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Interleukin -37 in rheumatoid arthritis: Correlation with clinical severity and genetic polymorphisms in Mosul city, Iraq

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Abstract

The contribution of anti-inflammatory cytokines to rheumatoid arthritis (RA) is not fully comprehended. In the current research we assessed the serum concentration of interleukin (IL)-37 anti-inflammatory cytokine in RA, studied its association to disease activity score 28 (DAS28) and investigated single nucleotide polymorphism (rs2723176) of IL-37 gene as a threat for RA development. The case-control study included 60 RA patients and 30 normal control individuals. Serum IL-37 was assessed by ELISA and genotyped by "sequence-specific primer-polymerase chain reaction (SSP-PCR)". The mean IL-37 was elevated in RA patients (69.42 ng /l ± 62.99) compared to control individuals (14.66 ng /l ± 23.58, $p < 0.001$). IL-37 tended to increase with age where highest levels were noted in patients more than 60 years (p = 0.037). No Gender influence was found on IL-37 level (p>0.05). At best cut-off value of 31.5 ng/ l, IL-37 had a sensitivity of 73.3% and specificity of 83.3%. No correlation of IL-37 with DAS 28 score was observed (r=0.1497, p=0.2535). For IL-37 (rs2723176) gene polymorphism, C/C genotype was prevailing in both RA (90%) and normal controls (93.3%) compared to A/C or A/A. Also, no variation was found between patents and controls in regard to C/C genotype (OR = 0.643, 95% CI (0.122-3.39, p=0.603). The mean IL-37 concentration in RA patients with C/C genotype (59.70± 67.92) was not different from AC genotype (80.54± 94.18, p=0.4748). We concluded that serum IL-37 had the implication as a diagnostic marker in RA. However, it did not correlate with clinical severity of the disease. Meanwhile, IL-37 (rs2723176) gene polymorphism did not seem to be as a risk factor for RA, nor contributed to the increase of IL-37 level among patients.

Keywords: Interleukin-37, rheumatoid arthritis, IL-37 single nucleotide polymorphism, Iraq.

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Introduction

Rheumatoid arthritis (RA) is a long-standing disabling joint disease with systemic extra-articular manifestations.[1] The pathoetiology of the disease is complicated and thought to be interplay between various immunological, genetic, familial, hormonal, environmental and infectious factors.[2] Age and sex - adjusted global annual incidence of RA is around 41 cases per

100,000 people with significant raise in the frequency of RA cases with negative rheumatoid factor (RF) compared to RF positive RA.[3]

Interleukin (IL) -37 is relatively a new cytokine member of IL-1family[4]. Its molecular weight around 17~25 kDa and is expressed in comparatively very low concentration by different types of normal cells and tissues.[5] However, IL-37 secretion is significantly elevated by the blood and tissue cells in regard to proinflammatory cytokines (IL1β, IL-18, IFN-γ, TNF-α and TGF-β1).[6] In such instance, blood mononuclear cells (PBMCs), macrophages, T lymphocytes, dendritic cells (DCs) and epithelial cells are the main sources of this cytokine.[6] IL-37 expression is also increased after stimulation by lipopolysaccharides (LPS) and synthetic bacterial Pam3CSK4.[5] After secretion, IL-37 modulates the inflammatory response due to its anti-inflammatory activity by directly suppressing the production of pro-inflammatory cytokines.[7] In addition, IL-37 has a natural inhibitory effect on both innate and adaptive immunity by suppressing pro-inflammatory cytokines secretion provoked by toll like receptors (TLRs) agonists.[7] Owing to its immune modulating functions, increased protein level of IL-37/or mRNA was linked to several inflammatory and auto-immune diseases.[8,9,10,11,12,13,14] In addition to protein level, different "single nucleotide polymorphisms (SNPs)" within IL-37 gene were also studied in various autoimmune and infectious diseases.[15,16,17]

The present study aimed to clarify the diagnostic potential of serum IL-37 in RA in a sample of Mosul city population in Iraq and to investigate its correlation with the clinical severity of the disease. On top, this study was the first to explore the impact of SNP within IL-37 gene namely IL-37 rs2723176 (CC/CA/AA) as a risk factor for RA susceptibility.

Subjects and Methods

Ethical considerations

The protocol of this research study was reviewed and approved by the Ethics Committee for Medical Research, College of Medicine, University of Mosul (Ref. no. UOM/COM/MREC/21-22 (52) on 11/3/2022). All participants gave written informed consents before involved in the study.

Subjects

The current case-control study was carried in the Department of Microbiology, College of Medicine, University of Mosul, Iraq during the period between March 2022 and September 2022. Totally, 90 participants were recruited in this study. Of these, 60 were RA patients collected from Ibn-Sena Teaching Hospital in Mosul City, Iraq. The diagnosis of RA in these patients and assessment of its clinical severity was confirmed by specialist rheumatologists in accordance with ACR/ EUROLAR 2010 specification.[18] The remaining 30 participants were apparently healthy blood donors without any history of co-morbidities as controls. They matched RA patients in terms of age and gender. Exclusion criteria included the presence of other diseases that might affect IL-37 level such as other autoimmune diseases, immune deficiencies, infections, pregnancy, liver and kidney diseases and cancers. All the available demographic, clinical and laboratory data were noted and documented. ESR levels in patients with RA were obtained and recorded from patients' files in hospital.

Blood sampling and collection

Whole blood samples (5 ml) were drawn from every participant by venipuncture in a septic syringe. Of these, one ml was poured into EDTA containing tube and stored at - 20 º C for genomic DNA extraction. The remaining 4 ml were transferred into a plain tube and centrifuged at 1795 xg for serum separation. All sera were frozen at -20ºC until used for measurement of IL-37 concentration by ELISA.

Quantification of serum IL-37 concentration by ELISA

IL-37 cytokine was quantified in sera of all participants by the Sandwich ELISA technique using 96-well human IL-37 ELISA kits (Cat. Number: E1947Hu, BT laboratory, China), according to the manufacturer's instructions. This kit has a minimum sensitivity of 4.56 ng/L.

Genotyping of IL-37 (rs2723176)

Genomic DNA was first extracted from the blood of both patients and controls using commercial kits (G-Spin ™ Total DNA Extraction Mini Kit, iNtRON Biotechnology, South Korea) according to manufacturer's instructions. The concentration and purity of DNA was then determined by spectrophotometry through measuring DNA absorbance at 260 and 280 nm wavelengths, respectively (BK -UV1800PC UV/VIS spectrophotometer, Biobase, China). The 260/280 ratios of ≥ 1.8 were taken to indicate good DNA purity.[19] The extracted DNA was then used as a template for genotyping of rs2723176 SNP (-6962 A/C) by the sequence-specific primer polymerase chain reaction (SSP - PCR) assay. Primers sequence for IL-37 (rs2723176) and internal control, human growth hormone (HGH) genotyping, were adopted from the leterature[17]. The following primer sequences were used: Forward primer (A allele): 5'-CTAGGGGCTGCTTTAACAAGA-3'; Forward primer (C allele): 5'-CTAGGGGCTGCTTTAACAAGC-3'; Common reverse primer: 5'-GCCAAGCCTCTGTCTTTCTGA-3'; HGH forward primer: 5'-GCCTTCCCAACCATTCCCTTA-3'; HGH reverse primer: 5'-TCACGGATTTCTGTTGTGTTTC-3'. Each PCR reactions included ~ 100 ng of extracted DNA, 1 µl of "MaximeTM PCR PreMix Kit (i-Taq)" (iNtRONS Biotechnology, South Korea), 1 µl equal to 10 pmole/µl of IL-37 allele-specific forward primer, 1 µl equal to 10 pmole/µl of IL-37 common reverse primer, 1 µl (10 pmole/µl) of HGH internal control forward primer and 1 µl (10 pmole/µl) of HGH reverse primer. The PCR volume was then completed to 20 µl with PCR grade distilled water. The PCR samples were placed into PCR thermocycler (MyCycler, BioRad, USA) and the following parameters were used for amplification [17]: 1 cycle at 94 ºC for 4 min (initial denaturation), 30 cycles each of denaturation at 94 ºC for 30 sec, annealing at 62 ºC for 40 sec and extension at 72 for º45 sec, followed by 1 cycle at 72 ºC for 4 min (final extension). Expected size of IL-37 rs2723176 PCR product was 604 bp and for HGH was 409 bp, respectively.

Statistical analysis

The MedCalc®20 software package (Belgium) was used to perform all statistical analysis in this study. Graphs were plotted using Microsoft Excel 10. The data are summarized as minimum, maximum, mean, median, standard deviation (SD) and 95% CI whenever applicable. Parametric "Student *t* test" and non-parametric "Mann - Whitney test" were used for to compare the means whenever indicated. The Kruskal-Wallis test was employed for inter-group analysis (more than 2 groups). The area under the Receiver Operating Characteristics (AUC-ROC) curve was utilized to test the validity of serum IL-37 to diagnose RA at different cut-off values. For IL-37 gene polymorphism, the genotypes and alleles were counted by direct counting. The Chi-squared (X^2) test was utilized to compare allele and genotype distribution. Hardy-Weinberg equilibrium (HWE) in control samples was tested by Chi-square (χ2) test. The odds ratio (OR) and 95% CI were calculated to measure the relative risks in both controls and RA patients. Pearson Correlations co-efficient evaluated correlations between studied parameters. The p values ≤ 0.05 were concerned to be statistically significant.

Results

Population characteristics

Table 1 illustrates the characteristics of the studied groups in regard to different demographical, clinical and laboratory parameters. Overall, 60 RA patients and 30 normal individuals were participated in the study. The two groups were adjusted in terms of sex (p = 0.185) and age (p = 0.358). Positive family history of RA was seen in 13 (21.7%) of patients. The RA patients had average disease duration of 8.55± 7.52 years, mean DAS score of 5.8±1.33 and mean ESR level of 43.26±17.93 mm/hr. About 68% of patient with RA were treated with steroids, 45% methotrexate, 75% etanercept and 6.7% chloroquine.

Table 1. Demographic, clinical and laboratory characteristics of the studied groups.

	RA (*n* =60)	Controls (*n*=30)		*p* value
Participants				
Female (73)	51 (85%)	22 (73.3%)		-
Male (17)	9 (15%)	8 (26.7%)		*NS
Age (years)				
Min	19	24		
Max	81	77		
Mean ± SD	48.47 ± 12.86	45.57 ± 16.16		**NS
Family history of RA				
Positive	(21.7%)	-		
Negative	47 (78.3%)	-		
IL-37 (ng/l)				
Min	21.15	13.0		
Max	369.61	38.72		
Means ± SD	61.79±69.70	25.96±6.68		***< 0.0001
Median	35.6	26.9638		
95% CI for the mean	43.78 - 79.80	23.46 - 28.45		
Skewness	2.8187	-0.2689		
Severity of RA	Mild	Moderate	Severe	All
Number of cases	5	17	38	60
Duration of disease (years)	2.97± 1.91	7.14± 7.38	9.92± 7.69	8.55± 7.52
DAS score*	3.1±0.01	4.66±0.39	6.79±0.44	5.8±1.33
ESR*	23.8±8.01	34.17±16.10	49.89±16.10	43.26±17.93
Medication				
Prednisolone	4/5 (80%)	10/17 (58.8%)	27/38 (71%)	41/60 (68.3%)
Methotrexate	2/5 (40%)	8/17 (47%)	17/38 (44.7%)	27/60 (45%)
Hydroxychloroquine	0/5 (0%)	2/17 (11.8%)	2/38 (5.3%)	4/60 (6.7%)
Leflunomide	0/5 (0%)	0/17 (0%)	2/38 (5.3%)	2/60 (3.3%)
Etanercept	2/5 (40%)	9/17 (53.0%)	34/38 (89.4%)	45/60 (75%)
Infliximab	0/5 (0%)	2/17 (11.8%)	2/38 (5.3%)	4/60 (6.7%)
SNP				
CC	5/5 (100%)	16/17 (94.1%)	33/38 (86.8%)	54/60 (90%)
AC	0/5 (0%)	1/17 (5.9%)	5/38 (13.2%)	6/60 (10%)
AA	0/5 (0%)	0/17 (0%)	0/38 (0%)	0/60 (0%)

RA= rheumatoid arthritis, n = number of participants, SD = Standard deviation, CI= Confidence interval, *DAS 28 (Disease activity score): mild < 3.2, moderate ≤ 3.2 – 5.1, severe ≥ 5.1, SNP = Single nucleotide polymorphism. IL-37 = Interleukin-37* Chi-squared test used to calculate the p value. ** Student -*t*- test was used to calculate the p value. ***Mann-Whitney test was used to calculate the *p* value. *P* > 0.05 is not significant (NS).

IL-37 level in RA group and normal controls

The average IL-37 concentration in the 60 sera of RA patients was significantly elevated (61.79±69.70 ng/l) compared to the 30 normal individuals (25.96±6.68 ng/l, $p < 0.0001$) (Figure 1, A). However, no difference was found in IL-37 level between RA females (62.79± 73.76, n=51) and males (56.10± 42.29, n = 9, p = 0.819) (Figure 1, B). The IL-37 tends to increase significantly with age 33.26± 7.55, n = 14 at the age group ≤ 40, 65.07± 67.88, n= 34 at the age group of 41-60 and 85.76±102.34, n=12 at the age group of > 60 years respectively, p = 0.037 (Figure 1, C).

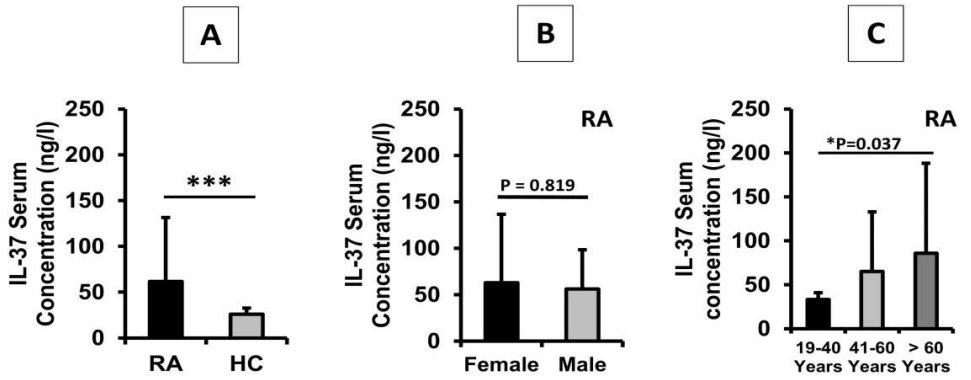

Figure 1. IL-37 serum level as determined by ELISA. Figure A) IL-37 was significantly higher in RA patients (61.79±69.70 ng/l, *n* = 60) compared to normal controls (25.96±6.68 ng/l, *n* = 30). Figure B) The IL-37 concentration was not different (62.79±72.76 in females and 56.10± 42.29 in males. Figure C) IL-37 concentration in RA patients increased with age (p = 0.037), *** *p* < 0.0001. *Mann - Whitney test* was used for comparison in Figures A and B and Kruskal–Wallis test was used in Figure C.

Validity of IL-37 for the diagnosis of RA

Different cut-off values for IL-37 ranged from 13.0-369.0 ng/l were generated and examined using AUC- ROC-curve (Table 2). In general, the IL-37 cytokine had a good validity to discriminate between RA and normal control individuals (AUC-ROC was 0.845, 95% CI: 0.754 - 0.913, *p*<0.001), (Figure 2). However, the cut-off value with best combination of sensitivity (73.3%) and specificity (83.3%) was 31.52 ng/l with an accuracy rate of 57 % (Youden index = 0.57).

Table 2. Sensitivity and specificity of IL-37 for diagnosis of RA at different cut-off values.

Criterion	Sensitivity	Specificity	Youden index (Accuracy rate %)
>13.077450962	100.00	3.33	0.03 (3%)
>18.569994978	100.00	13.33	0.13 (13%)
>21.154630808	98.33	30.00	0.28 (28%)
>24.680162002	96.67	43.33	0.40 (40%)
>27.42300445	90.00	53.33	0.43 (43%)
>30.047267522	80.00	63.33	0.43 (43%)
* >31.528068888	73.3	83.33	0.57 (57%)
>33.097884728	60.00	90.00	0.5 (50%)
>36.83167405	41.67	96.67	0.38 (38%)
>39.896354898	33.33	100.00	0.33 (33%)
>42.50895125	25.00	100.00	0.25 (25%)
>45.982123778	16.67	100.00	0.17 (17%)
>48.830437602	13.33	100.00	0.13 (13%)
>55.661874722	11.67	100.00	0.12 (12%)
>74.181944888	8.33	100.00	0.08 (8%)
>194.873984818	6.67	100.00	0.07 (7%)
>241.517399192	5.00	100.00	0.05 (5%)
>257.841874712	3.33	100.00	0.03 (3%)
>269.232174912	1.67	100.00	0.02 (2%)
>369.613913138	0.00	100.00	-

* Represents the optimal cut-off value.

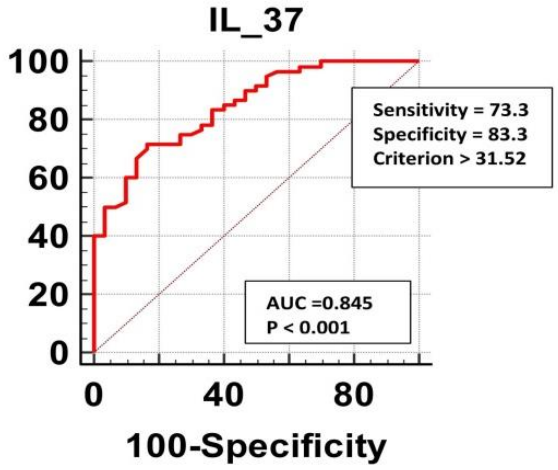

Figure 2. AUC-ROC curve of IL37 in RA compared to normal control individuals. At 31.5 ng/l cut-off, IL-37 has a sensitivity of 73.3% and a specificity of 83.33% and accuracy rate of 57% (AUC = 0.845, $p < 0.001$).

IL-37 correlation with DAS 28 score, ESR level and progression of RA

The degree of severity was categorized into mild (DAS28 score of ≤ 3.2), moderate (DAS28 score of 3.2 to ≤ 5.1) and severe (DAS28 score > 5.1). Accordingly, 5 cases were classified as mild with an average DAS score of (3.1±0.01), 17 moderate (4.67±0.39) and 38 severe (6.79±0.44). The mean IL-37 concentration was 31.16 ng/l ± 4.7 in the mild RA, 60.93 ng/l ± 71.45 in the moderate RA and 66.20 ng/l ± 73.42 in the severe RA. No statistical difference was established among all three groups ($p > 0.05$) (Figure 3A). To reach a better conclusion on the association of IL-37 serum concentration with the progression of RA, the Pearson correlation coefficient was employed to test whether IL-6 correlated with DAS 28 scores and ESR, and hence the severity of the disease. In support of the above results, there was no significant correlation between the overall IL-37 level and the overall DAS 28 score ($r = 0.1497$, $p = 0.2535$; Figure 3, B). Nearly, similar results were obtained with IL-37 and ESR levels ($r = 0.0774$, $p = 0.5567$; Figure 3, C).

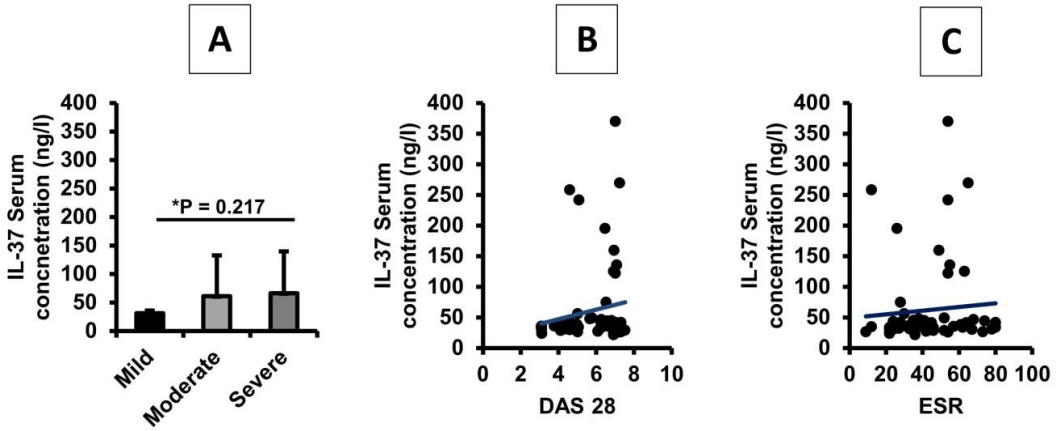

Figure 3. Correlation of IL-37 with the disease progression in RA according to DAS 28 criteria and ESR level. Figure A) Mean IL-37 serum level in mild RA (31.16 ng/l ± 4.7, $n = 5$) did not significantly differ from moderate (60.93 ng/l ± 71.45, $n = 17$) or severe RA (66.20 ng/l ± 73.42, $n = 38$). Moreover, no significant correlation was found between the IL-37 serum level and DAS 28 scores and ESR levels ($p > 0.05$). * Kruskal–Wallis test was used in Figure A, while Pearson correlation co-efficient was used in Figures B and C.

IL-37 rs2723176 (CC/CA/AA) genotyping and its association with IL-37 protein level

Figure 4 shows the results of IL-37 rs2723176 (CC/CA/AA) genotyping on agarose gel using SSP-PCR. Table 3 summarizes the frequencies of IL-37 genotype and alleles. For the control group, IL-37 rs2723176 (CC/CA/AA) polymorphism was in Hardy-Weinberg

equilibrium (χ2 = 0.037, *p* = 0.982). In both RA and normal controls, CC genotype was the most prevalent (90% in RA and 93.3% in the controls) compared to AC genotype (10% in RA and 6.7% in the controls). However, no difference was found between RA and control individuals in relation to CC genotypes as a risk factor for RA (OR= 0.643, 95% CI= 0.122-3.395, *p* = 0.603). Meanwhile, no AA genotype was detected in both RA and normal controls among our studied samples. Regarding allele frequencies, C allele was the most frequent in both RA (95.0%) and control individuals (96.7%). However, no statistical variation was found between the two groups (OR= 0.655, 95% CI= 0.128-3.48, *p* = 0.611). The mean IL-37 levels in RA patients with AC genotype (80.54± 94.18) was not different from the mean IL-37 level in patients with CC genotype (59.70± 67.92), *p* = 0.4748 (Figure 5).

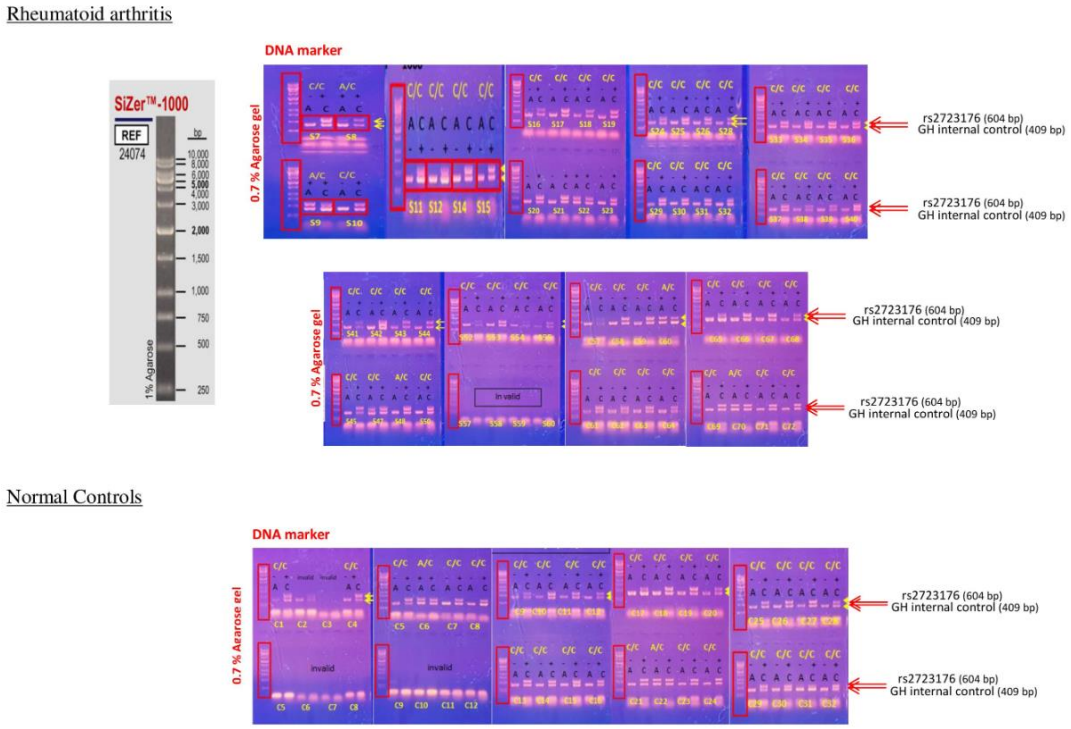

Figure 4. Results of SSP-PCR in RA patients and normal controls. Blood Genomic DNA from 60 RA patients and 30 normal controls were analyzed for IL-37 rs2723176 (CC/CA/AA) gene polymorphism by SSP-PCR using two PCR reactions for each sample (One reaction with A primer + common reverse and other with C primer + common reverse) together with internal control growth hormone (GH) primer pairs.

Table 3. Genotype distribution and allele frequency of IL-37 rs2723176 polymorphism among RA patients and normal controls.

Genotype	Patients (*n* = 60)	Frequency	Controls (*n* = 30)	Frequency	OR (95% CI)	*p value
CC	54	90.0%	28	93.30%		
AC	6	10.0%	2	6.70%	0.643 (0.122 - 3.395)	NS
AA	0	0.00%	0	0.00		
Allele						
C	114	95.0%	58	96.7%	0.655 (0.128 - 3.348)	NS
A	6	5.0%	2	3.3%		

OR: odds ratio; 95% CI: confidence interval. For genotype OR was computed using CC as a risk factor and AC + AA as the referent. * Chi -squared test was used to make comparison and calculate p value. *P* > 0.05 is not significant (NS).

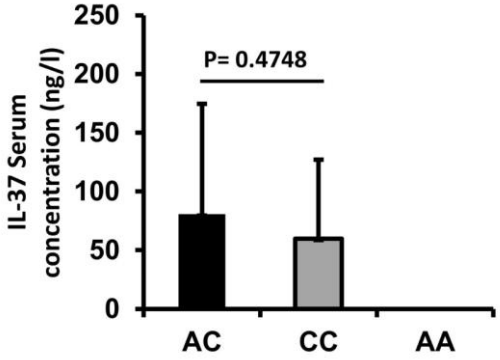

Figure 5. Effect of IL-37 rs2723176 (CC/CA/AA) gene polymorphism on serum IL-37 serum level. Overall, no significant difference in IL-37 level was found between RA patients with AC (80.54± 94.18) and CC (59.70± 67.92) genotypes. Results were presented as mean ± SD. *Mann-Whitney* test was employed for comparison.

Discussion

Cytokines and cytokine mediated pathways play crucial function in the pathogenesis of RA.[20] However, the cytokine network in RA is very complex, with dysregulation of both proinflammatory and anti-inflammatory cytokines in both the joint synovium and circulation.[21] While the role of proinflammatory cytokines such as TNF-alpha, IL-1 and IL-6 in RA is well established,[22,23,24,25] the role of anti-inflammatory cytokines is less ascertained and require more investigations. Understanding the anti-inflammatory cytokines involved, and the suppression pathways that are responsible for the resolution of the inflammation is of crucial importance in understanding the pathophysiology of the disease and in planning the treatment stratigies that enable long-term control of the disease.

Research in the past had studied the possible role of classical anti-inflammatory cytokines in RA like TGF-β, IL-4, IL-10, IL-11 and IL-13.[26,27,28,29,30,31,32] These studies suggested a protective role of these cytokines in RA. More recent research was carried out on new anti-inflammatory cytokines such as IL-33,[33] IL-34[34] and IL-37.[35,36,37,38,39] These studies further suggested a conceivable role of anti-inflammatory cytokines in RA. Our study was an additional ring in this series and tried to confirm or contradict the findings of others in an attempt to clarify the role of IL-37 anti-inflammatory cytokine as a diagnostic marker in RA and assess its prospective validity in the evaluation of diseases activity. Moreover, to the best of our knowledge, our study is the first research that studied the effect of IL-37 rs2723176 (CC/CA/AA) single nucleotide gene polymorphism (SNP) as a risk factor for RA and its correlation with IL-37 protein concentration and disease activity. In addition, our study is pioneering in studying this cytokine among a group of Iraqi population.

The main finding in the current research was the observation of higher serum levels of IL-37 cytokine in RA in comparison to normal control individuals together with the finding of increased IL-37 levels in older age groups (highest levels were observed in patients over 60 years). The elevated IL-37 serum level found in the current study was in agreement with the results of other researchers[10,11,12,35,36,37,38,39] and confirming its role as a diagnostic marker in RA. In addition, the ROC curve analysis further supported the diagnostic validity of IL-37 in RA as it clearly discriminated RA patients from normal control individuals. The increased IL-37 levels found in RA might be attributed to its possible protective role in these disease. IL-37 was increased in RA and several other autoimmune and inflammatory diseases such as SLE,[8,40] multiple sclerosis,[13] Graves disease,[9] ankylosing spondylitis[41] and Juvenile idiopathic arthritis.[14] Interestingly, the expression of IL-37 was found to be low in normal human cells and tissues but upregulated by inflammatory stimuli and pro-inflammatory cytokines[6]. Therefore, IL-37 might be increased as a counter regulatory mechanism in response to pro-inflammatory cytokines to inhibit or limit the inflammation

seen in these diseases. Several mechanisms might be suggested through which IL-37 attempts to inhibit or limit the inflammation in RA. First, IL-37 directly inhibit the production of proinflammatory cytokine (such as TNF-alpha and IL-6) by suppression of transcription of their proinflammatory genes[42]. Second, IL-37 inhibits the components of the innate and acquired immunity that thought to be nonregulated in RA and autoimmune diseases by inhibiting the production of these proinflammatory cytokine[43]. However, a question is still arised about the potency of IL-37 to dampen inflammation in RA as evidenced by continuous dedication of inflammation despite of this presumptive anti-inflammatory protective role. The exact answer for this question is still unknown, however, a positive shifting toward the pro-inflammatory cytokine milieu during the dysregulated inflammatory response in RA can be suggested. In support to this assumption, IL-37 was found to increase in RA, however its concentration was relatively low in comparison to the high concentration of pro-inflammatory cytoiknes.[39] In this setting, controlling the balance between these two groups of cytokines seems to be an attractive and important therapeutic goal in RA.

Despite the elevated IL-37 levels in RA, our data did not support its correlation with diseases activity as demonstrated by poor discriminative validity between mild, moderate and severe diseases and poor correlation with DAS28 scores and ESR level. This observation disagreed with findings of other studies who found such correlation.[11,12,35,37,38,39] Several explanations for this discrepancy can be proposed: 1) It might be due to technical issues related to the different sensitivities of the ELISA kits used. In favor of this, we found that the mean IL-37 level was varied greatly in both RA patients and normal individuals among different studies. This ranged from as low as (28.83 ng/l in RA and 11.84 in the controls) in one study[44] to as high as (712.40 ng/l in RA and 133.50 in the controls) in another study,[37] 2) Some elements of the DAS 28 scoring system such as the number of tender and swelling joints and the overall health condition of the patients are largely subjected to personal judgement of the physicians which might affect the calculation of DAS28 score, 3) Differences in cohorts is another possibility, so a larger cohort might explore more positive correlation, 4) Most of the studies that reveled a positive correlation between IL-37 and DAS28 were carried on Asian populations (China) and only one study was reported among Arab population (Egypt).[45] Therefore, ethnicity is an important suggested factor to be considered when interpreting IL-37 results, 5) The concentration of IL-37 was observed to be significantly higher in the aspirated synovial fluid of RA patients than the circulation[45] and therefore synovial IL-37 might be a better marker to be considered when correlated with DAS 28 score, 6) Finally, in the current study we did not take into consideration the effect of the drugs on the IL-37 level which might be considered one of the limitations of this study. Therefore, further studies are suggested with larger cohorts to precisely compare the correlation between activity score and IL-37 taken into consideration the effects of anti-rheumatic drugs in those patients.

In the current research, the association of IL-37 rs2723176 (CC/CA/AA) single nucleotide polymorphism (SNP) with RA susceptibility was also analyzed. Investigating the association of IL-37 SNPs and RA seems to be reasonable since SNPs of several cytokines were claimed to increase the susceptibility to RA. In addition, SNPs may affect the transcription rate of these cytokines and hence affecting both the structure and functions of the resulted proteins. IL-37 SNP in RA was investigated in a previous study.[46] They genotyped five selected SNP within IL-37 gene (rs2723186, rs3811046, rs4241122, rs4364030, and rs4392270) and concluded no association with increased risk of RA in their sample of Chinese population. Up to thebest our knowledge, our study is the first report that analyzed the effect of another IL-37 SNP namely rs2723176 (CC/CA/AA) on RA susceptibility. The result of the present study revealed that IL-37 CC genotypes was prevalent in both RA patients and controls (90% of RA and 93.3% in the controls). However, we did not observe differences in allele or genotype frequencies of IL-37 rs2723176 polymorphisms between RA and controls. Hence, these results

could not encourage the role of IL-37 rs2723176 as a predisposing genetic factor for the development of RA among patients in Mosul city. Moreover, we could not find any association between this type of polymorphism and IL-37 protein level. This observation might point out that this sort of polymorphism has no effect on the expression and / secretion of IL-37 among RA patients.

In conclusion, serum level of IL-37 was elevated in RA patients compared to control subjects and therefore, could be proposed as a diagnostic marker in this disease. However, our results did not support its correlation with disease severity and hence it could not predict the clinical course of the diseases among our patients. Meanwhile, IL-37 rs2723176 (CC/CA/AA) SNP did not seem to be a risk factor for RA development, nor affected its protein level.

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Author Contributions

FMDA, wrote and revised the manuscript. All authors contributed equally to the work and approved it for publication.

Declaration of Conflicting Interests

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Ethical approval

The protocol of this research study was reviewed and approved by the Ethics Committee for Medical Research, College of Medicine, University of Mosul (Ref. no. UOM/COM/MREC/21-22 (52) on 11/3/2022).

Informed consent

All participants gave written informed consents before involved in the study.

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