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Research Article

Serum Levels of IL-27 and IL-30 as Predictor of Disease Activity and Treatment Response in Patients with Rheumatoid Arthritis: A Case-Control Study

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Abstract

Background: Most individuals with inflammatory arthritis are diagnosed with rheumatoid arthritis (RA), an immunological disorder characterized by autoantibodies, particularly anti-cyclic citrullinated peptide antibodies (ACCP), which initiate inflammatory responses. Recent studies have shown that cytokines contribute to the progression and dissemination of RA, with elevated levels of serum IL-27 and IL-30. ***Objective***: To assess the utility of serum IL-27 and IL-30 levels as predictors of disease activity in RA patients and their correlation with response to treatment. ***Methods***: One hundred patients and fifty healthy controls made up the total number of participants in this case-control research. Baghdad Teaching Hospital conducted the study from November 2023 to February 2024. The American College of Rheumatology 2010 criteria were used for patient recruitment. An ELISA technique was used to evaluate the amounts of the biomarkers. ***Results***: The levels of IL-27 and IL-30 are significantly higher in rheumatoid arthritis patients compared to healthy controls. The mean serum IL-27 and IL-30 concentrations were highest in the severe, followed by moderate and low disease activity. Patients taking methotrexate had higher serum IL-27 and IL-30 concentrations, which were significantly higher than those on both etanercept and etanercept + methotrexate groups. ***Conclusions***: Elevated serum levels of IL-27 and IL-30 may serve as diagnostic markers for RA and as indicators of disease activity and response to treatment.

Keywords: Anti-CCP antibody, Etanercept, IL-27, IL-30, Methotrexate, Rheumatoid arthritis.

تقييم مستويات IL-27 و IL-30 في المصل كعلامة تشخيصية في التهاب المفاصل الرثوي

الخلاصة

الخلفية: يتم تشخيص معظم الأفراد المصابين بالتهاب المفاصل الالتهابي بالتهاب المفاصل الرثوي، وهو اضطراب مناعي يتميز بالأجسام المضادة الذاتية، وخاصة الأجسام المضادة للببتيد السيتروليني الحلقي، والتي تبدأ بالأستجابات الالتهابية. أن السيتوكينات تساهم في تطور وانتشار التهاب المفاصل الرثوي حيث تكون تركيزات انترليوكين 27 وانترليوكين 30 في المصل أعلى بكثيرلدى الاشخاص المصابين. **الاهداف:** تقييم دور الأنترلوكينات 27 و 30 في التقدم المرضي لالتهاب المفاصل الرثوي وقدرتهما التنبؤية للعلاج لدى المرضى العراقيين المصابين به. **الطرق:** اجريت الدراسة على مائة مريض وخمسين من الضوابط الأصحاء في مستشفى بغداد التعليمي من نوفمبر 2023 إلى فبراير 2024. تم استخدام معايير الكلية الأمريكية لأمراض الروماتيزم 2010 لتشخيص المرضى من أجل تقييم كميات المؤشرات الحيوية تم استخدام تقنية ELISA. **النتائج:** كانت مستويات الأنترلوكينات 27 و 30 أعلى بشكل ملحوظ في مرضى التهاب المفاصل الرثوي مقارنة بالضوابط الصحية. وكان متوسط تركيزهما في المصل أعلى في الحالات الشديدة، تليها الحالات المتوسطة والمنخفضة من نشاط المرض. كانت مستويات الأنترلوكينات 27 و 30 في المصل أعلى بشكل ملحوظ بين المرضى الذين يتناولون الميثوتركسيت، أكثر من كل من إيتانيرسيبت و ميثوتركسيت + إيتانيرسيبت. **الاستنتاجات:** إن التركيزات المرتفعة من الأنترلوكينات 27 و 30 في دم المرضى قد تصلح كعلامات تشخيصية لالتهاب المفاصل الرثوي ومؤشرات لنشاط المرض واستجابة العلاج.

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INTRODUCTION

Rheumatoid arthritis (RA) is a prevalent autoimmune disease characterized by persistent inflammation of the joints. The condition impacts around 1% of the global population, with females accounting for 75% of the affected individuals [1]. The pathogenic mechanisms of RA are affected by both genetic and environmental factors. However, the precise cause of the illness remains uncertain. High levels of inflammatory cytokines (IL-1, IL-6, and TNF-α) have been found in both the synovial fluid and blood of people with RA, which suggests a possible link to the cause of the disease [2]. In addition, the existence of anti-cyclic citrullinated peptide antibodies (ACCP), which include IgG, IgM, and IgA isotypes, is associated with the deterioration of joints and raises the probability of developing diseases [3]. Disease-modifying anti-rheumatic drugs (DMARDs) are the first approach in the treatment plan for rheumatoid arthritis. These drugs make the illness less severe. If the DMARDs failed to induce remission, biological therapy started. These are monoclonal antibodies that target specific molecules, such as IL-6 and TNF-α

[4]. Interleukin-27 (IL-27) is a member of the IL-12/IL-23 cytokine family and is made up of two distinct subunits: Epstein-Barr virus (EBV)-induced gene 3 (Ebi3) and IL-27p28, which is also referred to as IL-30. The IL27 receptor complex comprises IL-27ra and glycoprotein 130 (gp130) [5]. Some immune cells express IL-27 and its receptor, including monocytes, macrophages, dendritic cells (DCs), neutrophils, T cells, and B cells [6]. Recent research has shown that interleukin-30 (IL-30), a part of IL-27, belongs to the IL-6 cytokine family and has distinct biological functions independent of IL-27. Generated by immune cells of myeloid origin, including dendritic cells, macrophages, monocytes, and microglia, in response to different immunological and microbiological stimuli. Furthermore, it is produced by endothelial, epithelial, plasma, and neutrophil cells [7,8]. Prior studies suggest that inflammatory substances, including IL-6, IL-11, and IL-27, have the potential to stimulate fibroblast-like synoviocytes (FLS) and lead to the production of additional cytokines [9]. Elevated levels of IL-27 have been seen in individuals with RA, and it may impact the advancement of the illness by regulating synovial fibroblasts and other immune cell responses [10]. IL-30 regulates T cells to mitigate inflammation-induced acute and chronic liver damage. In contrast, individuals with sepsis have increased concentrations of IL-30 in their bloodstream [7]. Increased IL-30 serum levels have also been reported in some autoimmune diseases, such as psoriasis [8]. This study aimed to assess the utility of IL-27 and IL-30 as biomarkers for disease activity in rheumatoid arthritis patients and their correlation with therapeutic response.

METHODS

Study design and setting

The study design is a case-control study conducted in the Rheumatology Consultation Clinic at Baghdad Teaching Hospital, Medical City, from November 2023 to February 2024.

Inclusion criteria

Patients with RA who were clinically diagnosed by a rheumatologist using the American College of Rheumatology 2010 criteria were divided into three groups: those who were treated with disease-modifying antirheumatic drugs (DMARDs) such as Methotrexate 2.5 mg, those who were treated with biological DMARDs such as Etanercept 50 mg, and those who received a combination of both.

Exclusion criteria

Patients refused to participate in this study: patients with autoimmune illnesses other than RA, pregnant women, patients under the age of 18, rheumatoid arthritis patients who have received biological DMARDs rather than etanercept 50 mg, and patients who have received disease-modifying antirheumatic medications (DMARDs) rather than 2.5 mg MTX.

Sampling and outcome measurements

One hundred patients with RA were included in the study. Among them, 84 were females and 16 were males. The patients were between the ages of 25 and 75 years. A specialist rheumatologist diagnosed RA based on clinical examination, which was further verified by laboratory tests and radiographic imaging. Fifty healthy persons were used as a control sample and paired with patients based on sex and age. Using sterile techniques, 5 mL of venous blood samples were taken from both patients and controls. The blood samples were incubated at room temperature for 30 minutes, followed by centrifugation at 3000 revolutions per minute for 15 minutes. Sample serum was kept at -20 °C in Eppendorf tubes until testing by ELISA for ACCP and IL-27 (Sunlong Biotech, China) and IL-30 (ELK Biotechnology, USA). ESR was measured using the conventional Westergren technique.

Ethical consideration

The study adhered to the Helsinki Declaration's principles. We informed all participants about the purpose and recorded their willingness to participate. The Ethical Committee of the College of Medicine, University of Baghdad, approved this study under document number 0244 dated September 29, 2023.

Statistical analysis

Except for the receiver operating characteristic (ROC) curve, statistical analysis was carried out using SPSS Statistical software (Version 26; SPSS, IBM) and Microsoft Office Excel 2010. For comparisons of quantitative variables between study groups, independent samples of analysis of variation (ANOVA), the least significant difference (LSD) F test, and Students' t-test were used. Normally distributed data is expressed as mean±SD. The Pearsons' correlation test identified correlations between immunological testing and other factors. Statistical significance was determined at $p< 0.05$.

RESULTS

Table 1 displays the distribution of RA patients based on DAS28 mean scores: mild < 3.2, moderate > 3.2, and severe > 5.1. Severe cases had a mean ESR of 53.65±31.83 mm/hr, which was significantly higher than moderate cases (41.13±25.13 mm/hr) and low cases (26.64±18.38 mm/hr) (p=0.001). The severe stage had a higher mean anti-CCP antibody level of 22.25±6.64 U/mL compared to the moderate (17.78±3.79 U/mL) and low stages (16.23±2.68 U/mL) (p=0.009). IL-27 level in severe stage was 67.28±19.65 pg/mL and significantly greater than the intermediate (54.34±5.61) and low stages (43.63±8.30 pg/mL), p=0.002.

Table 1: Distribution of biomarkers according to DAS28 outcomes of disease severity

Assays	Severity of RA disease			*p*-value
	Low (DAS28 ≤3.2)	Moderate (DAS28 > 3.2)	Severe (DAS28 > 5.1)	
ESR (mm/hr)	26.64±18.38	41.13±25.13	53.65±31.83	0.001
Anti-CCP (U/mL)	16.23±2.68	17.78±3.79	22.25±6.64	0.009
IL-27 (pg/mL)	43.63±8.3	54.34±5.61	67.28±19.65	0.002
IL-30 (pg/mL)	43.61±8.17	65.73±6.62	85.49±17.9	0.007

Data is expressed as mean±SD.

Similarly, IL-30 levels in the severe stage were 85.49±17.9 pg/mL, which were greater than both moderate (65.73±6.62) and low stages (43.61±8.17 pg/mL), *p*=0.007. Table 2 shows that RA patients had higher mean levels than the healthy control group, indicating a statistically significant difference. The ESR in RA patients was 43.71±29.03 mm/hr, while in the control group was 8.21±4.99 mm/hr (*p*=0.005). Anti-CCP antibody levels in RA patients were 19.49±5.74 U/mL, while the control group had 10.03±2.83 U/mL, *p*=0.002. The IL-27 level in RA patients was 57.94±17.04 pg/mL, while in the control group it was 30.67±6.29 pg/mL (*p*=0.001). The IL-30 level in RA patients was 69.95±21.11 pg/mL, while the control group was 33.11±7.69 pg/mL (*p*=0.007). Table 3 shows that there is no statistically significant difference in the response to various drugs among RA patients when the types of therapies are compared in terms of biomarkers, ESR, and anti-CCP antibodies.

Table 2: Serum levels of the markers studied in RA patients and controls

Assays	Groups		*p*-value
	Control	RA Patients	
ESR (mm/hr)	8.21±4.99	43.71±29.03	0.005
Anti-CCP (U/mL)	10.03±2.83	19.49±5.74	0.002
IL-27 (pg/mL)	30.67±6.29	57.94±17.04	0.001
IL-30 (pg/mL)	33.11±7.69	69.95±21.11	0.007

Data is expressed as mean±SD.

However, there was a highly significant difference in IL-27 level in RA patients receiving methotrexate (63.945±22.313 pg/mL), which is raised greater than both etanercept (56.664±14.882 pg/mL) and etanercept+ methotrexate (52.677±9.025 pg/mL), *p*=0.007.

Table 3: Distribution of markers studied in RA patients according to the response to treatment

Assays	Treatments			*p*-value
	Etanercept	Methotrexate	Etanercept + Methotrexate	
ESR (mm/hr)	38.58±27.64	48.29±30.52	43.97±28.804	0.391
Anti-CCP (U/mL)	19.83±4.86	19.62±5.49	19.01±6.88	0.751
IL-27 (pg/mL)	56.66±14.88	63.94±22.31	52.68±9.02	0.007
IL-30 (pg/mL)	68.52±19.1	76.67±25.55	64.10±15.58	0.009

Data is expressed as mean±SD.

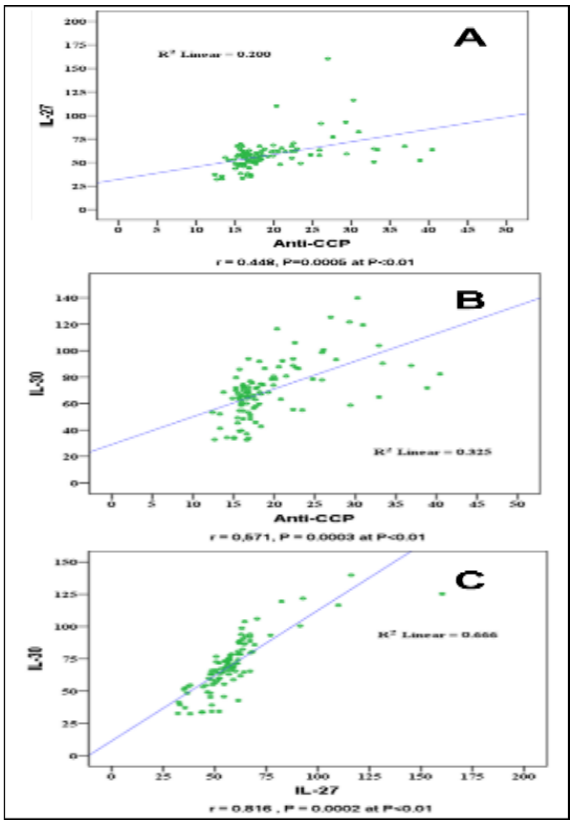

Figure 1: Correlation between levels of Anti-CCP with IL-27 (A), IL-30 with Anti-CCP (B), and IL-30 with IL-27 (C) in sera of RA patients.

RA patients on methotrexate have significantly higher levels of IL-30 (76.67±25.55 pg/mL) compared to those on etanercept (68.52±19.1 pg/mL) or etanercept + methotrexate (64.10±15.58 pg/mL) (*p*=0.009). Strong positive correlations were predicted between anti-CCP antibodies and IL-27 (*r*=0.448, *p*<0.005), anti-CCP antibodies and IL-30 (*r*=0.571, *p*<0.003), and IL-27 and IL-30 (*r*=0.816, *p*<0.002) (Figure 1). The performance characteristics of serum IL-27, IL-30, and anti-CCP validity as diagnostic assays in RA patients were determined. Figure 2 showed that IL-27 was highly valid with a sensitivity of 98%, specificity of 72%, area under the curve (AUC) of 0.974, accuracy of 89.34%, a cutoff value of 33.8, and *a p-value* of 0.005. IL-30 has an AUC of 0.964, sensitivity 96%, specificity 68%, accuracy 86.67%, cutoff value 33.8, and *p*= 0.005. The anti-CCP had an AUC of 0.968, sensitivity 96%, specificity 84%, accuracy 92%, cutoff value 13.5, and *p*= 0.003.

DISCUSSION

One reliable indicator of rheumatoid arthritis is the presence of antibodies against cyclic citrullinated peptides (CCPs). In addition to ACCP, antibodies that stimulate the enzyme involved in citrullination may amplify the disease's erosive effects [11]. Our findings support prior studies by demonstrating that the levels

of ACCP in the serum of rheumatoid arthritis patients rise as the disease progresses.

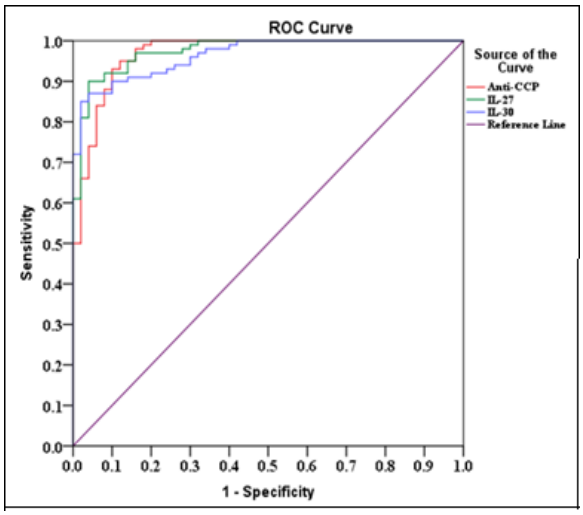

Figure 2: Validity tests of Anti-CCP antibody, IL -27 and IL -30 by using ROC test in sera of RA patient and control.

We discovered that ACCP levels were greater in individuals with severe disease activity than in those with moderate activity. Furthermore, we observed that ACCP levels were consistently greater in all patients with rheumatoid arthritis than in healthy controls [12]. Inflammation exacerbates rheumatoid arthritis (RA), and as inflammation progresses, so does the erythrocyte sedimentation rate (ESR), a laboratory measurement that is not unique to any condition. The results of our study corroborate a previous local investigation, which demonstrated that patients with rheumatoid arthritis (RA) exhibited elevated erythrocyte sedimentation rate (ESR) levels in comparison to healthy controls [13]. This study found that RA patients have substantially elevated serum IL-27 levels compared to healthy controls. This finding is consistent with previous research that also observed increased levels of IL-27 in the blood of rheumatoid arthritis compared to healthy controls [14,15]. The serum IL-27 levels are positively correlated with DAS28, and this has already been confirmed by others [16-18]. In contrast, IL-27 stimulates Th1, CD8, NK, and B cell proliferation and activity while suppressing Treg and Th2 cells, hence exerting pro-inflammatory functions. However, IL-27 also reduces inflammation by increasing the production of IL-10 and inhibiting DC activity [19, 20]. Studies have shown that IL27 and IL12 work in concert to enhance the production of IFNγ by CD4, CD8 T cells, and NK T cells in adaptive immune systems. Intrinsic immunity stimulates the production of IL1, TNF-α, IL18, and IL12 in monocytes, as well as IL1 and TNF-α in mast cells [21]. IL-27 influences the progression of RA through stimulating synovial fibroblast activation and Th1 cell differentiation. These mechanisms can worsen RA synovitis, intensify inflammation, and stimulate various immune cells [22]. Recent studies have shown that interleukin (IL)-30, a component of the IL-27 cytokine, may exhibit biological effects even without binding to EBI3 [7]. Data show that IL-30 and IL-27 possess overlapping pro-inflammatory properties in human monocytic cells. Several reasons may explain the two cytokines' differing responses. For instance, IL-30 and IL-27 may have different gp130/WSX-1 affinities. Additionally, variations in the IL-27p28 protein structure, whether coupled to or freed from EBI3, may potentially hinder or promote receptor binding, respectively [23]. However, no previous study has provided information on the serum IL-30 level in individuals with rheumatoid arthritis. This study aimed to address the lack of information by conducting a comparison of the demographic and clinical laboratory characteristics between patients with rheumatoid arthritis (RA) and healthy individuals. The results of this investigation showed that RA patients had considerably greater blood IL-30 levels than healthy groups. Furthermore, our results demonstrate a favorable association between the DAS28 score, which measures the severity of RA, and blood levels of IL-30. As a result, RA diagnosis and therapy may be drastically improved if the cytokines involved in RA pathogenesis can be identified. More in vitro and in vivo research is necessary to understand the role of IL-30 in RA completely. The highest levels of serum IL-27 and IL-30 were in the group that took methotrexate, followed by the group that took etanercept, while the lowest were in the group who took both etanercept and methotrexate. While the use of methotrexate enhances the production of adenosine, it will inhibit the divided immune cells and the production of cytokines, which promotes remission of the disease [24]. The use of etanercept inhibits TNF-α, resulting in a reduction of cell death and activation of other inflammatory mediators [25], which had a bigger effect on IL-27 and IL-30. When compared to methotrexate or etanercept used individually, the combination of the two was significantly more effective in reducing disease activity, improving functional deficits, and decreasing disease progression, as shown on x-rays.

Conclusion

There is a strong correlation between RA severity and serum levels of IL-27 and IL-30 (subunit 27). A positive correlation exists between elevated levels of serum IL-27, IL-30, anti-CCP, ESR, and DAS28. Serum IL-27 and IL-30 are important in the development of RA and can be used to predict how well methotrexate and etanercept will work as treatments. This means that serum IL-27 and IL-30 are not only important in understanding disease pathophysiology; they could also be used as biomarkers and treatment targets in the future.

Conflict of interests

No conflict of interest was declared by the authors.

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The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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