% UI: 83.8- 115.7), and the incidence was 4763 cases (95% UI: 4071, 5605) with the annual incidence rate of 4.9 (4.2, 5.7).[3]

The etiology of RA is unknown, and several factors including genetic, immunological, and environmental factors are involved. The immunological mechanism of RA inflammation is initiated by formation of autoantibodies as anti-citrullinated peptide antibodies (ACPAs).[4] T cells recognize self-antigens presented by dendritic cells. $CD4^+$ T cells differentiate into T helper (Th) cells, among which T helper 17 (Th17) cells develop. Th17 secrete interleukin-17A (IL-17) which play a key role by mediating the proliferation of the sub lining synovial fibroblasts and innate immune cells such as neutrophils and macrophages and induce production of pro-inflammatory cytokines, like tumor necrosis factor (TNF), interleukin-6 (IL-6), IL-1 and chemokines, such as chemokine (C-C motif) ligand 20 (CCL20) and C-C Motif Chemokine Ligand 2 (CCL2), which enhance cell migration resulting in inflammation and bone destruction.[5] In addition, Th17 cells control the activity of autoantibodies by regulating antibody glycosylation which in turn affect the onset and progression of the RA.[6] The cytokine IL-23 is responsible for maintenance and expansion of Th17 cells. It enhances secretion of IL-17 by Th17 cells. IL-23R, is expressed on activated Th17 cells and not naïve T-cell.[7] Binding of IL-23 with its receptor complex activates the signal transducer and activator of transcription 3 (STAT3) signaling in Th17 cells that induce Th17 differentiation to gain effector functions including expression of proinflammatory cytokines IL-17, granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon gamma (IFN-γ).[4]

Polymorphisms of genes of the IL-23/Th17 immune axis may alter their shapes and expression, hence might have a role in the development and pathogenesis of RA.[8] Single nucleotide polymorphism (SNPs) of the IL-17A gene include IL-17 rs 763780, rs 2275913 and rs 8193036 SNPs may affect IL-17 expression.[9] The SNP rs 2275913 (G 197 A) is located at IL-17A gene promoter region which might affect gene transcription, increasing IL-17A secretion. Many studies linked this SNP to susceptibility to RA,[10–12] systemic lupus erythematosus (SLE),[13] inflammatory bowel disease[14] and other autoimmune diseases. IL-23R gene SNP rs 11209026 GA result in missense variant and were linked to several autoimmune conditions such as psoriasis[15], Ankylosing spondylitis[16] inflammatory bowel disease[17], and SLE[18]. Also, several studies have suggested its relationship with susceptibility to RA.[11,19–21]

Studies in gene polymorphisms are contradicting in different populations due to high genetic diversity. In Egypt, there was few studies on genetic polymorphisms with RA. Therefore, the aim of the current study was to investigate the association of IL-17A *(rs 2275913),* IL-23R *(rs 11209026)* polymorphisms and serum level of IL-17A with susceptibility to RA and disease activity in a sample from Upper Egypt population.

Subjects and Methods

Study design

This was a case-control study, conducted in the Departments of Medical Microbiology and Immunology, Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Sohag University Hospital during the period from January 2020 to January 2022.

The study included 92 patients with rheumatoid arthritis, and a control group of 74 volunteers, age and sex matched to the patient's group. Exclusion criteria included patients less than 18 years or with other autoimmune diseases.

Ethical consideration

The study protocol was reviewed and approved by the Medical Research Ethics Committee, Faculty of Medicine, Sohag University. An informed written consent was obtained from

each study participant, before included in the study.

Data collection

Data were collected from the rheumatoid arthritis patients' group, included full history taking including age, sex, disease duration, age of disease onset, duration of morning stiffness, presence of extra-articular manifestations and current treatment in addition to full clinical and musculoskeletal examination. Routine laboratory tests were done for all patients at the hospital laboratory and recorded. These included rheumatoid factor (RF), anti-cyclic citrullinated peptide (AntiCCP) antibodies, erythrocyte sedimentation rate (ESR) mm/1st hour, serum C-reactive protein (CRP).

The diagnosis of RA was done according to diagnostic criteria of the American College of Rheumatology (ACR) [22]. Severity was assessed by disease activity score 28 (DAS28) which depend on assessment of 28 joints, CRP or ESR tests. The overall score was calculated by a mathematical formula and scores greater than 5.1 suggested highly active disease, between 3.2 and 5.1 indicated moderate disease activity, less than 3.2 low disease activity, and less than 2.6 indicated a state of remission[23]. As for, the control group demographic and clinical data as age, sex and complete medical history were recorded in a preformed study sheet.

Laboratory methods

The following tests were done to all the study groups.

Quantitative detection of serum IL-17A by enzyme linked immunosorbent assay (ELISA)

Under complete aseptic conditions a blood sample (5 ml) was obtained from each subject in a plane tube. Samples were transported directly to the laboratory and centrifuged at 2000 xg for 20 minutes. Serum was collected from the upper part of the tube then transmitted to a microcentrifuge tube (1.5 ml) and stored at -20°C until used. The ELISA technique was done by a commercial kit (Catalog # BMS2017, Invitrogen; Thermo Fisher Scientific Inc., USA), according to the manufacturer instruction. Absorbance was read at 450 nm using a microplate reader (Stat Fax 2100 model, Awareness Technology, Inc. USA).

Detection of IL-17A rs2275913 and IL-23R rs11209026 genes polymorphism by Taq Man SNP genotyping assays

Another blood sample (5 ml) was withdrawn in EDTA tube from each subject under aseptic conditions, used for detection of genes polymorphism. For DNA extraction, blood samples were immediately transported to the laboratory and centrifugated at 2000 xg for 20 minutes at room temperature. After centrifugation, the buffy coat was aspirated by micropipette, adjusted to 200 µl and transformed to a microcentrifuge tube (1.5 ml), and used for DNA extraction. DNA was extracted using a commercial kit (QIAamp DNA Mini Kit, QIAGEN, Germany), according to the manufacturer's instructions. The extracted DNA was stored at -60° C until the genotyping assay was done.

The SNP genotyping assay was carried out using a commercial kit (Taq Man SNP genotyping assays supplied by Applied Biosystems, Inc., Foster City, CA, USA). The TaqMan™ SNP Genotyping Assay for *197 G/A IL-17A (rs2275913)* used SNP primers with context sequence (VIC/FAM): TGCCCTTCCCATTTTCCTTCAGAAG [A/G]AGAGATTCTTCTATGACCTCATTGG. The TaqMan™ SNP Genotyping Assay for IL-23R rs11209026 used SNP primers with context sequence [VIC/FAM]: ATTGGGATATTTAACAGAT CAT TCC[A/G]AACTGGGTAGGTTTTTGCAGAATTT. In both assays one probe was labeled with VIC dye to detect the A allele and the other labeled with FAM dye to detect the G allele.

The total volume of the genotyping assay reaction was 25 µL. For each genotyping assay the following were used; Taq Man universal PCR Master Mix (2X) (12.5 µL) supplied by (Applied Biosystems, Foster City, CA, USA), working stock of TaqMan™ SNP Genotyping assay (20X) (1.25 µL) supplied by (Applied Biosystems. Foster City, CA, USA), DNase free water (7.25 µL) and four µL of the extracted genomic DNA was added to each tube. The tubes were capped and inverted several times to mix. The real- time PCR

amplification was performed by Step One™ Real-Time PCR System (Applied Biosystems ™, Thermo Fisher, USA). The cycling conditions were as follows: 10 minutes at 95 °C for Ampli Taq Gold enzyme activation, followed by 40 cycles each of denaturation step at 95 °C for 15 seconds, primer annealing at 60 °C for 1 minute and a final primer extension at 72 °C for 5 minutes. The sequence detection system (SDS) software (Applied Biosystems, CA, USA) was used. The fluorescence measures made during the plate reading was used to plot the fluorescence (rn) values based on the signals from each well to draw the allelic discrimination plot automatically. The horizontal axis indicates an allele AA homozygote, the vertical axis indicates allele GG homozygote, and the diagonal is allele AG heterozygote.

Statistical analysis

Data were analyzed using the Stata Statistical Software (STATA version 14.2, Release 14.2 College Station, TX: Stata Corp LP.). Quantitative data were represented as mean, standard deviation, median and range. Student t-test was used to compare the means of two groups and ANOVA for comparison of the means of three groups or more. For non-normally distributed data, Kruskal Wallis test for comparison of three or more groups and Mann-Whitney test was used to compare two groups. For qualitative data, they were presented as number and percentage and compared using Chi square test or fisher exact test. The receiver operating characteristic (ROC) curve analysis was used to detect the best cutoff. Also, the sensitivity, specificity, positive predicted value, and negative predictive value were calculated. Spearman's correlation tests were used. The logistic regression analysis was used to calculate Odds ratios. Graphs were produced by using Excel or STATA program. *p* value was considered significant if it was less than 0.05.

Results

Demographic characteristics of the study groups

RA patients included 84 females and 8 males; their ages ranged from 23 to 80 years. The control group included 66 females and 8 males, with ages ranged from 25 to 60 years and a mean of 45.75±8.97 years. The two groups were age and sex matched.

Clinical characteristic of rheumatoid arthritis patients

The mean age of RA patients at disease onset was 39 years and 56 (60.87%) showed severe disease activity, 47.8% had deformity, 88 (95.65%) were rheumatoid factor positive and 78 (84.78%) were AntiCCP positive. The mean serum level of IL-17A in RA patients was 39.07±10.47 pg./ mL, higher than in the controls group 15.23±1.88) pg/ mL (*p* <0.0001). Detailed clinical features of RA patients are shown in Table 1.

Table 1. Demographic, clinical and laboratory characteristics of the rheumatoid arthritis patients and controls.

	RA patients N=92	Controls N=74	*p value*
Age/year			
Mean ± SD	47.09±10.57	45.75±8.97	NS
Median (range)	50 (23:80)	50 (25:60)	
Gender			
Female	84 (91.30%)	66 (89.18%)	NS
Male	8 (8.70%)	8 (10.81%)	
Age at disease onset /year			
Mean ± SD	39.0±10.47	-	
Median (range)	39.5 (17:76)		

Table 1. Continued.

	RA patients N=92	Controls N=74	*p value*
Disease duration/year			
Mean ± SD	7.92±5.55		
Median (range)	6.5 (0.25:20)	–	
RF (IU/ml)			
Mean ± SD	312.67±1044.25		
Median (range)	195.2 (0.5:7200)	–	
AntiCCP (U/ml)			
Mean ± SD	263.41±330.69		
Median (range)	128 (8:1280)	–	
Serum IL-17A level			
Mean ± SD	39.07±10.47	15.23±1.88	<0.0001
Median (range)	40 (13.9:55)	14.4 (10.8:18.0)	
DAS28			
Mean ± SD	4.55±1.20		
Median (range)	5.22 (2.01:5.86)		
Disease activity			
Remission	6 (6.52%)		
Low activity	12 (13.04%)		
Moderate	18 (19.57%)	–	
Sever	56 (60.87%)		
Deformity			
No	48 (52.17%)		
Yes	44 (47.83%)	–	
Skin nodule			
No	84 (91.30%)		
Yes	8 (8.70%)	–	
Raynaud's phenomena			
No	68 (73.91%)		
Yes	24 (26.09%)	–	
Sjogren syndrome			
No	66 (71.74%)		
Yes	26 (28.26%)	–	
Morning stiffness (> 1hr)			
No	44 (47.83%)		
Yes	48 (52.17%)	–	
Treatment			
Methotrexate,	86 (93.48%)		
Hydroxy chloroquine, NSAID	6(6.52%)	–	
Diabetes			
No	42 (45.65%)		
Yes	50 (54.35%)	–	
Hypertension			
No	40 (43.48%)		
Yes	52 (56.52%)	–	

P > 0.05 is not significant (NS).

Genotypic distribution and allele frequency of IL-17A rs2275913 and IL-23R rs11209026 polymorphisms in the RA patients and controls groups

There was a statistically significant difference between RA patients and controls in genotypic distribution and allelic frequency of IL-17A (rs2275913) (p <0.0001). Subjects carrying the GG genotype were significantly associated with increased risk of rheumatoid arthritis about 7 times than controls (OR=7.04, 95% CI: 2.11-23.46, p=0.001). Also, there was a statistically significant difference between RA patients and control in genotypic distribution of IL-23R rs 11209026 (p<0.0001) but there was no difference between the G allele and the A allele in RA patients and controls (p=0.08). Table 2

Table 2. Genotypic distribution and allele frequency of IL-17A rs2275913 polymorphisms and IL-23R rs11209026 polymorphism in the RA patients and controls groups.

Genotype	RA patients no. (%)	Controls no. (%)	*p* value*	OR (95% CI)	*p* value
IL-17A rs2275913					
AA	6 (6.52%)	8 (10.81%)		1	
AG	12 (13.04%)	52 (70.27%)	<0.0001	0.31 (0.09:1.05)	NS
GG	74 (80.43%)	14 (18.92%)		7.04 (2.11:23.46)	0.001
G allele vs. A allele	160 (86.96%) vs. 24 (13.04%)	80 (54.05%) vs. 68 (45.95%)	<0.0001	3.0 (1.61:5.62)	0.0001
IL-23R rs11209026					
AA	4 (4.35%)	6 (8.11%)		1	
AG	6 (6.52%)	8 (10.81%)	<0.0001	1.13 (0.22:5.86)	NS
GG	82 (89.13%)	60 (81.08%)		2.05 (0.55:7.58)	NS
G allele vs. A allele	170 (92.39%) vs. 14 (7.61)	128 (86.49%) vs. 20 (13.51%)	NS	1.90 (0.87:4.22.91)	NS

P* from X^2, OR odds ratio, CI confidence interval, p value from logistic regression analyses, $P > 0.05$ is not significant (NS).

Relation between IL-17A rs2275913 polymorphisms demographic and clinical features of RA patients

There was an increase in the mean of the DAS28 score for the patients carrying the GG genotype (4.99±0.84) compared to patients with GA (2.73±0.52, p<0.0001) and patients with AA genotypes (2.67±0.41, p<0.0001). Also, the mean serum level of IL-17A was significantly higher in patients with GG genotype than AG and AA genotypes. However, patients with AG genotype had more deformity than GG genotype (p=0.01). Also, skin nodules and morning stiffness were significantly higher in patients with IL-17 AA genotype than those carrying AG and GG genotypes (Table *3*).

Table 3 Relation between IL-17A rs2275913 polymorphisms with demographic and clinical features of RA patients.

	IL-17A rs2275913 polymorphisms			*p* value
	AA (N=6)	AG (N=12)	GG (N=74)	
Age/year				
Mean ± SD	51.66±9.24	41.83±8.70	47.57±10.86	NS
Median (range)	57 (41:57)	38.5 (33:55)	50 (23:80)	
Gender				
Female	6 (100%)	12 (100%)	66 (89.19%)	NS
Male	0	0	8 (10.81%)	

Table 3 Continued.

	IL-17A rs2275913 polymorphisms			*p* value
	AA (N=6)	AG (N=12)	GG (N=74)	
Age at disease onset /year				
Mean ± SD	45.0±8.0	33.03±11.27	39.35±10.40	NS
Median (range)	45 (37:53)	34.5 (17:50)	40 (20:76)	
Disease duration/year				
Mean ± SD	10.0±8.72	8.10±7.96	7.72±5.01	NS
Median (range)	6 (4:20)	6.5 (0.25:20)	8 (0.25:20)	
RF (IU/ml)				
Mean ± SD	256±0	169.33±188.53	279.27±360.59	NS
Median (range)	265 (256:256)	96 (24:512)	128 (8:1280)	
AntiCCP (U/ml)				
Mean ± SD	175.2±157.07	189.03±109.07	343.87±1164	NS
Median (range)	220 (0.6:305)	200 (29.2:305)	195 (0.5:7200)	
ESR (mm/1st h)				
Mean ± SD	58.33±32.53	40.17±23.66	48.73±24.57	NS
Median (range)	60 (25:90)	40 (13:70)	41 (13:115)	
Serum IL-17A				
Mean ± SD,	22.53±7.54,	27.02±3.87, 27.75	42.36±8.58,	0.0001
median (range)	25.9 (13.9:27.8)	(20:30.9)	40.8 (30:55)	
*p*1= 0.69, *p*2 <0.001, *p*3<0.001				
DAS28				
Mean ± SD	2.67±0.41	2.73±0.52	4.99±0.84	<0.0001
Median (range)	2.41 (2.41:3.2)	2.76 (2.01:3.28)	5.28 (2.46:5.86)	
*p*1=1.00, *p*2<0.0001, *p*3<0.0001				
Deformity				
No	4 (66.67%)	2 (16.67%)	42 (56.76%)	0.03
Yes	2 (33.33%)	10 (83.33%)	32 (43.24%)	
*p*1=0.11, *p*2=1.00, *p*3=0.01				
Skin nodule				
No	4 (66.67%)	10 (83.33%)	70 (94.59%)	0.04
Yes	2 (33.33%)	2 (16.67%)	4 (5.41%)	
*p*1=0.57, *p*2=0.06, *p*3=0.20				
Raynaud's phenomena				
No	4 (66.67%)	10 (83.33%)	54 (72.97%)	NS
Yes	2 (33.33%)	2 (16.67%)	20 (27.03%)	
Sjogren syndrome				
No	4 (66.67%)	8 (66.67%)	54 (72.97%)	NS
Yes	2 (33.33%)	4 (33.33%)	20 (27.03%)	
Morning stiffness				
No	0	6 (50.00%)	38 (51.35%)	0.053
Yes	6 (100%)	6 (50.00%)	36 (48.65%)	
*p*1=0.054, *p*2=0.03, *p*3=0.93				

Pairwise comparison was done if $p < 0.05$. *p*1 indicates comparison between AA & AG, *p*2 indicates comparison between AA & GG, and *p*3 indicates comparison between AG & GG. $P > 0.05$ is not significant (NS).

Relation between IL-23R rs11209026 polymorphisms with demographic and clinical features of RA patients

There was an increase in the mean of disease duration in patients with IL-23R AG (12.0±4.73) and GG (7.37±5.25) genotypes (p=0.04). There was decrease in the frequency of skin nodules in patients with IL-23R AA genotype (50%) comparatively higher to those carrying GG (7.32%, p=0.04). Also, DAS28 score was significantly lower in AA genotype than AG and GG genotypes (p=0.002 and p=0.009, respectively) Table 4.

Table 4. Relation between IL-23R rs11209026 polymorphisms with demographic and clinical features of RA patients.

	IL-23R rs11209026 polymorphisms			*p* value
	AA N=4	AG N=6	GG N=82	
Age/year				
Mean ± SD	46.5±12.12	51.0±5.87	46.82±10.75	NS
Median (range)	46.5 (36:57)	50 (45:58)	50 (23:80)	
Gender				
Female	4 (100%)	6 (100%)	74 (90.74%)	NS
Male	0	0	8 (9.76%)	
Age at disease/year				
Mean ± SD	33.5±4.04	38.67±1.37	39.29±10.93	NS
Median (range)	33.5 (30:37)	39 (37:40)	40 (17:76)	
Disease duration/year				
Mean ± SD	13.0±8.08	12.0±4.73	7.37±5.25	0.048
Median (range)	13 (6:20)	10 (8:18)	6 (0.25:20)	
*p*1=1.00, *p*2=0.13, *p*3=0.04				
RF (IU/ml)				
Mean ± SD	148.0±124.71	202.67±240.04	273.49±341.09	NS
Median (range)	148 (40:256)	64 (32:512)	128 (8:1280)	
AntiCCP (U/ml)				
Mean ± SD	110.25±126.73	123.7±79.56	336.37±1097.90	0.47
Median (range)	110 (0.5:220)	145.1 (26:200)	195.4 (0.5:7200)	
ESR (mm/1st h)				
Mean ± SD	52.5±43.30	72.67±25.87	46.24±22.70	NS
Median (range)	52.5 (15:90)	83 (40:95)	41 (13:115)	
Serum IL-17A				
Mean ± SD, median (range)	29.5±4.16, 29.5 (25.9:33.1)	36.93±6.02,40 (30:40.8)	39.21±10.74,40 (13.9:55)	NS
DAS28				
Mean ± SD	2.81±0.46	5.41±0.35	4.57±1.17	0.002
Median (range)	2.81 (2.41:3.2)	5.23 (5.13:5.86)	5.28 (2.01:5.86)	
*p*1=0.002, *p*2=0.009, *p*3=0.25				
Deformity				
No	2 (50.00%)	2 (33.33%)	44 (53.66%)	NS
Yes	2 (50.00%)	4 (66.67%)	38 (46.34%)	
Skin nodule				
No	2 (50.00%)	6 (100%)	76 (92.68%)	0.009
Yes	2 (50.00%)	0	6 (7.32%)	
*p*1=0.13, *p*2=0.04, *p*3=1.00				

Table 4. Continued.

	IL-23R rs11209026 polymorphisms			*p* value
	AA N=4	AG N=6	GG N=82	
Raynaud's phenomena				
No	4 (100%)	4 (66.67%)	60 (73.17%)	NS
Yes	0	2 (33.33%)	22 (26.83%)	
Sjogren syndrome				
No	4 (100%)	4 (66.67%)	58 (70.73%)	NS
Yes	0 (%)	2 (33.33%)	24 (29.27%)	
Morning stiffness				
No	2 (50.00%)	2 (33.33%)	40 (48.78%)	NS
Yes	2 (50.00%)	6 (66.67%)	42 (51.22%)	

Pairwise comparison was done, significant if *p* value <0.05. *p1* indicates comparison between AA & AG, *p2* indicates comparison between AA & GG and, *p3* indicates comparison between AG & GG. *P* > 0.05 is not significant (NS).

Serum level of IL-17A in rheumatoid arthritis patients

The mean serum level of IL-17A concentrations were increasing in patients with more disease activity. The concentrations of IL-17 in the severe and moderate groups were higher than in mild and remission groups (p <0.0001) Table 5.

Table 5. Relation between DAS28 and serum IL-17 level.

Serum IL-17 level	Remission	Mild	Moderate	Severe	*p* value
Mean ± SD	27.97±19.32	27.65±1.85	41.6±10.23	41.89±8.39	<0.0001
Median (range)	20 (13.9:50)	27.7(25.8:30.9)	40(29.55:55)	40.4 (30:55)	

*$P \leq 0.05$ is significant.

Also, there was significant correlation between DAS28 and serum IL-17A and the Spearman correlation coefficient (r) = 0.42 (p<0.0001) Figure 1.

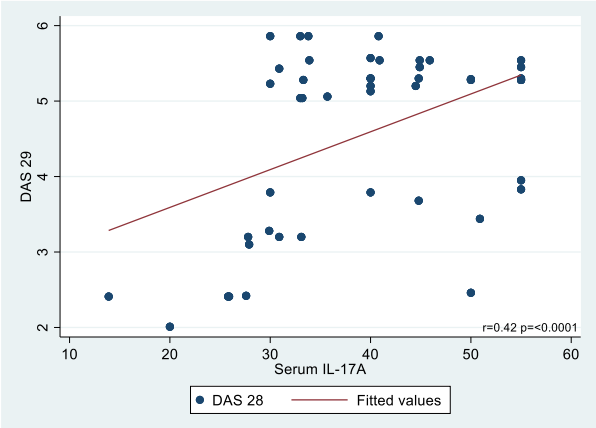

Figure 1. Scatter diagram showing correlation between DAS28 and serum IL-17A.

The ROC curve was used to determine the ability of serum IL-17 level in identifying RA patients. For a 100% specificity, the maximum of sensitivity was 97.8% at a cut-off value of 18.0 pg/ml with an area under the curve of 0.982 (95% CI 0.948:0.996), p<0.0001 Figure 2.

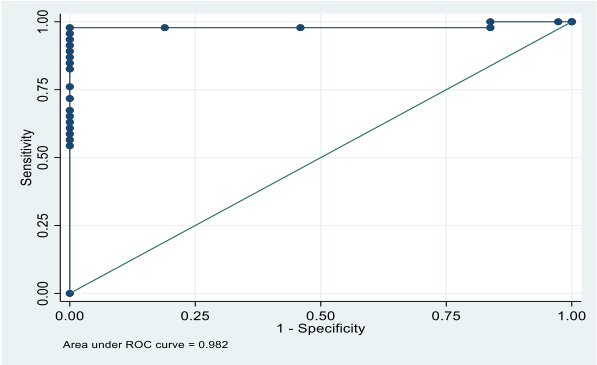

Figure 2. Receiver Operator Characteristic (ROC) curve to evaluate serum IL-17A in diagnosis of RA patients. The area under curve (AUC) was 0.982 (95% CI 0.948:0.996), p<0.0001. A cutoff point of >18.0, showed senstivity=97.8%, specicity=100%, postive predictive value (PPV)=100% negative predictive value (NPP)=97.4.

Logistic regression analysis of risk factors of RA

Univariate logistic regression analysis of risk factors of RA in patients revealed that age, female sex, serum IL-17 level, and AG, GG genotypes of IL-17A (rs 2275913) were the factors significantly associated with RA development. Multivariate logistic regression analysis of predictor variables of RA in patients confirmed that serum level of IL-17A was a predictor for development of RA in patients (odds ratio = 2.68, 95% confidence interval = 1.20- 6.01, $p = 0.02$).

Discussion

In the present study, we investigated the association of IL-17A rs2275913 and IL-23R rs11209026 polymorphisms and serum level of IL-17A with rheumatoid arthritis in Egyptian patients. In this study, the genotypic distribution of IL-17A (rs2275913) showed that GG genotype was significantly high in RA patients compared to the control group (OR=7.04: 95% CI 2.11:23.46, $p = 0.001$). Such data suggest that GG genotype could be a risk factor for RA in our population. Recently similar results were reported by *Silva et al*., *2017* who found that GG genotype was significantly higher in Brazilian patients with RA.[4] Also, Nordang et al., 2009 found that GG allele was significantly higher in RA patients from New Zealand.[25] A recent meta-analysis study by *Shao et al., 2020* found that the rs2275913 G allele was significantly associated with RA in Caucasians [26]. Also, Amin et al., 2021 found that the G allele at rs2275913 polymorphic site was associated with RA patients in sample from Pakistani population.[27]

However, Marwa et al., 2017 found that the risk of RA was associated with IL-17A gene rs2275913 A allele not the G allele in sample from Tunisian population.[28] Also, two studies from Egypt[12] and Tunisia[29] found no significant difference in genotypes and alleles frequency between controls and RA patients, indicating that polymorphism was not linked to RA susceptibility in their studies. The difference between data obtained in the previously mentioned studies can be attributed to different ethnic groups, different detection methods, and small studied sample sizes.

The role of IL-17A rs2275913SNP, located at the promoter region of IL-17 at position -197, was controversial. It was suggested that the wild GG genotype produced more cytokines due to enhanced promotor activity.[8] Data obtained in our study support this point, as we found that there was significant difference between different genotypes as regard the IL-17A serum level and the GG genotype has higher level of IL-17A compared to AA and GA ($p<0.001$).

Furthermore, in our study, there was significant relation between IL-17 (rs2275913) genotype and DAS28 score in RA patients ($p<0.0001$). This agreed with data of a study by Fahmy et al., 2022, who found that RA patients with more active disease (DAS28> 5.1) were carrying wild type *GG* genotype.[30] Also, similar results were reported by Bogunia-Kubik et al., 2015, who found that patients with the IL-17 GG wild-type genotype had more active disease.[31] However, Dhaouadi et al, 2018, reported no relationship between the IL-17A rs2275913 GA polymorphism and the disease activity.[32]

In the present study the GG genotype of IL-23R rs11209026 was the predominant variant in RA patients (89.13%) and in the control group (81.08%). However, the AA genotype was less frequent in RA patients (4.35%) and the control group (8.11%) indicating significant difference between genotype distributions between both groups ($p <0.0001$). However, the difference was not significant at the logistic regression analysis ($p>0.05\%$). These results agreed with those of Soysal et al., 2022, who concluded that GG genotype was detected more frequently in RA patients (92%) and AA genotype less frequently detected in RA patients (2%) with significant variation between cases and controls and G allele more detected in cases and A allele less detected in cases.[33] The same results were observed in an Iranian study in which the AA genotype was less frequently detected in RA patients (2%).[34] Also, in a study conducted in Poland found that IL-23R A variant was rarely detected.[31] And the study by Paradowska-Gorycka et al., 2018, found that the A allele accounted for 5.33%.[35]

An Egyptian study by Hamdy et al., 2015, reported controversial data, they found that the frequency of the AA genotypes of rs11209026 was significantly increased in the patients (95%) compared with the controls (61.7%) (p = 0.001) and suggested that AA genotype variant of rs11209026 would contribute to RA etiology.[36] The same results were reported in a Spanish study, found that *A* allele of IL-23R rs11209026 polymorphism was associated with increased predisposition to RA.[37] Also, *Du et al., 2020*, found that IL23R rs11209026 AA genotype was a risk for RA in Caucasians.[38]

In this study, DAS28 score was significantly lower in AA genotype than the GG and AG genotypes (p=0.002 and 2=0.009, respectively). This may be explained by understanding that this SNP rs11209026 confers a guanine (G) to adenine (A) substitution at DNA level which results in an arginine (R) to glutamine (Q) substitution in position 381 (R381Q) within the cytoplasmic domain of the IL-23R.[19] This rare mutation resulted in functional changes in the IL-23R signal transduction affecting the interaction between IL-23R and its associated Janus kinase 2. This might lead to a reduction in the cellular response to IL-23 and could explain the protective effect of the rare AA allele.[39]

In the present study, serum level of IL-17A was significantly elevated in RA patients (39.07 ± 10.47 pg/ml) than in the control group (15.23 ± 1.88 pg/ml, p<0.0001) and this supports the key role of IL-17 in the pathogenesis of RA. Our result agreed with an earlier Egyptian study that found IL-17 serum level was significantly higher in the RA group than the controls.[30] In the same line, a Tunisian study reported that plasma IL-17A levels were significantly higher in RA cases (55.07 pg/ml) than in controls (4.75pg/ml).[32] Furthermore, the same observation was reported by many studies from different populations.[40, 41,42,43]

In this study there was a significant positive relation between IL-17 serum level and DAS28 (r = 0.42, p<0.0001), the higher serum IL-17 level coincided with higher disease activity in RA, which reflected its contribution in inflammatory process, responsible for RA. This result agreed with several studies that detected a significant correlation between serum levels of IL-17 with disease activity DAS28.[41,42,44] Also, other studies considered serum levels of IL-17 as an indicator for high disease activity and suggested it as a treatment target for patients with poor prognosis.[45 46]

In the present study, by the ROC curve analysis we evaluated the ability of serum IL-17A to detect RA and demonstrated that a cutoff point of ≥ 18.0 pg/ml conferred a sensitivity of 97.8%, with a 100% specificity. These results indicated the possible prognostic and predictive value of IL-17 serum level. This agreed with a study by Altamemi & Alkhafaji, 2018, who found that according to the ROC curve analysis, IL-17 had 100% specificity and sensitivity.[42] Also, Dhaouadi et al., 2018, found that the ROC curve showed 100% specificity and 61.7 % sensitivity at a cut-off value of 18.25 pg/ml.[32] Also, Marwa et al., 2017, found that at cut-off value of 23 pg/ml of serum IL-17, the sensitivity and specificity were 55.56% and 100%, respectively.[28] Also, Siloşi et al., 2016, found that the cut-off value for discrimination between patients with RA and controls was 9.40 pg /mL with 86.67% sensitivity and 100% specificity.[47] However, the use of IL-17 serum level in diagnosis of RA should be combined with other clinical characteristics as it was frequently high in other autoimmune diseases or inflammatory conditions.

The present study had certain limitations including the small sample size. The genotype distributions of IL-17 and IL-23 were not in agreement with Hardy-Weinberg equilibrium. We did not study all polymorphisms associated with each gene which may together affect RA.

In conclusion, data of the current study demonstrated that IL-17A rs2275913 gene polymorphism might have a role in susceptibility to RA in the studied population. The GG genotype IL-17A rs2275913 gene polymorphism could be a risk factor for RA. Also, GG genotype IL-17A could be related to disease activity in RA patients. The serum IL-17A may have a role in RA development and disease activity.

Author Contributions

HAAA wrote the protocol, collected the data, did laboratory work, statistical analysis, shared in writing the manuscript. MFM reviewed the protocol,

laboratory work, reviewed the manuscript. MAE approved the protocol, clinical assessment of the patients, reviewed the manuscript. AMG reviewed the protocol, laboratory work, wrote and submitted the manuscript as the corresponding author.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Medical Research Ethics Committee, Faculty of Medicine, Sohag University.

Informed consent

An informed written consent was obtained from each study participant, before included in the study.

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