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The Role of Interleukin-22 in The Diagnosis and Evaluation of Disease Activity in Rheumatoid Arthritis

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Abstract

Background: Rheumatoid Arthritis (RA) is a chronic autoimmune disease that primarily affects small joints, resulting in joint inflammation, pain, and limited mobility. The identification of novel biomarkers specific to RA could enhance early diagnosis and treatment and also improve the monitoring of the disease activity and treatment response. In this study, the levels of IL-22 were measured in 133 patients with inflammatory arthritis using the ELISA method with the aim of assessing the diagnostic value of IL-22 in the diagnosis of RA and evaluating the disease activity. The findings revealed that serum IL-22 levels were significantly higher in RA patients compared to patients with other types of inflammatory arthritis. Additionally, positive correlations were observed between IL-22 and disease activity scores as determined by CDAI, DAS-28 ESR, and DAS-28 CRP. Notably, when IL-22 was used as a diagnostic tool, with a cut-off value of ≥ 26.9 pg/ml, it exhibited a sensitivity of 83%, specificity of 75%, and an AUC of 0.838. Furthermore, significant associations were found between IL-22 levels and the age of RA patients, while no significant correlations were observed with gender, BMI, and smoking index. It is noteworthy that IL-22 levels were significantly correlated with ESR, RF titer, and ACPA levels. Consequently, IL-22 can be regarded as a valuable biomarker for diagnosing RA and evaluating disease activity. **Subjects and method:** This cross-sectional study was conducted from September 2022 to January 2023, involving 133 patients with inflammatory arthritis, including RA and other forms of inflammatory arthritis. All patients were recruited from As-Sader Teaching Hospital in Najaf. The ages of the patients included in the present study range from 20 to 70 years, with a sample comprising 116 females and 17 males. **Results:** The higher median (IQR) value of IL-22 was observed among the RA patients [36.9 (28.6-63.7) pg/ml] compared to that of the other patients with inflammatory arthritis [23.6 (19.8-26.8) pg/ml]. The difference between the two groups of patients was highly significant ($P < 0.0001$). Among the RA patients, a highly significant difference in the median (IQR) level of IL-22 was observed among the disease activity groups according to CDAI, DAS-28 ESR, and DAS-28 CRP ($P < 0.0001$). **Conclusion:** Interleukin-22 can be regarded as a reliable biomarker for the diagnosis of RA and holds potential for assessing disease activity.

Keywords: Arthritis (RA), Clinical Disease Activity Index, (CDAI), DAS-28 CRP, DAS-28 ESR, Interleukin 22 (IL-22), Rheumatoid.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic multi-system inflammatory autoimmune disease of uncertain etiology. The primary target of this condition is synovial joints, which leads to persistent inflammation and subsequent destruction of both cartilaginous and bony components. Consequently, individuals with RA experience pain and functional impairment. Furthermore, the disease presents a variety of extra-articular manifestations that affect multiple systems [(1)]. Rheumatoid arthritis is the most prevalent autoimmune inflammatory arthritis, with an estimated prevalence of approximately 0.24% to 1% in the population. In Iraq, the incidence of RA among patients attending rheumatology units has shown a significant increase from 1.1% in 2014 to 10% in 2019. [(2)]. Rheumatoid arthritis is a disease of unknown origin; however, in recent years, it has become evident that RA arises due to genetic and epigenetic components [(3)]. Substantial progress has been made in the past decade concerning the understanding of RA's pathogenesis. Nevertheless, studies still lack a comprehensive understanding of the molecular network that predisposes individuals to develop the disease, exacerbates symptoms, or leads to a favorable response to specific treatments. [(4)]. The heterogeneity of clinical manifestations and the variability of therapeutic response serve as evidence of the intricate nature of this disease. The advancements made in comprehending the pathogenesis of RA have sparked a surge of interest in investigating the biomarkers implicated in various disease stages. Consequently, novel biomarkers continue to be identified and studied in this field [(5)]. Biomarkers play a crucial role in guiding the clinical and therapeutic management of all stages of RA. They offer valuable insights by aiding in the prediction of disease development in individuals at risk, bridging the serological gap to enhance diagnosis, providing prognostic information to inform therapeutic decisions, assessing treatment responses and outcomes, and enabling the monitoring of disease activity and progression [(6)]. The major role of biomarkers can be elucidated by comparing the diagnostic criteria. The sole biomarker in the American College of Rheumatology (ACR) 1987 criteria is the rheumatoid factor (RF). In contrast, the updated ACR/EULAR 2010 criteria for early diagnosis of RA incorporate four serological tests, namely Rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) [(7).] The biomarkers currently available for the diagnosis, activity, prognosis, and management of rheumatoid arthritis (RA) have several limitations. Rheumatoid factor lacks specificity, as positive RF testing can be triggered by any condition that causes chronic antigenic stimulation. While anti-citrullinated protein antibodies (ACPA) are more specific, both tests fail to identify 20%-25% of patients with seronegative RA[(8)]. Although analyzing the fine specificity of ACPA does not provide additional information regarding disease activity or the calculation of the progression score, it may contribute to predicting extra-articular involvement. Due to the lack of adequate biomarkers for this purpose, disease activity monitoring still relies on clinical evaluation. Moreover, ESR and CRP are nonspecific acute-phase reactants that can be elevated for various reasons [(9)]. There is a subset of patients diagnosed with RA who exhibit erosive disease despite testing negative for RF, ACPA, and human leukocyte antigen (HLA). The absence of serological markers in these individuals poses a challenge in initiating early and appropriate treatment [(10)]. Therefore, there is a critical need for more reliable predictors of disease outcomes, such as soluble biomarkers that can be detected at an early stage of the disease progression.

Interleukin 22 (IL-22) is a member of the IL-10 family, synthesized by various cell types, predominantly CD4+ T-cells and natural killer (NK) cells. It plays a critical role in tissue protection, regulation of inflammation, and autoimmunity. Interleukin 22 binds to a unique receptor composed of IL-10R2 and IL-22R1 subunits, with IL-22R1 being highly expressed in synovial fibroblasts. Upon binding to IL-22R1, IL-22 triggers the activation of signaling pathways, including STAT3 and protein kinase cascades. Notably, IL-22 is involved in the synthesis of anti-inflammatory proteins such as IL-11; it also influences the expression of pro-inflammatory cytokines like IL-6 and acute-phase reactants cytokines. Elevated serum levels of IL-22 have been observed in patients with rheumatoid arthritis (RA), serving as an indicator of disease severity [(11)]. This research aimed to study the diagnostic value of interleukin-22 (IL-22) as an ideal biomarker for the diagnosis of RA and differentiate it from other types of inflammatory arthritis in addition to studying their roles in the evaluation of RA activity.

MATERIALS AND METHODS

A cross-sectional study on 133 patients with inflammatory arthritis, rheumatoid arthritis and other inflammatory arthritis, was conducted from September 2022 to January 2023. All the patients were recruited from As-Sader Teaching Hospital in Najaf. The age of the patients enrolled in the current study range from 20 to 70 years. The sample included 116 females and 17 males. Patients at the time of their clinic visit were randomly selected and classified into:

- Group 1 that consists of patients with RA who are classified as remission, mild, moderate, or severe based on their disease activity score (CDAI, DAS-28 ESR, and DAS-28 CRP).
- Group 2 that consists of patients with other types of inflammatory arthritis.

Inclusion criteria:

1. Patients who are diagnosed with RA by a rheumatologist according to the 2010 ACR/EULAR criteria get a score > or = 6 on these criteria and their ages are between 20 and 70 years.
2. Patients with other inflammatory arthritis.

Exclusion criteria:

Patients with other autoimmune diseases, central nervous system diseases, cardiovascular diseases except for hypertension, immunodeficiency disease, malignancy, and chronic infections, patients who have had recent surgery, wound, or acute local inflammation, and patients older than 70 years and younger than 20 years. The serum levels of IL-22 and ACPA in patients with inflammatory arthritis were measured using the Enzyme-Linked Immunosorbent Assay technique (ELISA) (Sunlong, China). Additionally, ESR was measured using the Westergren method, while RF and CRP were assessed using the sandwich immunodetection method (Boditech/Korea). All data from both study groups were collected and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 26, developed by Inc. in Chicago, USA.

THE RESULTS

The higher median (IQR) value of IL-22 was observed among the RA patients [36.9 (28.6-63.7) pg/ml] when compared to that of the other inflammatory arthritis patients [23.6 (19.8-26.8) pg/ml] with a highly significant difference (P = <0.0001) between the two groups of patients as shown in Table 1. In the RA patients, a highly significant difference in the median (IQR) level of IL-22 was observed among the disease activity groups according to CDAI, DAS-28 ESR, and DAS-28 CRP (P= <0.0001) as shown in table (2). Furthermore, a

highly significant positive correlation was observed between the disease activity score according to CDAI, DAS-28 ESR, and DAS-28 CR and the level of IL-22 (P= <0.0001), as shown in Figures (1, 2 and 3). A statistically significant correlation was observed between the level of IL-22 and age (p-value = 0.001). Nevertheless, no statistically significant correlations were identified between IL-22 levels and gender, BMI, and smoking index, as evidenced by the respective p-values [(P = 0.787), (P = 0. 317), (P = 0.103), respectively], as presented in Table (3). There was a statistically significant correlation of IL-22 value with ESR level, RF titer, and ACPA level (P = 0.013, P = 0.0001, and P = 0.0001, respectively), but there was no significant correlation between IL-22 level and the CRP titer (P = 0.099) as shown in Table (4). A receiver operating characteristic (ROC) curve was used to quantify the diagnostic utility of IL-22 and the other two routine biomarkers (RF and ACPA) in the RA patients proven by ACR criteria (the current gold standard) and to differentiate RA patients from the other inflammatory arthritis patients, as shown in Figure.(4). At 26.9 pg/ml as a cutoff value, IL-22 was considered a good predictor of RA because of an area under the curve of more than 0.8 and highly significant P values (P = 0.0001) and the level of accuracy was more than 70%. In addition, the sensitivity of IL-22 for the diagnosis of RA was 83% which is higher than the sensitivity of RF but lower than the sensitivity of ACPA, while the specificity was 75% which is lower than the specificity of RF and ACPA. The positive and negative predictive values were clarified in the Table (5).

Table No. 1: Comparison of IL-22 Values between the RA Patients and other Inflammatory Arthritis Patients.

Characteristic	RA patients	Other Inflammatory Arthritis Patients	P- value
IL-22 (pg/ml), median (IQR)	36.9 (28.6-63.7)	23.6 (19.8-26.8)	<0.0001

Table No. 2: Comparison of IL-22 Values among Disease Activity Groups of the RA Patients According to CDAI, DAS-28 ESR, and DAS-28 CRP.

	CDAI				P- value
	Remission n = 1	Low Activity n = 28	Moderate Activity n = 50	High Activity n = 30	
IL-22 (pg/ml), median	20.7 (20.7-20.7)	26.4 (21.7-28.8)	36.1 (29.4-46.8)	69.8 (65.0-74.2)	<0.0001
	DAS-28 ESR				
	Remission n = 1	Low Activity n = 28	Moderate Activity n = 50	High Activity n = 30	
	25 (22.1-27.9)	28.6 (23.7-36.1)	34.5 (28.6-46.8)	68.7 (63.5-74.9)	

(IQR)	DAS-28 CRP				
	Remission n = 19	Low Activity n = 20	Moderate Activity n = 50	High Activity n = 20	
	25 (20.7-29.1)	29 (26.5-34.8)	43.2 (31.4-63.8)	68.7 (63.5-76.1)	

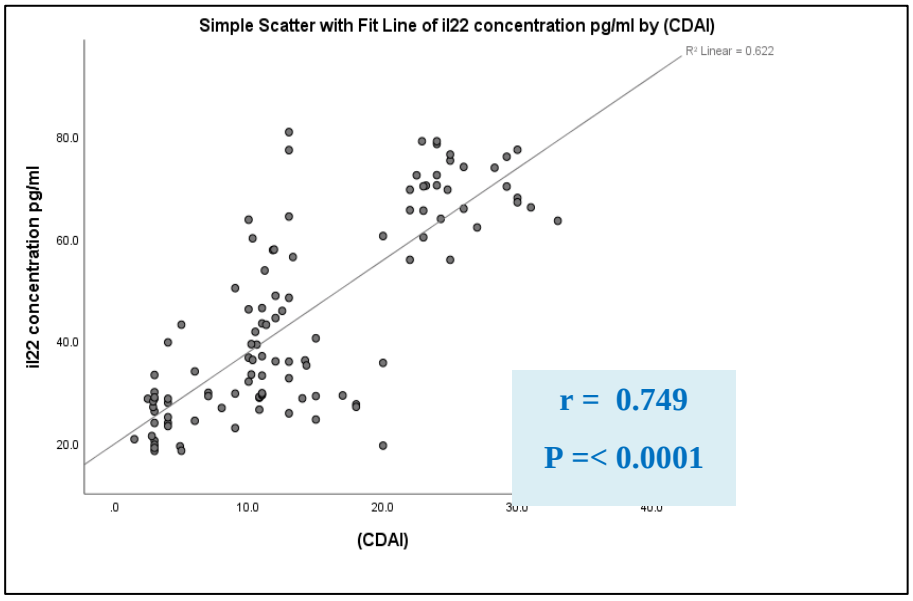

Figure No. 1: The Correlation between IL-22 level and RA Activity score According to CDAI. Results for IL-22 are Expressed as picograms per milliliter.

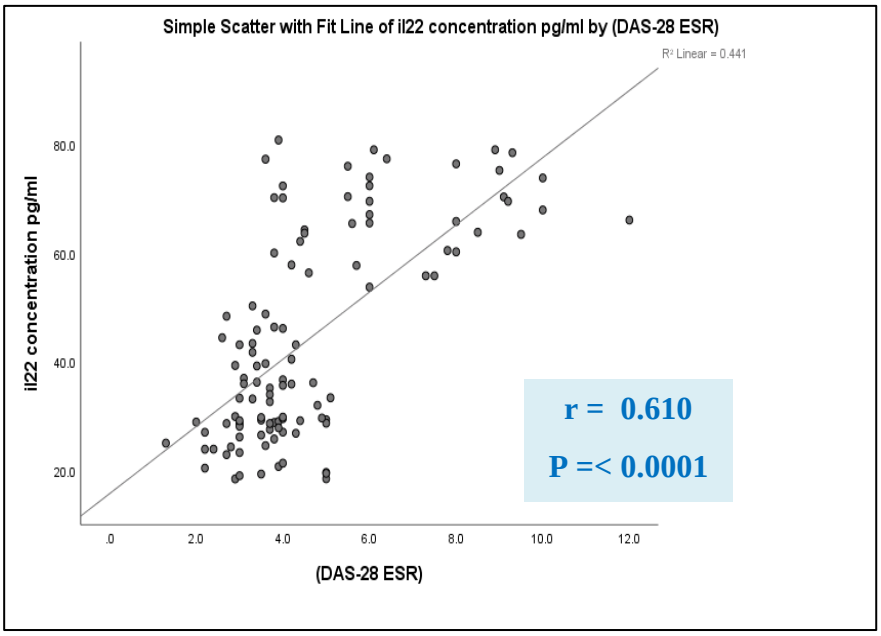

Figure No.2: Correlation between IL 22 level and RA Activity score According to DAS-28 ESR. Results for IL-22 are Expressed as picograms per milliliter.

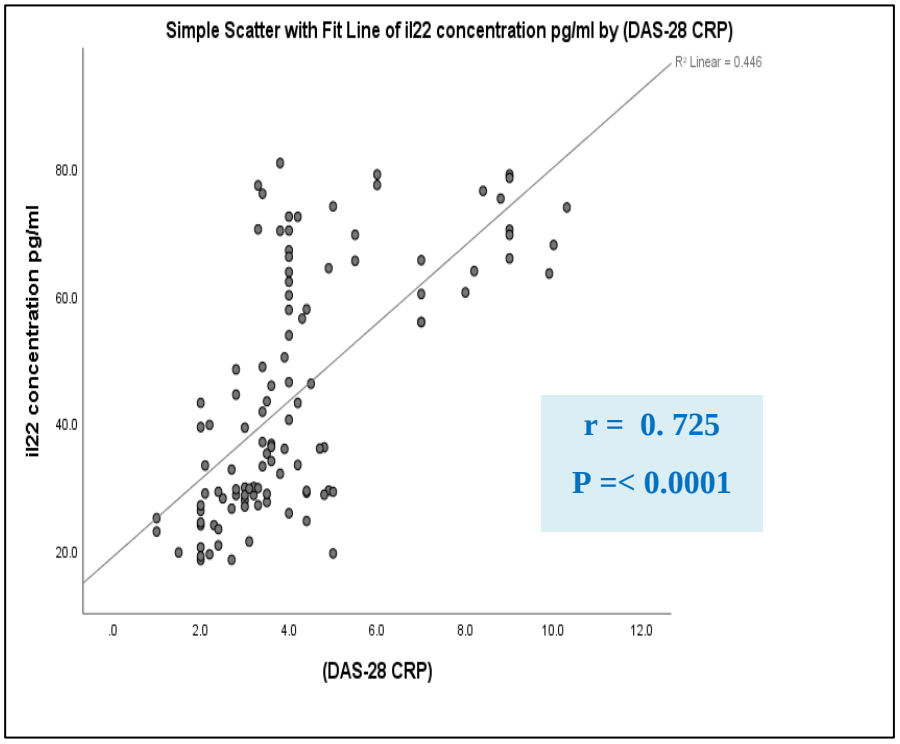

Figure No.3: The Correlation between IL-22 level and RA Activity Score According to DAS-28 CRP. Results for IL-22 are Expressed as picograms per milliliter.

Table No. 3: The Correlations of Demographic Characteristics and BMI with IL-22 Value in the RA Patients.

Characteristic		IL-22
Age (years)	Correlation Coefficient (r)	0.319
	P-value	0.001
Gender	Correlation Coefficient (r)	0.026
	P-value	0.787
BMI	Correlation Coefficient (r)	0.097
	P-value	0.317
Smoking index, (pack per year)	Correlation Coefficient (r)	0.341
	P-value	0.103

Table NO. 4: Correlations between IL-22 level and levels of other Biomarkers in the RA patients.

Parameters		IL-22 (pg/ml)	ESR (mm/h)	CRP titer (mg/L)	RF titer (IU/mL)	ACPA (U/ml)
IL-22 (pg/ml)	r	1				
	P					
ESR (mm/h)	r	0.237	1			
	P	0.013				
CRP	r	0.159	0.463	1		
	P	0.099	0.0001			
RF (IU/mL)	r	0.456	0.111	0.122	1	
	P	0.0001	0.248	0.205		
ACCP (U/ml)	r	0.402	0.143	0.155	0.416	1
	P	0.0001	0.138	0.107	0.0001	

Table No.5: Characteristics of Receiver Operator Characteristic (ROC) Curve of the Inflammatory Arthritis Patients.

Characteristic	IL-22	RF	ACPA
AUC	0.838	0.820	0.908
SE	0.043	0.048	0.032
Sig.	0.0001	0.0001	0.0001
95% Confidence Interval	0.753- 0.922	0.727- 0.914	0.846- 0.971
Optimal Cut-Point Value	26.9 pg/ml	18.1 IU/mL	19.0 U/ml
Sensitivity (%)	83	70	84
Specificity (%)	75	83	95
PPV (%)	93	95	98
NPV (%)	50	38	57

Diagnostic effectiveness (accuracy)	**82**	**72.9**	**86.4**
Youden's index	**0.60**	**0.70**	**0.87**

AUC, Area under the Curve; SE, stander error, PPV (Positive Predictive Value), NPV, (Negative Predictive Value); Youden's index is a measure for evaluating the biomarker effectiveness.

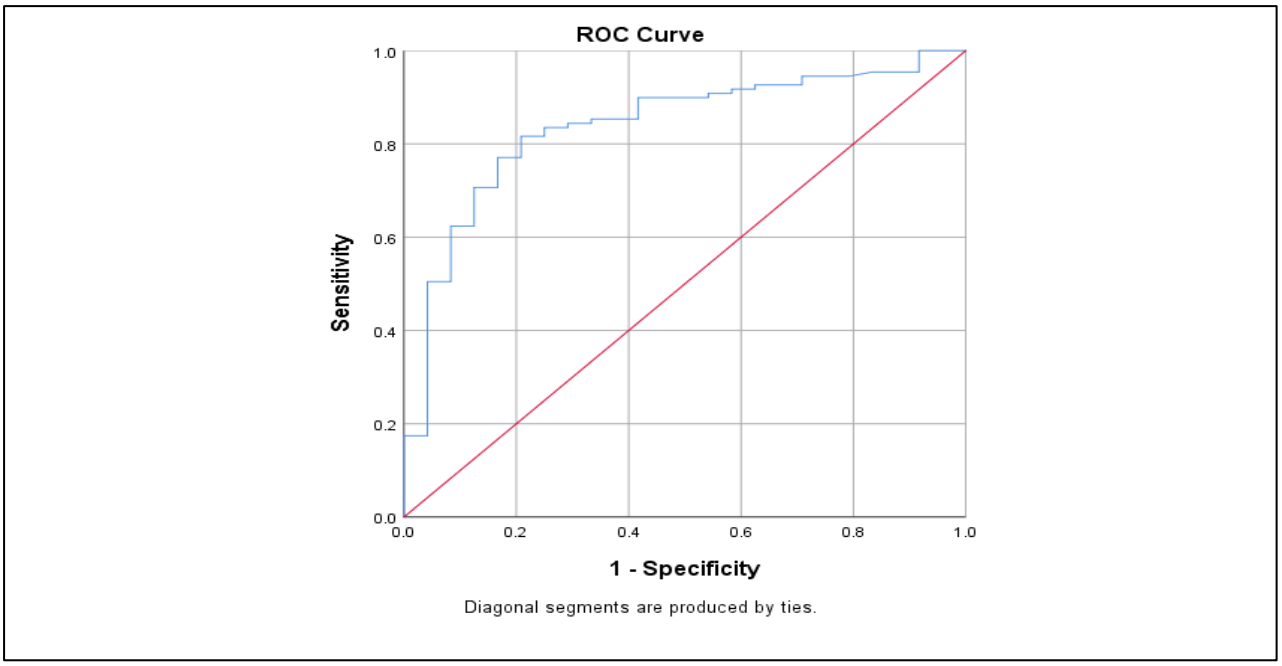

Figure (4): Receiver Operator Characteristic (ROC) Curve Analysis to Find the Best IL-22 Cutoff Value that can Diagnose RA, and Differentiate it from Other Inflammatory Arthritis.

DISCUSSION

According to CDAI, DAS-28 ESR, and DAS-28 CRP, the present study showed significant differences in IL-22 among the four groups of disease activity. Rheumatoid arthritis patients in the high-activity group had significantly the highest median IL-22 level followed by the moderate activity group, then the intermediate activity group, while the lowest median IL-22 level was in the low-activity group. So, IL-22 level had significant associations with the disease activity group progression, and this finding was confirmed by the presence of a highly positive correlation between IL-22 level and the disease activity score. This finding is compatible with Khater & Sheik [(11)]. The potential explanations for this higher median IL-22 of the RA patients in the high-activity group may be related to the increased production and activation of Th17 cells and other immune cells in the synovial tissue, contributing to the inflammatory and destructive processes that occur in active RA [(11)].

Based on the results of the present study, the increase in the level of IL-22 is considered an important marker for the increase in the activity of the disease in patients with RA.

Furthermore, the level of IL-22 may be used to monitor disease activity and response to therapy. Therefore, monitoring this biomarker can be useful in the management of RA as they provide important information about disease activity. Regular monitoring of this biomarker can help guide treatment decisions and optimize disease control. The present study showed that serum IL-22 levels had a significant positive correlation with age. This is consistent with the results of Yousef [12]. This may be because IL-22 is involved in the chronic inflammation associated with RA. Interleukin-22 is involved in the recruitment of immune cells and the production of inflammatory cytokines, which can contribute to tissue damage in RA. Older patients with RA may have more chronic inflammation and tissue damage, leading to higher levels of IL-22 [12]. According to this study, there was no significant correlation of IL-22 level with gender, BMI, and smoking index. The present result is similar to the results of Yousef [12]. These findings of the current study demonstrate many ideal features of IL-22 as a diagnostic biomarker for RA because this biomarker is not affected by gender, BMI, and smoking index. Based on the findings of this study, there was a significant correlation between the IL-22 level and the ESR level, RF titer, and ACPA level. The current result aligns with the findings of Yousef (12). This suggests that IL-22 may be involved in the production of RF and ACPA, or that these autoantibodies may influence the production or activity of IL-22. One proposed mechanism is that IL-22 stimulates the production of citrullinated proteins, which are then targeted by ACPA. Additionally, IL-22 has been shown to promote the survival and activation of B cells, which are responsible for producing RF and ACPA [12]. Interleukin-22 had a high sensitivity for the diagnosis of RA compared to RF and it was almost identical to ACPA. It had higher AUC and accuracy compared to RF but lower than that ACPA. Additionally, IL-22 has a lower specificity compared to RF and ACPA. This is consistent with the result of Aubaid [13]. Although IL-22 has a lower specificity, its relatively high sensitivity and accuracy can make it a potential biomarker for the diagnosis of RA. Additionally, it is an inexpensive test.

CONCLUSION

Interlukin-22 can be considered a good biomarker for the diagnosis of RA and can be used to evaluate the disease activity.

Approval of the Ethical Committee

Before commencing the research project, this study received ethical approval from the Faculty of Medicine's ethical committee at the University of Kufa. Informed consent was obtained from all participating patients, and permission was acquired from the Rheumatology Unit.

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