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Exploring the Correlation between Interleukin-17A Promoter Polymorphism at its -197 G/A and Rheumatoid Arthritis: Impact on Disease Severity and Activity

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Abstract

T helper 17 (Th17) cells have been reported to be the most powerful factor in autoimmune disorder pathogenesis, which points to the Th17 master cytokine, interleukin (IL)-17A, as the crucial mediator. We aimed to determine the impact of IL-17A polymorphism in the -197 G/A promoter region on level of IL-17 and intensity of rheumatoid arthritis (RA) disease symptoms. This case-control study was conducted at the Department of Clinical Rheumatology of Aswan university Hospital and included 35 people suffering RA and 30 volunteer controls, matched for age and sex. Rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, erythrocyte sedimentation rate (ESR), serum IL-17, and C-reactive protein (CRP) were measured in the RA patient group. Restriction fragment length polymorphism (RFLP) was conducted by polymerase chain reaction (PCR) amplicon obtained by IL-17A -197 G /A primers. Of the 35 RA patients, RF was positive in 33 (94.29%) and anti-CCP antibodies in 25 (71.43%), CRP in 31 (88.57%). Of the 35 RA patients, 5 (14.29%) patients carried the G/G genotype, 18 (51.43%) G/A and 12 (34.29%) A/A. IL-17 serum level was significantly greater in the more active RA (DAS28 >5.1) group than the less active (DAS28 ≤5.1) group. Of the RA patients carrying wild type G/G genotype, 60% had more active disease (DAS 28> 5.1), as compared to those with lower activity (DAS 28 ≤5.1), 40% carried the wild type G/G genotype. In conclusion, the study findings imply that IL-17A gene polymorphism is connected to RA clinical severity rather than with RA susceptibility.

Keywords: rheumatoid arthritis, polymorphism, interleukin -17, RFLP.

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Introduction

Rheumatoid arthritis (RA) is an autoimmune connective tissue disease that affects nearly 1% of the adults around the world in which women are triplicates influenced greater than men. The disease primarily affects the peripheral joints and is characterized by symmetrical, chronic, invasive synovitis, and multiple joints involvement. It progressively ends by cartilage damage, joint destruction, and numerous extra-articular complications leading to joint deformity, functional status subsidence, and early morbidity and mortality.[1]

A recent study has indicated that interleukin (IL)-17 is predominantly elevated in synovial fluid and serum in RA individuals, regardless of whether they are in the early stages of the disease or have not yet undergone treatment.[2] IL-17 was initially recognized as a product of T helper 17 (Th17) cells, a member of CD4+ T cells population that is clearly distinguished from Th1 and Th2. Th17 cells have a crucial function in immune reactions versus extracellular fungi and bacteria. The pathogenesis of psoriasis, inflammatory bowel disease, multiple sclerosis, and spondyloarthropathies is characterized by excessive activation of Th17 cells. [3]

The IL-17 super family comprises six ligands (IL-17A to IL-17F) and five receptor subtypes (IL-17RA to IL-17RE), with the IL-17A being the prototypical ligand. Upon attaching to a receptor, IL-17A induces de novo gene transcription or stabilizes the pro-inflammatory chemokines and cytokines' mRNA to increase the expression of inflammatory genes.[4] Numerous polymorphisms have been found in the IL-17A gene that could potentially impact the expression of IL-17.[5]

Evidence suggested that IL-17A, the primary cytokine of Th17 cells, plays a crucial role in the development of autoimmune diseases due to its superior effectiveness compared to other cells of the immune system. The promoter region of the IL-17 gene contains a SNP rs2275913 (G-197A), which could potentially act as a significant transcriptional regulator.[6]

Positive outcomes have been observed in treating two joint diseases, active ankylosing spondylitis and psoriatic arthritis with IL-17 inhibitors, suggesting that blocking IL-17 may reduce bone degradation and inflammation. Therefore, it is essential to identify patients with RA who would benefit from targeted therapy aimed at IL-17.[7] Consequently, the goal of this study was to determine the impact of IL-17A polymorphism in the -197 G/A promoter region on level of IL-17 and intensity of rheumatoid arthritis (RA) disease symptoms.

Subjects and Methods

This case control study comprised 35 RA patients ranging in age from 18 to 80 years old who were registered at the Department of Clinical Rheumatology, Aswan University Hospital. Patients were classified according to 2010ACR/EULAR criteria.[8] In addition, a control group consisted of 30 normal volunteers, age and sex matched to the patient group were included. A participant was allocated to the control group if his/her medical history revealed no infectious diseases, RA, or other autoimmune disorders. He/she was removed from the control group if one of these conditions existed.

Participants filled out a questionnaire on their social and demographic factors including gender, age, illness duration. Serological and other laboratory tests results including rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were collected from the patient's medical records. We created a Disease Activity Score in 28 Joints (DAS-28) for each patient by mathematical tool including the 28 specified joint assessment along with visualizing global function of the patient and serum inflammatory markers ESR and CRP. A DAS28 score higher than 5.1 is indicative of high disease activity, whereas a DAS28 below 5.1 indicates moderate to low disease activity.

Patients with another systemic connective tissue or autoimmune disease, obvious hepatic or renal functional impairment, hepatic viral infections resistant to therapy, pregnant or breastfeeding women, subjects with a history of

cancer with no cure, uncontrolled diabetes, or alcohol abuse were all excluded from the study.

Methods

Peripheral venous blood samples (5 mL) were collected aseptically from each study subject and divided in two portions. The first aliquot was collected in EDTA tubes and maintained at -80°C until DNA were isolated for genetic analysis. The remaining volume of blood was collected in plain tubes to extract the serum for investigation of IL-17 levels.

Estimation of serum IL -17 level

Serum IL-17 was determined using commercial kits (Human Interleukin 17 ELISA Kit, Bioassay Laboratory Technology (96 wells), China), according to the manufacturers' instructions. A semi-automated ELISA reader system (Tecan, Infinite F-50 microplate absorbance reader, Czech Republic) was used to process the ELISA and to determine optical densities of final products.

Determination of the IL-17A -197 G/A polymorphisms

Genomic DNA was isolated from blood specimens using commercial kits (catalogue # K0781, Gene JET Whole Blood Genomic DNA Purification Mini Kit, Thermo Scientific, USA), according to the manufacturers' instructions. The identification of polymorphisms in the promoter region of IL-17A rs2275913 (-197 G/A) was achieved through a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) approach, utilizing specific primers as previously reported by Zandi *et al*., 2013.[9] These included:

IL-17A -197 G/A forward–TM=72, nucleotide sequence: 5'AACAAG TAAGAATGAAAAGAGGA CATGGT3', and IL-17A -197 G/A reverse – TM = 75, nucleotide sequence: 5'CCCCCA ATGAGG TCATAGAAGAATC3', (Thermo Fisher Custom Primers, USA).

The PCR amplification was performed in a total volume of 20 µl mixture containing: 8 µl genomic DNA, 1 µl of each primer, 10 µl My Red Taq. The sample amplification was performed by using a thermal cycler (VeritiPro thermal cycler, Applied Biosystem, Thermo Fisher, USA). The PCR circumstances comprised initial denaturation cycle of at 94 °C for 7 min, followed by 35 cycles, included a denaturation step at 95 °C for one minute, annealing at 60°C for 60 s, and 50 s extension at 72 °C. The last extension step included one cycle at 72 °C for 7 min.

Digestion of PCR products was performed by incubating 10 µl of PCR products with 1 µl of XagI enzyme (Lot No 00675907," XagI" FastDigest enzyme, Lithuania) and 2.5µl FastDigest Buffer 10 X at 37°C for 1 hour. The amplified PCR products and the restriction fragments were extracted by electrophoresis in a 2 % agarose gel and visualized after staining with Ethidium bromide solution. Electrophoresis power supply (code No.020-400, Biometra Whatman company) was adjusted at 80 V for 1h. We were capable of having distinct banding patterns demonstrated in similar studies by Zandi *et al*., 2013.[9] The amplified PCR products were 102 base pair (bp), GG showing two bands (68 and 34 bp), GA showing three bands (102, 68, and 34 bp), and AA (102 bp) (Figure 4).

Statistical Analysis

The Stata Statistical Software: Release 14.2 (STATA 14.2 College Station, TX: StataCorp LP) was used to analyze the data. The mean and standard deviation (SD) were used to express quantitative data. The Kruskal Wallis test was utilized to compare three or more groups since the data were not normally distributed, and the Mann-Whitney test was utilized to compare two groups. The Chi square test was utilized to compare qualitative data, presented as numbers and percentages. The receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff for many indicators that predict the existence of RA and the specificity, sensitivity, positive and negative predictive values. The ROC curve was also utilized to distinguish between more active and less active illness. From the analysis of logistic regression, odds ratios were derived. Graphs were created with the STATA or Excel tools. A *p* value less than 0.05 was considered significant.

Results

Out of the 35 rheumatoid arthritis patients, 30 (85.71 %) were females, significantly higher than males 14.29 % (p=0.004). The control group consisted of 30 individuals, with 16 females (53.33 %) and 14 males (46.67 %). The mean age of RA patients was 51.37 ± 12.83 years, while that of controls was 41.3 ± 13.63 years (Table 1).

Table 1 shows the ongoing medications, laboratory, and clinical features of RA patients. RF was positive in 33 patients (94.29 %), anti-CCP antibodies positive in 25 patients (71.43 %), and CRP positive in 31 patients (88.57%). DAS-28 varied between 2.7 to 7.1 with a mean of 5.28 ± 1.24. Patients with more active disease (DAS28 >5.1) were 14 patients (40%).

Table 1. Demographic data of study subjects and, biological and clinical parameters of the rheumatoid arthritis patients.

Variable	Rheumatoid arthritis N=35	Controls N=30	*p value
Age/year (Mean±SD)	51.37 ± 12.83	41.3 ± 13.63	0.006
Gender			
Female	30 (85.71%)	16(53.33%)	0.004
Male	5 (14.29%)	14(46.67%)	
RF n (%)			
Positive	33 (94.29%)	---	---
Anti-CCP antibodies n (%)			
Positive	25 (71.43%)	---	---
ESR mn/h (Mean ± SD)	52.97 ± 27.78	---	---
CRP n (%)			
Negative	4 (11.43%)	---	---
Positive	31 (88.57%)		
DAS – 28 (Mean ± SD)	5.28 ± 1.24	---	---
DAS - 28 n (%)			
Less active disease (DAS 28 ≤5.1)	21 (60.00%)	---	---
More active disease (DAS 28>5.1)	14 (40.00%)		
IL-17 serum level pg/ml (Mean±SD)	77628.01±185630.9	127.96±164.07	<0.0001

RF rheumatoid factor; Anti-CCP: antibody to cyclic citrullinated peptides; ESR: erythrocyte sedimentation rate; CRP: c-reactive protein; DAS: disease activity score 28; N: number; SD: standard deviation. *$p \leq 0.05$ is significant.

The RA group had a significantly higher mean (±SD) IL-17 serum level (77628.01 ± 185630.9) compared to the control group (127.96 ± 164.07) (p<0.0001) (Table 1). There was no correlation between IL-17 serum level and demographic or laboratory features of RA, such as gender, RF positivity, anti-CCP antibodies, and CRP. However, IL-17 serum level was significantly higher in the more active RA group (DAS28 >5.1) (193543.8 ± 256916.8) compared to the less active group (DAS28 ≤5.1) (350.88 ± 246.8) (p= 0.0001) (Figure 1).

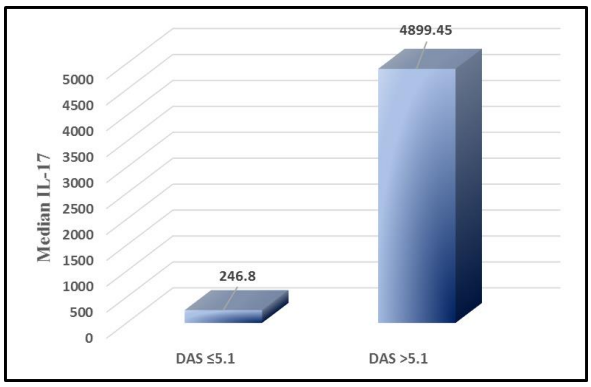

Figure 1. Distribution of serum IL-17 by disease activity disease activity score in 28 Joints (DAS-28).

Associations between the mean IL-17 serum levels and age, ESR levels, and DAS-28 were analyzed in RA patients. A moderate positive correlation was found between IL-17 serum levels and DAS-28 (correlation coefficient r = 0.76, $p < 0.0001$ (Table 2).

Table 2. Relationship between serum IL-17 levels, age, erythrocyte sedimentation rate (ESR), and disease activity score in 28 Joints (DAS -28) in patients with rheumatoid arthritis.

Variable	Correlation coefficient (r)	*p value
Age/year	0.15	NS
ESR	0.30	NS
DAS-28	0.76	<0.0001

*p > 0.05 is not significant (NS).

To determine whether serum IL-17 concentration can be used for diagnosis of RA, we used the ROC curve analysis. The analysis showed that IL-17 at a cutoff value of > 157 (pg/ml) had a sensitivity of 82.86%, a specificity of 86.67% with an area under the curve of 0.902; p <0.0001, CI 95% = 0.803±0.962 (Figure 2).

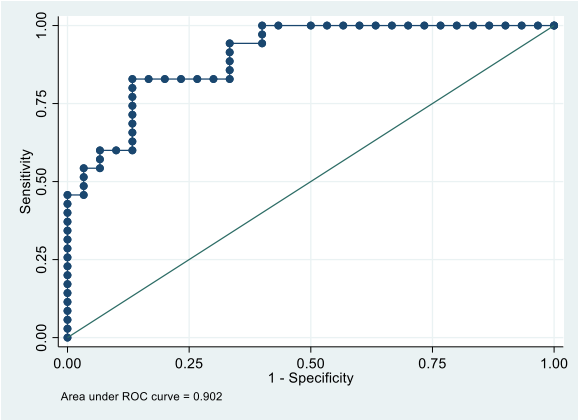

Figure 2. Receiver operating characteristic (ROC) curve analysis to determine the sensitivity and specificity of serum IL-17 levels for diagnosis of rheumatoid arthritis (RA).

Another ROC curve was plotted (Figure 3) to determine the ability of serum IL-17 levels to distinguish between more and less active RA. The results indicated that a cutoff value of 506.7 (pg/ml), IL-17 has a sensitivity of 90.48% and a specificity of 100%, with an area under the curve of 0.990, and the confidence interval at 95% was between 0.881 and 1.00 (p<0.0001).

17 A -197 G/A polymorphism banding patterns in rheumatoid arthritis patients (Figure 4).

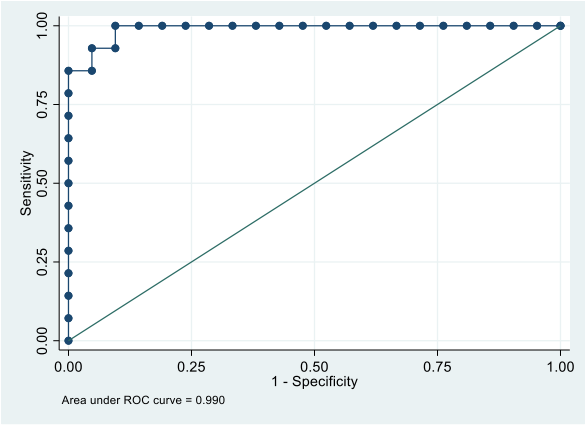

Figure 3. Receiver operating characteristic (ROC) curve to determine the ability of serum IL-17 level to distinguish between more active and less active rheumatoid arthritis (RA).

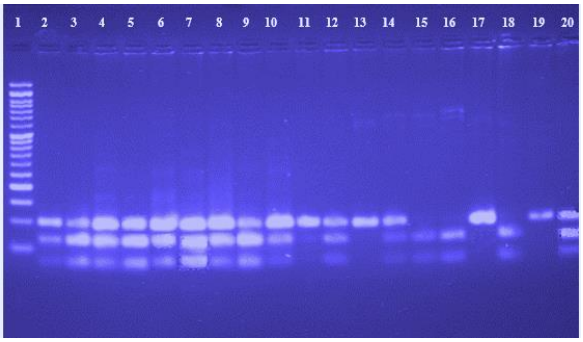

Figure 4. Photograph of polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) gel electrophoresis of IL-17A -197 G/A polymorphisms in rheumatoid arthritis patients after digestion using XagI enzyme. The figure shows fragments of IL-17A -197 G/A, Lane 1: 50 bp DNA ladder, lanes: 2–10, 20 (GA =102, 68, and 34 bp), lanes: 11, 13, 17, 19 (AA = 102 bp) and lanes: 15, 16, 18 (GG= 68, 34 bp) genotypes.

IL-17A -197 G/A genotyping results in RA patients and controls is demonstrated in (Table 3). There were no differences in genotyping between the two groups.

Table 3. IL-17A -197 G/A genotype in rheumatoid arthritis (RA) patients and controls.

Variable	Rheumatoid arthritis N=35	Controls N=30	*p value	Odds ratio (95% CI)
Genotype				
G/G	5 (14.29%)	5 (16.67%)		
G/A	18 (51.43%)	18 (60.00%)	NS	
A/A	12 (34.29%)	7 (23.33%)		
G/A+A/A	30 (85.7%)	25 (83.33%)	NS	1.20 (0.31:4.67)

$p > 0.05$ is not significant (NS).

There was no relation between IL-17A -197 G/A polymorphism and different studied parameters as shown in (Table 4). However, there was a relation between polymorphism and disease activity, as 60% of rheumatoid arthritis patients were with more active disease (DAS 28> 5.1), carried wild type G/G genotype compared to 40% of those with lower activity (DAS 28 ≤5.1 (Figure 5).

Table 4. Relation of the three IL-17A -197 G/A genotypes with different studied variables in the rheumatoid arthritis patients.

Variable	G/G N=5	G/A N=18	A/A N=12	p value	Odds ratio (95% CI)
Age/ year (Mean ± SD)	53.8±6.01	48.89±13.59	54.08±13.75	NS	
Gender					
Female	4 (80.00%)	16 (88.89%)	10 (83.33%)	NS	
Male	1 (20.00%)	2 (11.11%)	2 (16.67%)		
RF					
Negative	1 (20.00%)	1 (5.56%)	0	NS	
Positive	4 (80.00%)	17 (94.44%)	12 (100%)		
ACCP					
Negative	1 (20.00%)	7 (38.89%)	2 (16.67%)	NS	
Positive	4 (80.00%)	11 (61.11%)	10 (83.33%)		
ESR (Mean ± SD)	65.4±17.98	46.17±31.24	58.0±24.20	NS	
CRP					
Negative	0	4 (22.22%)	0	NS	
Positive	5 (100%)	14 (77.78%)	12 (100%)		
IL-17 (Mean ± SD)	133639±294299	69237±175457	66876±160528	NS	
DAS (Mean ± SD)	6.07±1.03	4.82±1.23	5.63±1.13	0.047	
DAS					
≤5.1	2 (40.00%)	14 (77.78%)	5 (41.67%)	NS	0.2 (0.03:1.06) p=0.03
>5.1	3 (60.00%)	4 (22.22%) [a]	7 (58.33%)		

RF: rheumatoid factor; ACCP: antibody to cyclic citrullinated peptides; ESR: erythrocyte sedimentation rate ;
CRP: c-reactive protein; DAS: disease activity score 28; N: number; SD: standard deviation. $p > 0.05$ is not significant (NS).

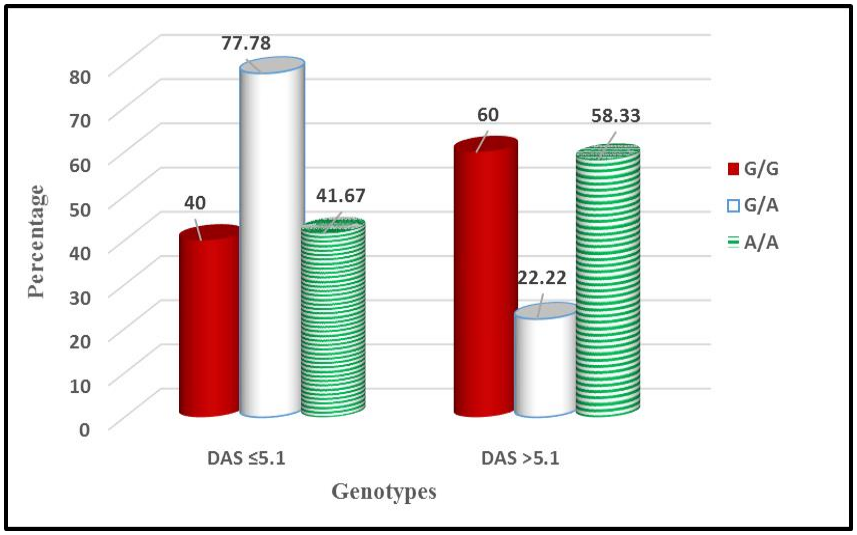

Figure 5. Comparison of the three IL-17A -197 G/A genotypes in rheumatoid arthritis patients according to disease activity.

Discussion

The present study aimed to determine the impact of IL-17A polymorphism in the -197 G/A promoter region on level of IL-17 and intensity of RA disease symptoms. Our study on genotyping IL-17A -197 G/A in RA patients and controls did not show any significant variations in the distribution of genotypes or frequencies of alleles between the two groups. The percentages of G/G, G/A, and A/A genotypes in RA patients were 14.29 %, 51.43 %, and 34.29%, respectively, they were not different in the controls as they were 16.67 %, 60.00 %, and 23.33% (p= 0.63).

Our results are consistent with those reported by Elfasakhany et al*.,* 2018,[10] who found no difference in allele frequency or genotype between controls and RA patients for the IL-17 A or IL-17 F gene polymorphisms. This suggests that these polymorphisms may not be associated with susceptibility to RA in the Egyptian population. Similarly, Dhaouadi et al., 2018,[11] found no significant correlation between vulnerability to disease and IL-17A rs2275913 in the Tunisian population.

Carvalho et al., 2016,[12] found no correlation between the allelic frequency and genotypes of IL-17A (-197 G/A) and IL-17F (7488T/C) genes in the Brazilian population with susceptibility to RA or Sjogren's disease. The study did not yield statistically significant results regarding how these polymorphisms affect clinical and laboratory markers of the diseases. Similarly, Erkol et al., 2015,[13] found no discernible difference in the allelic frequency and genotype distributions of IL-17A G-197A, IL-17F (7383A/G, and 7488A/G) polymorphisms between RA patients and controls in the Turkish population.

Contrary to our initial search, Osman et al., 2021,[14] found a significant correlation between the IL-17A AA genotype and RA disease. In their study, 95 (93.1%) of the control group and 7 (6.9%) of the case group had the IL-17A homozygote AA genotype, (p=0.001). However, there were no differences in the GG and AG genotypes between the patient and control groups. Shen et al., 2015,[15] also confirmed the presence of the AA genotype in the Chinese RA patients and observed a reduced risk for RA associated with this genotype.

Nordang et al., 2009,[16] conducted a study on patients with RA from Norway and New Zealand to investigate the link between the IL-17A gene's promoter region SNP rs2275913 and RA. A weak correlation was observed among Norwegian RA patients, but this link was not confirmed in the New Zealand RA cohort. However, the IL-17A G allele was found to be associated with a modest risk (OR = 1.17, 95 percent CI = 1.02-1.34). According to Gomes da Silva et al., 2015,[17] the -197 G/A IL-17A polymorphisms are linked to RA etiology but not

to clinical parameters. The GG genotype is strongly associated with higher susceptibility to RA, as people with this genotype have a three-fold greater chance of developing RA compared to controls (OR 3.18; 95 percent CI=1.13-9.95; *p*= 0.033). The contradictory results between different studies may be due to genetic variation among different populations, and population differences could be the fundamental explanation for this variability.

The current study observed a relationship between disease activity and polymorphism. Patients with higher disease activity (DAS 28> 5.1) showed a larger percentage of G/G genotype (60%) than those with lesser activity (DAS 28 5.1), who had a lower percentage (40%) of wild type G/G genotype. Bogunia-Kubik et al., 2015,[18] found a weak relationship between the -197 G/A IL-17A polymorphism and illness activity score in a Polish population. Female patients carrying the G/G homozygous genotype represented the most severe disease while under anti-tumor necrosis factor (TNF) treatment with a poor response to it. In their study, Dhaouadi et al., 2018,[11] observed a connection between the IL-17A A/A genotype and subcutaneous nodules, as well as the IL-17A G/G genotype and osteoporosis but did not observe any relationship between the IL-17A polymorphism and RA activity. Meanwhile, Pawlik et al., 2016,[19] did not identify any association between the -197 G/A IL-17A and +7488 A/G IL-17F polymorphisms and age of onset of disease, rheumatoid factor, or erosion incidence in RA patients. The RA group exhibited a notably higher level of IL-17 serum level (77628.01±185630.9) in comparison to the control group (127.96±164.07). Among the RA group, the active subgroup (DAS28 >5.1) had a significantly higher IL-17 serum level (193543.8±256916.8) than the non-active (DAS28 ≤5.1) group (350.88±246.8) with positive association between IL-17 serum level and DAS-28 (correlation co-efficient r = 0.76, p< 0.0001). No association was found between the IL-17A polymorphism with serum IL-17 concentration, which is consistent with findings of Dhaouadi et al., 2018,[11] that there is no link between the IL-17A polymorphism and plasma IL-17A concentration. However, higher IL-17 plasma concentrations were observed in individuals compared to controls, and there was a significant correlation with RA activity based on analysis of IL-17 plasma levels, as also noted by the study of Azab et al., 2020.[20] Lee & Bae, 2017,[21] meta-analyses confirmed this correlation between RA and elevated levels of circulating IL-17 as well.

In conclusion, this study findings suggest that IL-17A gene polymorphism is not associated with RA susceptibility, but rather with severity of the disease. However, based on our study IL-17A gene polymorphism may be useful in identifying individuals with high active disease.

Author Contributions

EMF, MMA; participated in designing the study, managed the practical part, read and approved the final manuscript. HMN; performed the practical part, wrote the first draft of the manuscript. AS; supervised the research. FHE; formulation of the research question and statement of hypothesis. LIA; data analysis, helped in collection of clinical samples.

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 protocol of the study was reviewed and approved by the Ethics Committee Board of the Faculty of Medine, Aswan University (Ref. No. Asw. U. /301/10/18).

Informed consent

Each study participant signed a written informed consent form before being included in the study.

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