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The Role of IL-37 as an Anti-Inflammatory Biomarker in some Iraqi Rheumatoid Arthritis Patients and Its Correlation with DAS28

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Abstract

Interleukin-37 (IL-37) is a new anti-inflammatory cytokine that inhibits immunological response and inflammation. The purpose of this study is to look into the role of IL-37 and if it correlates with disease activity in some Iraqi RA patients. The scope of the investigation comprised of 76 females with RA who are aged in the range of (45.38 ±1.23 years) and 40 individuals in the control group with age of (46.48 ± 2.02years). When compared to controls, the level of serum IL-37 was shown to be considerably elevated in RA patients. (49.16 ± 3.25pg/ml vs. 32.04 ± 2.22pg/ml; $p \leq 0.05$). However, the DAS28 score results showed a non-significant difference between the mild and severe rheumatoid arthritis patients. There was a positive association, statistically significant between the DAS28 score and anti-CCP Abs, RF. A positive correlation between DAS28 and ESR. While non-significantly correlated between DAS28 and CRP, IL-37.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease of connective tissues that typically affects synovial joints and causes joint swelling and inflammation, stiffness of the joints, as well as deterioration of the synovial membrane of the bones and joints, which ultimately results in severe disability and untimely death [1]. The progression of RA may be broken down into four distinct phases. Stage 1 is an early stage of RA, the synovial membrane is inflamed, but there is no evidence of damage to the cartilage or bone at this time. Stage 1 is considered an early stage. The synovial inflammation that occurs in stage 2, Stage 2 is a moderate level of RA, causes damage to the articular cartilage. Stage 3 at the stage of the disease, both the articular cartilage and the bones that are located behind it are badly afflicted, the second stage of RA, joint X-rays show evidence of thinning bones around the joint and some bone destruction underneath cartilage of the joints. While the last stage of RA is known as stage 4, during which the joint is completely disabled and the inflammation has subsided. When patients reach this point in the disease process, it is not uncommon for them to have diminished muscle strength, structural damage to the joint, and bone fusion [2].

Autoantibodies that circulate in the blood are used as biomarkers to diagnose RA in patients. Rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) testing are the two most popular methods for diagnosing RA [3]. Because seropositive and seronegative RA have significant phenotypic overlap. However, there are differences in immune complexes, complement fixation inside the joint, synovial T cell populations, and genetic linkage between these diseases [4]. Hepatocytes are the primary generators of C-reactive protein (CRP), an acute-phase protein that is also used to diagnose RA as an inflammatory biomarker [5].

Rheumatoid arthritis can be recognized by utilizing the disease activity score 28, also known as DAS28, in conjunction with the erythrocyte sedimentation rate, also known as ESR [6]. Previous studies have shown that interleukin-37 (IL-37) plays an important role in the regulation of the inflammatory response. In particular, it inhibits the production, synthesis, and activity of cytokines that are considered to be pro-inflammatory. IL-37 is abundantly expressed in a variety of cell types, tissues, and organs, including dendritic cells, plasma cells, and monocytes. It is hypothesized that IL-37, through inhibiting immunological responses and inflammatory processes, has a role in the development of autoimmune illnesses [7].

The anti-inflammatory cytokine interleukin-37 (IL-37), which is generated as a protein to release its mature form, is a member of the cytokine's family. Its function inhibits the inflammatory response's many cytokine productions in many *in vivo* and *in vitro* models. It has been established that inflammatory cytokines and the onset of RA are directly related to higher serum IL-37 levels.

Nomenclature			
ACPAs	Anti-citrullinated protein/peptide antigens	SE	Standard error
ACR	American College of Rheumatology	SD	Standard deviation
Anti-CCP	Anti-cyclic citrullinated peptide antibody	r	Pearson correlation
CRP	C-reactive protein	ELISA	Enzyme linked immune assay
DAS28	Disease activity score	µl	Microliter
ESR	Erythrocyte sedimentation rate	TLRs	Toll like Receptors
IL-37	Interleukin -37	IL-1β,	Interleukin- 1 beta
P value	Probability value	TNF-α	Tumor necrosis factor-α
Pg	Picogram	IFN-γ	Interferon-γ
RA	Rheumatoid arthritis	TGFβ1	Transforming growth factor-β1
RF	Rheumatoid factor	LPS	lipopolysaccharide

There is still a lot of mystery about the connection between the levels of IL-37 in the body and the severity of rheumatoid arthritis. Recent studies have shown that interleukin 37 (IL-37) plays an important role in the regulation of the inflammatory response, specifically in preventing the expression, synthesis, and activity of cytokines that promote inflammation. It is also induced and upregulated in response to a variety of inflammatory stimuli, including IL-1, tumor necrosis factor (TNF), interferon (IFN), IL-18, transforming growth factor (TGF), and lipopolysaccharide. IL-37 is expressed by a wide variety of cell types, organs, and tissues, including monocytes, plasma cells, dendritic cells, uterus-stimulated B cells, and keratin (LPS) [8]. It is thought that IL-37 may contribute to autoimmune disorders by reducing immune responses and inflammation (7). The current study aimed to investigate the role of IL-37and whether it was correlated with the disease activity (DAS28) in some Iraqi RA patients [6].

2. Patients and Methods

This study comprised of 76 female RA patients, ranging in age from 26 to under 60 years old, who met the criteria established by the American College of Rheumatology (ACR) in 2010 for rheumatoid arthritis [7]. These RA patients are registered attendances of rheumatology at the consultation Clinic /Baghdad and Teaching Hospital and Al-Yarmouk teaching in Baghdad during the period from July 2020 - May 2021 and they are previously diagnosed by rheumatologists. From non-relatives, 40 healthy, their age-matched with RA patients. During the clinical investigation, in addition to collecting data for ESR, CRP, & RF, the length of time that the ailment had been present was also taken into consideration. The DAS28 score of the 76 RA patients was compared, and the patients were divided into two groups: those with mild disease ($2.6 < DAS28 \leq 3.2$) and those with severe disease (<5.1 DAS28) [8].

2.1 Estimation of Anti-CCP antibodies

All of the participants had three milliliters of blood drawn from them, and then the blood was centrifuged at 5000 revolutions per minute for ten minutes. Serum was stored at -80 ºC until it was needed for the detection of anti-CCP antibodies, serum was employed. (Elabscience Biotech, China).

2.2 Measurement of IL-37

In order to measure IL-37 (Human IL-37, Invitrogen™, China), a quantitative sandwich enzyme-linked immune sorbent assay was carried out in accordance with the instructions provided by the manufacturer. The ELISA reader was used to read the absorbance, and the findings were interpreted using the standard curve that was designed specifically for this kit.

2.3. Measurement of DAS28 score

The Illness Activity Score of 28 Joints, more commonly abbreviated as DAS28, is a measure for determining the severity of disease. It is frequently utilised as an indicator of the disease activity of rheumatoid arthritis as well as the patient's response to treatment [7]. The 76 people diagnosed with RA were separated into two groups according to their DAS28 scores: mild ($2.6 < DAS28 < 3.2$), and severe ($5.1 < DAS28$) [8].

2.4. Statistical analysis

The findings of this research were then incorporated into a database organization using a computer. Statistical analysis was carried out, and IBM SPSS version 28 was utilised to determine the mean and standard error ± (SE) of the mean. Using cross-tabulation, we investigated whether or not there was a connection between two category variables. The Chi-square (X2) test was utilized to investigate the level of statistical significance associated with such correlations for the non-Parametric data. In order to analyses the significance of the difference in means between two continuous numeric variables, a T-test was carried out.

3. Results

3.1. Characteristics of the clinical and demographic profiles of the study groups

According to the demographical description of studied groups, it was clear from Table 1 that there were non-significant differences between the mean age for RA patients (45.38 ±1.23) and control group (46.48 ± 2.02).

Table 1 summarizes, the mean of disease duration was (6.31 ± 0.48years) among RA patients. So, the mean of serum CRP level in the RA patients and control groups were (29.37±2.0pg/ml and 3.12 ± 0.9, respectively) with significantly difference (P < 0.001). As well as, mean of ESR was highly significant difference (P < 0.001) between RA patients and apparently heathy control groups (42.50 ± 1.46 mm/hr; 7.48 ± 0.82mm/hr, respectively). The present study showed a highly significant association of family history with RA patients. In general, the ratio of patients that had a family history (Yes 39, 51.3% and No 37, 48.7%), in comparison with the control group (Yes 0, 0.0%) and No 42,100%) with a highly significant difference. Additionally, the findings of this study revealed a correlation between morning stiffness and the presence of RA in the patients. In general, there was not a significant gap between the patients who complained of morning stiffness as well as the other groups. (Yes, 76, 100%; No, 0,00%) and the healthy control group w (Yes, 0, 0.0%; 42, 100%), listed in (Table1).

Table 1 Clinical features at characteristics of people diagnosed with RA and a control group

Parameters	RA Patients	Control	P value
Age (Mean±Sd)	45.38 ± 1.23	46.48 ± 2.02	0.785
CRP	29.37 ± 2.0	3.12 ± 0.19	7.0×10^{-10}
ESR	42.50 ± 1.46	7.48 ± 0.82	1.21×10^{-21}
Duration years	6.31 ± 0.48	-	-
Family history Yes No.	 39 (51.3%) 37 (48.7%)	-	0.000022^{**}
Morning stiffness Yes No.	 76 (100%) 0(0.0)	-	$6.93 \times 10^{-23**}$
Smoking Yes No.	 4 (5.3) 72 (94.7%)	 0 (0.0) 42 (100%)	0.283 NS

3.2. Assessment of autoantibodies

The serum level of Anti-CCP was considerably highly significant in RA patients compared to controls (63.04 ± 2.82 U/mL vs. 14.02 ± 0.30U/mL; $p \leq 0.05$). Additionally, the levels of RF were highly significant in the groups under study (37.30 2.50 mg/L vs. 4.91 0.53 mg/L; $p \leq 0.05$), as observed in Table 2.

Table 2 Estimation of the mean values of RF & Anti-CCP in patients with RA compared to healthy controls

AutoAs	Mean ± SE		P- value
	Patients	Controls	
Anti-CCP	63.04 ± 2.82	63.04 ± 2.82	1.50×10^{14}
RF	37.30 ± 2.50	4.91 ± 0.53	9.86×10^{-10}

3.3. Measurement of serum IL-37

Patients with rheumatoid arthritis had a blood level of IL-37 that was considerably higher as compared to controls ((49.16 ± 3.25) vs. (32.04 ± 2.22); P<0.05) (Table 3).

Table 3 Estimation of the mean levels of serum IL-37 in individuals with RA compared to healthy controls

Interleukin	Mean ± SE (U/L)		P- value
	Patients	Controls	
IL-37	49.16 ± 3.25	32.04 ± 2.22	0.05

3.4. Estimation of disease activity

The DAS28 score of RA patients was estimated for rheumatoid patients as 6 Mild (22.67 ± 5.66) and 70 severe (23.11 ± 0.77). From the results non significantly difference between mild and severe (Table 4).

Table 4 Mean of DAS28 between sever and mild RA patients

DAS28 mean ± SE		P- value
Mild (6)	Sever (70)	
22.67 ± 5.66	23.11 ± 0.77	0.884

There was a substantial positive association between serum anti-CCP Abs, RF, and DAS28, according to the statistical analysis. (r= 0.487, P < 0.001 and r= 0.314, P < 0.001). The significant correlation between inflammatory markers and DAS28 including ESR (r= 0.290, P < 0.05). While, non-significantly correlated CRP and DAS28 (r=0.031). Finally, there were significant correlation between serum IL-37 and DAS28 (r=0.986, P < 0.001), Table 5.

Table 5 Correlation between DAS28 score and Biomarkers of studied groups

Biomarkers DAS28	Anti - CCP	RF	CRP	ESR	IL-37
r	0.487**	0.314**	0.031	0.290*	0.012

**. Correlation is significant at the 0.01 level (2-tailed), *. Correlation is significant at the 0.05 level (2-tailed), r. Pearson Correlation

4. Discussion

Rheumatoid arthritis is a common chronic autoimmune illness that stands out for its variability and unknown cause. This study aims to investigate the role of IL-37and whether it correlated with the disease activity (DAS28) in some RA patients. The findings agree with those of another study, which found that RA typically affects patients over the age of 40 and typically begins after middle age [9]. After 40 years, rheumatoid arthritis develops as a result of a variety of factors including stress, weakened immunity, and exposure to numerous antigens like tobacco, which activate autoreactive lymphocytes. In the current investigation, there were more patients with family histories than there was no family history. The findings concur with Somers and his coworkers, found that female offspring with a mother who had RA had a high rate of the disease [10]. While, current findings disagree with those that indicated a substantial percentage of RA patients had no a family history, (24, 40 %). According to the current study, nonsmokers had a higher rate of arthritis than smokers of the patients. These results were consistent with studies carried out in Iraq [11]. According to a prior study, smoking increased the likelihood of developing RA [12].

Nonspecific inflammatory markers known as serological indicators of CRP have been shown to have diagnostic importance for rheumatoid arthritis). They have been applied to the examination of inflammatory reactions throughout the system. One example of a serological marker is the anti-CCP antibody, which is quite well known. [13]. Assessing the anti-CCP antibody and RF, it increases the diagnostic accuracy in the early RA diagnosis [14]. Anti-CCP markers should be extremely specific autoantibodies for the RA and able to differentiate RA from other arthritis that mimics RA [15].

The results of the current study were in agreement with those which found that anti-CCP levels in the sera of RA patients were significantly increased [16]. The correlation between RA and anti-CCP revealed that RF was strongly related to this antibody [17]. These tests, are helpful in the identification of the disease, include biomarkers linked to RF and positive ACPAs in people with chronic rheumatoid arthritis. An anti-CCP antibody is a primary indicator for the serological diagnosis of RA. In addition, RA patients' serum levels of IL-37 were markedly higher than those of controls.

The findings concur that there was a considerable increase in IL-37 levels in RA patients compared to healthy controls [18]. Recent research has demonstrated that RA patients' serum and synovium IL-37 concentrations may be quantified [19]. These findings lend credence to the prior findings obtained from a sample of fifty RA patients who were treated in northeast China between 2011 and 2012[20]. It leads us to hypothesize that IL-37 might be a possible biomarker for RA diagnosis, disease activity evaluation, or measurement of therapeutic effects [21- 23]. Although the higher level of IL-37 in RA can be understood as the underlying mechanism for reducing joint inflammation and disease severity, it is still insufficient to counteract the negative impacts of the RA's progressing pro-inflammatory cytokines [24].

Interleukin-37 is a critical anti-inflammatory cytokine that plays a role in the regulation of inflammation. It possesses the potential to suppress the production and activity of pro-inflammatory cytokines, making it an important player in the control of inflammation [25].

IL 37 has been described as an anti-inflammatory cytokine in many inflammatory disorders other than RA. as well as, systemic lupus erythematosus (SLE) [26], inflammatory bowel disease (IBD) [27], graves' disease (GD) [28], and ankylosing spondylitis (AS) [29].

The CDAI mean values for mild and severe were not statistically significant (P>0.05. The findings are in accordance with those of Choe et al., who calculated the association of DAS28 activity indexes in RA patients, and found no statistically significant association [30].

5. Conclusion

Rheumatoid arthritis patients had higher IL-37 blood levels than the individuals in the healthy control group. This shows that IL-37 may be significant in RA diagnosis and progression. More studies will be required in the future to explain this correlation.

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Ethical Approval

Ethical approval for current study was granted from the ethical committee of the Iraqi Ministry of Health (no. 12601).

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