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A novel Link of Serum IL-39 Levels in Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is an inflammatory condition causing joint pain and stiffness, with often debilitating and life-limiting consequences. Recently, a new B-cell secreted cytokine, IL-39, was identified in mice. The most up-to-date research indicates that although IL-39 is expressed in murine models of lupus and has a role in mediating the inflammatory response in this context, there is no solid, replicated evidence of the existence of IL-39 in humans. This study aimed to clarify the existence and role of IL-39 in the human body and to elucidate whether it plays a role in rheumatoid arthritis. Accordingly, serum samples were collected from 66 patients with rheumatoid arthritis who were under therapy and from 66 healthy controls attending the Baghdad Teaching Hospital Rheumatology Unit. Enzyme-linked immunosorbent assay (ELISA) was used to detect serum levels of IL-39. Our results showed that mean ages of RA patients were 46.48 ± 10.17 years, and for healthy controls 44.97 ± 11.658 years. The results revealed that serum IL-39 levels were significantly lower in RA patients (p = 0.016) (4.95 ± 1.1001) compared to healthy controls (5.55 ± 1.762). Corresponding sensitivity of IL-39 was 56.1%and specificity was 60.6% at cutoff values of ≤ 4.99 ng/ml. In Conclusion, IL-39 is found in humans but is downregulated in rheumatoid arthritis patients. This suggests either that IL-39 can have both pro- or anti-inflammatory functions based on the underlying disease or that the role of IL-39 is masked by the effects of treatment.

Keywords: IL-39**,** rheumatoid arthritis, autoimmune condition, inflammation

رابط جديد لمستويات مصل IL-39 في مرضى التهاب المفاصل الروماتويدي

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الخلاصة:

التهاب المفاصل الروماتويدي (RA) هو حالة التهابية تسبب آلامًا وتيبسًا في المفاصل ، وغالبًا ما يكون لها عواقب منهكة ومحددة للحياة. في الآونة الأخيرة ، تم التعرف على خلية B جديدة تفرز السيتوكين ، IL-39 ، في الفئران. تشير أحدث الأبحاث إلى أنه على الرغم من التعبير عن IL-39 في نماذج الفئران من الذئبة وله دور في التوسط في الاستجابة الالتهابية في هذا السياق ، لا يوجد دليل قوي مكرر على وجود IL-39 في البشر . هدفت هذه الدراسة إلى توضيح وجود ودور IL-39 في جسم الإنسان وتوضيح ما إذا كان

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يلعب دورًا في التهاب المفاصل الروماتويدي. وعليه ، تم جمع عينات مصل الدم من 66 مريضا مصابين بالتهاب المفاصل الروماتويدي كانوا يخضعون للعلاج ومن 66 من الاصحاء الذين حضروا الى وحدة الروماتيزم في مستشفى بغداد التعليمي. تم استخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) للكشف عن مستويات المصل من IL−39. أظهرت نتائجنا أن متوسط أعمار مرضى التهاب المفاصل الروماتويدي كان 46.48 ± 10.17 سنة ، وللأصحاء 44.97 ± 11.658 سنة. أظهرت النتائج أن مستويات IL−39 في الدم كانت أقل بشكل ملحوظ في مرضى RA (p = 0.016) (4.95 ± 1.1001) مقارنة بالضوابط الصحية (5.55 ± 1.762). كانت الحساسية المقابلة لـ IL−39 56.1٪ وكانت النوعية 60.6٪ عند قيم القطع ≥ 4.99 نانوغرام / مل. في الختام ، تم العثور على IL−39 في البشر ولكن يتم تقليله في مرضى التهاب المفاصل الروماتويدي. يشير هذا إما إلى أن IL−39 يمكن أن يكون له وظائف داعمة أو مضادة للالتهابات بناءً على المرض الأساسي أو أن دور IL−39 محجوب بتأثيرات العلاج.

Introduction

Among the different types of inflammatory arthritis, a group of conditions characterised by an autoimmune-mediated process that leads to damage and swelling of the joints known as rheumatoid arthritis (RA), is the most common. The main symptom of RA is a progressive, life-limiting pain and stiffness of the joints, mainly affecting the hands, wrists, fingers, and feet, although other joints can also be affected[1, 2]. These symptoms can have significant negative consequences on the overall quality of life of the affected individuals. There are several risk factors for rheumatoid arthritis including gender, with females being more affected than males; ethnicity; lifestyle habits such as smoking; and genetic factors such as possession of the HLA-DRB1 gene [3]. Overall, the pathophysiology of RA is mediated by autoimmune and inflammatory-related processes that result in an erosion of the bone and cartilage of the joints [4]. With a worldwide prevalence of 2% and 1% in the United Kingdom, RA affects millions of people each year, causing a significant human and economic burden on society and public health systems all over the world [5]. Based on this, a large amount of scientific research efforts has been dedicated to identify certain biomarkers of this disease, both for the purpose of diagnosis as well as prognosis prediction. Specifically, cytokines, small proteins involved in signalling in the immune system, and with proven functions in the pathophysiology of rheumatoid arthritis have received particular attention. Cytokines, which are produced by immune cells such as T-cells and B-cells, infiltrate the liquid surrounding and lubricate the joints, also known as synovial liquid, and mediate the erosion of bone and cartilage, which in turn causes inflammation and pain [6]. Previous research has identified several cytokines with important functions in the pathophysiology of arthritis, including cytokines such as TNFα, IL-1, and IL-17 [7, 8]. Indeed, such research has identified that some of these cytokines have great potential as not only diagnostic and predictive tools but even as therapeutic targets.

More recently, IL-39 was identified as a new member of the IL-12 family of cytokines in mice, specifically in a mouse model of systemic lupus erythematosus, an autoimmune disease in which the person's immune system attacks several bodily tissues, including lungs, heart, kidneys, skin and other organs [9, 10]. IL-39 is secreted by B-cells and is composed of two subunits, IL-23p19 and Ebi3 [9]. Furthermore, the researchers described that IL-39 is having a role in the mediation of inflammation in the mouse model of lupus and thus highlighted its potential role as a therapeutic target for this condition [9]. However, since this initial study, little to no research has been carried out on IL-39. This is mainly due to the fact that more recent publications have indicated that IL-39 has a murine-specific expression and is thus not expressed in humans. Indeed, with the exception of a publication from 2017 reporting the presence of IL-39 in the serum of patients with acute coronary syndrome [11], no other

publication since has found any evidence of IL-39 expression from human cells [12, 13]. The findings that IL-39 is involved in the pathophysiology of lupus in mice, which is an autoimmune and inflammatory condition, suggest it could have interesting functions in RA. However, research into any potential link between IL-39 expression and RA is hampered by the lack of clarity around its expression from human cells with literature yielding contradicting results. Thus, while recent research has identified several cytokines such as IL-40 and IL-17 as having potential as diagnostic and predictive tools for rheumatoid arthritis, the usefulness of IL-39 in this aspect remains to be elucidated. The present study focuses on confirming the presence of IL-39 in humans and aims to understand whether it is specifically expressed in the context of RA or not.

1. Materials and Methods

1.1. Patients and Controls

From November 2021 to January 2022, blood samples were collected from 66 RA patients (55 females, 11 male; age range 23-69 years) and 66 healthy controls (49 females, 17 males; age range 22-69 years) attending the Baghdad Teaching Hospital Rheumatology Unit. Exclusion criteria included RA with comorbidities with other connective tissue diseases, seronegative spondyloarthritis, malignancy, pregnancy, or patient refusal to participate. Inclusion criteria included patients with RA who had been diagnosed according to the 2010 ACR/EULAR classification criteria [14], any gender, age >18yrs, and an active disease state. For each patient, an information sheet was completed under the supervision of the rheumatologist. The information included gender, age, disease duration, disease activity score-28 (DAS-28), and type of current therapy. The following laboratory tests were also included: rheumatoid factors (RFs), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), haemoglobin (Hb), white blood cell (WBC) count, and anti-cyclic citrullinated peptide (ACCP) antibodies. The study objectives and procedures were provided to all participants and formal consent was obtained before the onset of the study. The study was approved by the scientific ethics committee of the College of Medicine, University of Baghdad. The immunological tests were performed in the Medical Research Unit at the College of Medicine, Al-Nahrain University.

RA patients were under therapy, with five different protocols followed for different treatments. Patients were either treated with: 1) methotrexate (MTX; a single oral weekly dose of 5 – 15 mg); 2) etanercept (single weekly subcutaneous dose of 25 mg) or adalimumab (20 mg); 3) corticosteroids (oral methylprednisolone: single daily dose of 50 mg); or 4) disease-modifying anti-rheumatic drugs (DMARDs), which included leflunomide (daily oral dose of 5 mg), sulfasalazine (daily oral dose of 500 – 1000 mg), azathioprine (daily oral dose of 50 mg), or hydroxyl chloroquine (daily oral dose of 200 – 500 mg). In a further group of patients, the therapy included a combination of the four protocols (combined group).

1.2. Immune assays

The serum was extracted by centrifugation at 1000-3000 rpm for 10 minutes after blood samples were taken in gel tubes, following which it was frozen at -20°C. Antibodies to IL-39 were detected in human serum using an enzyme-linked immune sorbent assay (ELISA) (Sun Long Biotech Company, China), as directed by the manufacturer. A plate reader was used to measure the absorbance at 450 nm.

1.3. Statistical analysis

Statistical analysis was performed by using the SPSS Statistical package (Version 21;

SPSS, IBM) and Microsoft Office Excel (2010) for drawing the figures except for the receiver operating characteristic (ROC) curve.

Independent samples of students (t-test) were performed for comparisons of quantitative variables between studied groups (Age / Year & serum IL – 39 ng/ml level). Normally distributed data is expressed as (Mean±SD) with Pearson chi-square test (χ^2) for comparisons of qualitative variables between studied groups (i.e., Age groups / Year, gender}& a binomial Z-test was performed for comparison of anti-CCP, RF, CRP assay, duration / Year, and smoking & treatment data. The validity of ELISA test was estimated with a ROC curve, cut-off value, area under curve (AUC), sensitivity (%), specificity (%), positive predictive value % (NPV), negative predictive value % (NPV), and accuracy. The statistical significance threshold (*P* – value) was accepted at P<0.05 & *P*<0.01.

2. Results and Discussion

Rheumatoid arthritis is a complex disease with inflammatory and autoimmune pathophysiology. Based on this, a potential avenue of research for therapeutic development involves the use of agents able to downregulate the immune response, and with reducing the progressive erosion of joints, and with it alleviate inflammation and pain. In this context, significant focus has been placed on cytokines, small proteins that are tightly linked to the inflammatory and autoimmune processes underlying RA, the usefulness of which for diagnostic, prognostic, and therapeutic purposes has already been described [7, 15].

2.1. Characteristics of patients

A demographic comparison of the control (n=66; age: 23-68 years) and RA group (n=66' age: 21-69 years) was performed.

As shown in Table 1, no significant differences were noted between the groups under study for all demographics parameters including: gender (RA: n_{female} =55 (83.3%) vs. control: n_{female} =55 47 (71.2%); (p = 0.097, p > 0.05)], age groups / year, (p = 0.601, p > 0.05); and mean age / year (p = 0.428, p > 0.05).

Table 1: Demographics distributions according to studied groups

Demographics			Studied groups		*P*-value
			Controls N = 66	RA Patients N = 66	
Gender	Male	N	19	11	*P* = 0.097 Non-significant (*P*>0.05)
		%	28.8%	16.7%	
	Female	N	47	55	
		%	71.2%	83.3%	
Age groups / Year	20 - 30	N	8	3	*P* = 0.601 Non-significant (*P*>0.05)
		%	12.1%	4.5%	
	31 - 40	N	14	15	
		%	21.2%	22.7%	
	41 - 50	N	23	27	
		%	34.8%	40.9%	
	51 - 60	N	15	16	
		%	22.7%	24.2%	
	61 - 70	N	6	5	
		%	9.1%	7.6%	
Age / Year	Mean		44.97	46.48	*P*=0.428 Non-significant (*P*>0.05)
	Std. Deviation		11.658	10.170	

As shown in Table 2; highly significant differences (P<0.01) was reviewed within study of RA patients parameters for: disease duration / Year {1 – 10 Year 44 (66.67%) two fold more than 11 – 20+ 22 (33.33%), P = 0.009, P<0.01}; smoking {non smoker 62 (93.94%) hardly increased than smoker 4 (6.06%), P = 0.00, P<0.01}; C – reactive protein (CRP) assay{positive 50 (75.76%) and negative 16 (24.24%), P = 0.00, P<0.01}; steroids drugs intake {NO 52 (78.79%) while intake (Yes) 14 (21.21%), P = 0.00, P<0.01}; finally, biologics drugs intake {Enbrel 54 (81.82%), amgivita 3 (4.54%) & NO 9 (13.64%), P = 0.00, P<0.01}.

Non-significant difference (P>0.05) were observed for: Anti - CCP assay {positive 41 (62.12%) and negative 25 (37.88%), P = 0.229, P>0.05}; RF assay {positive 43 (65.15%) and negative 23 (34.85%), (p = 0.091, p > 0.05)}; steroids drugs intake {MTX 37 (56.06%) while NO 29 (43.94%), P = 0.389, P>0.05}.

Table 2: Study of RA patient's parameters (duration/year, smoking, immunological assays & treatments.

Parameters		N = 66	%	P-value
Duration / Year	**1 - 10**	**44**	**66.67**	***P* = 0.009 Highly significant (*P*<0.01)**
	11 – 20+	**22**	**33.33**	
Smoking	**Non-Smoker**	**62**	**93.94**	***P* = 0.00 Highly significant (*P*<0.01)**
	Smoker	**4**	**6.06**	
Anti- ccp	**Positive**	**41**	**62.12**	***P* = 0.229 Non-significant (*P*>0.05)**
	Negative	**25**	**37.88**	
RF	**Positive**	**43**	**65.15**	***P* = 0.091 Non-significant (*P*>0.05)**
	Negative	**23**	**34.85**	
CRP	**Positive**	**50**	**75.76**	***P* = 0.00 Highly significant (*P*<0.01)**
	Negative	**16**	**24.24**	
Steroids	**NO**	**52**	**78.79**	***P* = 0.00 Highly significant (*P*<0.01)**
	Yes	**14**	**21.21**	
DMARDS	**NO**	**29**	**43.94**	***P* = 0.389 Non-significant (*P*>0.05)**
	MTX	**37**	**56.06**	
Biologics	**Enbrel**	**54**	**81.82**	***P* = 0.00 Highly significant (*P*<0.01)**
	Amgivita	**3**	**4.54**	
	NO	**9**	**13.64**	

Results of the demographic study showed non-statistically significant differences in the distributions of RA Patients age groups / Year according to their gender, (p = 0.351, p > 0.05) as male {31 – 40 year, raised 12 (45.5%) and 20 – 30 year, lowermost (0, 0%)}, and female {41 – 50 year, higher (24, 43.6%) and 16 – 20 year, condensed (3, 5.5%)}.

Table 3: Distributions of RA patients' age groups/year according to their gender.

Age groups / Year		Gender		Chi-Square Test (*P*-value)
		Male	**Female**	
20 - 30	**N**	**0**	**3**	***P* = 0.351 Non-significant (*P*>0.05)**
	%	**0%**	**5.5%**	
31 - 40	**N**	**5**	**10**	
	%	**45.5%**	**18.2%**	
41 - 50	**N**	**3**	**24**	
	%	**27.3%**	**43.6%**	
51 - 60	**N**	**2**	**14**	
	%	**18.2%**	**25.5%**	
61 - 70	**N**	**1**	**4**	
	%	**9.1%**	**7.3%**	
Total	**N**	**11**	**55**	
	%	**100%**	**100%**	

2.2. IL-39 serum levels

The mean study (see Table 4) confirmed significant differences ($p = 0.016$, $p < 0.05$) for IL - 39 levels, which was decreased in the sera of RA patients (4.95 ± 1.1001 ng/ml) compared to healthy controls (5.55 ± 1.762 ng/ml).

Table 4: Mean distributions of immunological assay IL – 39 ng/ml among studied groups

IL-39 level							
Studied groups	**N**	**Mean**	**Std. Deviation**	**Std. Error**	**Range**		**T - Test (*P*-value)**
					Mini.	**Maxi.**	
Control	**66**	**5.55**	**1.762**	**0.216**	**1.237**	**12.829**	***P*=0.016 Significant (*P*<0.05)**
RA Patients	**66**	**4.95**	**1.001**	**0.123**	**3.320**	**9.237**	
Total	**132**						

As shown by the ELISA results, the expression of IL-39 was detected in the serum of both healthy and RA patients. This is a significant result since the studies by both Bridgewood [12]and Ecoeur et al. [13] failed to detect IL-39 in human cells. This, however, raises the question as to what the source of such contradicting results is. Therefore, a closer look at the methodological protocols and samples should be carried out, taking a closer look at the sensitivity and specificity of the immunoassays used to detect IL-39. Notably, rather than displaying increased levels of IL-39, RA patients had significantly decreased levels of IL-39. Although further research would be required to determine the cause of this, such results hint toward IL-39 having an anti-inflammatory rather than inflammatory role in RA. This would be similar to the anti-inflammatory function that has been reported for IL-10 in a number of diseases, including some of inflammatory and/or autoimmune nature, such as inflammatory bowel disease (IBD), colitis-associated cancer (CAC) and systemic lupus erythematosus, for example [16, 17]. In the context of arthritis, reduced levels of IL-10 are associated with more severe disease, as exemplified by Il-10 KO murine models [18]. If confirmed this would be in stark contrast with the results obtained by Wang et al. (2016)[13] which showed that IL-39 mediates the inflammatory process in lupus as well as those of Luo et al. (2017)[7] which also linked IL-39 with inflammation and cardiac disease. It is however possible that the baseline levels of IL-39 in RA patients were masked by the treatments that the patients were

undergoing (e.g., steroids, biologics and DMARDs). Indeed, while the distribution of IL-39 levels across the different treatments was analysed, we did not specifically look at differences between the levels of IL-39 between rheumatoid arthritis patients with and without treatment. This, therefore, presents both a limitation as well as a future course of research. Other limitations of this study include the small number of patients, which limits the statistical power of the analysis, as well as the lack of functional analysis of IL-39. Further, a more in-depth analysis of treatment-induced changes in IL-39 levels would have been necessary to determine the link between IL-39 and inflammation.
Furthermore, Table 5 shows non-significant differences ($p > 0.05$) between the mean of level IL - 39 in the sera of RA patients when considered for all parameters across the study.

Table 5: Mean distributions of immunological assay IL – 39 ng/ml among RA patients.

Parameters (RA Patients)		N	IL-39 level	
			Mean	**SD**
Duration / Year	**1 - 10**	**44**	**4.84**	**1.001**
	11 - 20+	**22**	**5.18**	**0.984**
	***P*-value**		***P*=0.196 NS**	
Smoking	**Smoker**	**4**	**4.02**	**0.453**
	Non-Smoker	**62**	**5.01**	**0.998**
	***P*-value**		***P*=0.055 NS**	
Anti - CCP	**Positive**	**41**	**5**	**0.828**
	Negative	**25**	**5.17**	**1.446**
	***P*-value**		***P*=0.664 NS**	
RF	**Positive**	**43**	**4.84**	**0.693**
	Negative	**23**	**5.43**	**1.553**
	***P*-value**		***P*=0.131 NS**	
CRP	**Positive**	**50**	**5.05**	**1.054**
	Negative	**16**	**4.69**	**0.803**
	***P*-value**		***P*=0.213 NS**	
Steroids intake	**Yes**	**14**	**5.31**	**1.415**
	NO	**52**	**4.85**	**0.851**
	***P*-value**		***P*=0.137 NS**	
DMARDS	**Yes**	**37**	**4.86**	**0.739**
	NO	**29**	**5.07**	**1.264**
	***P*-value**		***P*=0.401 NS**	
Biologics intake	**Enbrel**	**54**	**4.83**	**0.819**
	Amgivita	**3**	**5.45**	**1.410**
	NO	**9**	**5.51**	**1.631**
	ANOVA Test (*P*-value)			
	***P*=0.115 NS**			
	LSD Test (P-value)			
Enbrel	**Amgivita**		***P*=0.292 NS**	
	NO		***P*=0.061 NS**	
Amgivita	**NO**		***P*=0.931 NS**	

NS = Statically non-significant difference (*P*>0.05).

Although the lack of significant correlation observed between smoking habits and levels of IL-39 is surprising, the result is nearing the threshold for statistical significance of $p<0.05$. This is key as there is a large body of literature that shows that smoking is an important risk factor in the development of rheumatoid arthritis, mainly through the mediation of increased inflammation in the body [19]. Thus, the finding that levels of IL-39 are reduced in patients who smoke compared to those who do not further supports the hypothesis that IL-39 may have anti-inflammatory effects in the context of rheumatoid arthritis. If this were true one would likely expect to see higher levels of IL-39 in patients with negative anti-CCP, RF and CRP, all of which are related to autoantibody production and inflammation. In this study, anti-CCP and RF negative patients had higher levels of IL-39. However, given the lack of statistical significance, the implications of such findings cannot be ascertained before further research is carried out. Similarly, one would expect higher levels of IL-39 in patients taking anti-inflammatory treatments such as steroids, DMARDs or biologics such as Enbrel or Amgivita. However, such correlation was not observed, again indicating the need for future research to address these questions.

A weak correlation between patient parameters and serum IL – 39 levels with a non-significant difference ($p > 0.05$) was shown (Table 6).

Table 6: Correlation study between immunological assay IL – 39 ng/ml levels & other RA patient's parameters.

Pearson correlations		IL-39 level
Age / Year	**r**	**.057**
	***P* - value**	**.520**
Disease Duration / Year	**r**	**.149**
	***P* - value**	**.233**
WBC	**r**	**-.200**
	***P* - value**	**.107**
Hb	**r**	**.105**
	***P* - value**	**.403**
ESR	**r**	**-.017**
	***P* - value**	**.893**
DAS 28	**r**	**-.048**
	***P* - value**	**.700**

When looking at any potential correlation between the levels of IL-39 cytokine and some of the different parameters obtained from the group of RA patients, including age, disease duration, disease activity score-28, ESR, Hb, and WBC count, non-significant correlations were observed. The lack of a significant correlation between IL-39 and the disease-activity score is unexpected. If IL-39 had a significant role in the pathomechanisms of rheumatoid arthritis one would expect a correlation between the two, whether positive if it has a pro-inflammatory function, or negative if IL-39 has an anti-inflammatory function. While this finding does not rule out a function of IL-39 in this condition it does support the need for replication studies. Additionally, the lack of correlation with other key markers of RA disease supports this. Despite the lack of significant correlations for the above-mentioned parameters, given that for some of these parameters a weak (although yet still not significant) correlation was observed future studies should consider including a larger number of patients, which could perhaps help achieve greater statistical power. Further, consideration should be taken

when selecting the patient sample. In this study, the distribution of patient demographics was non-significant, and the distribution of other key parameters among the RA population was highly significant including disease duration, smoking habit, CRP, steroid use, and biologics. A more equal distribution of patient-related parameters may allow for a more stringent analysis of the results. Finally, we looked at the validity of using IL-39 as a diagnostic tool for RA. Overall, the results showed poor performance of IL-39 as a diagnostic marker, with specificity and sensitivity of around 60% which is well under the threshold to be considered adequate for clinical use[20].

2.3. ROC curve analysis:

Valuation of serum IL – 39 tests was performed. Low validity was observed on the basis of: cut-off value {≤ 4.99} ng/ml, area under curve (AUC) {0.622}, sensitivity (True positive %) {56.1 %}, specificity (True negative %) {60.6%}, positive predictive value (PPV) {58.7%}, negative predictive value (NPV) {58%}, and finally, accuracy {58.34%}, with significant difference (P=0.016, P<0.05) as shown in Figure 1.

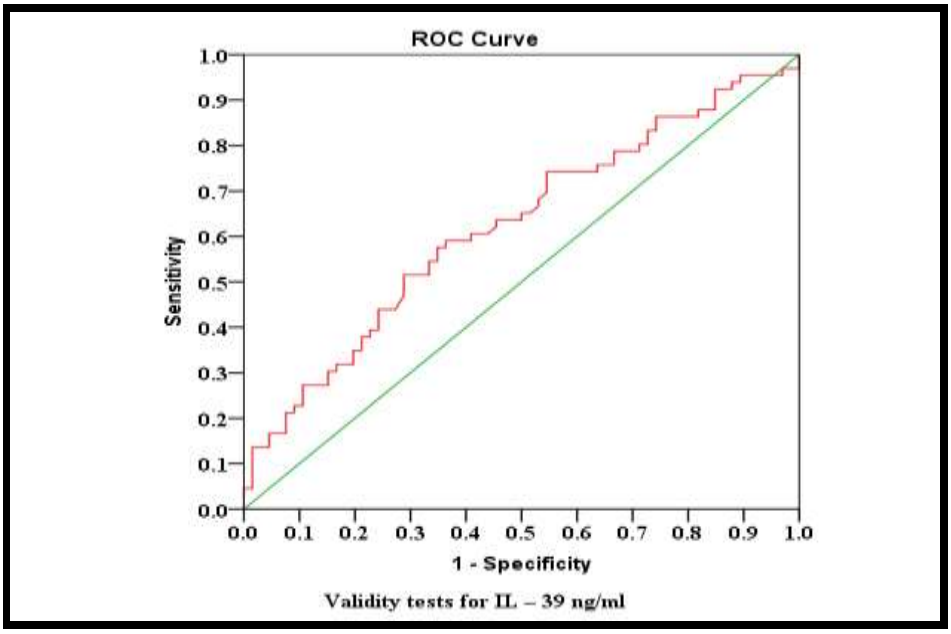

Figure 1: Validity tests of IL – 39 ng/ml by using ROC test

Although this finding does not rule out the potential role of IL-39 in the pathology of rheumatoid arthritis it does indicate that it might have limited usefulness in a diagnostic context. Given however the contradicting results present in the literature, and the small number of patients included in this study, there are enough indications to suggest repetition of this work. Further, the lack of a diagnostic utility does not exclude the possibility that, if proved to have anti-inflammatory properties like other cytokines, like IL-10, IL-39 could have potential as a therapeutic option for this condition. If on the other hand it was confirmed to have pro-inflammatory functions, its utility as a marker of disease activity and severity should be further investigated.

3. Conclusion

The results from this study provide another layer of evidence of the existence of IL-39 in humans. This together with the one previous study indicating the expression of IL-39 in cardiac patients, suggests that IL-39 may indeed be expressed in humans. However, given the contradicting evidence, further work is necessary to support these findings. Further, given the

fact that IL-39 was shown to mediate inflammation in the context of lupus in mice and cardiac disease in humans, the present result showing it was actually downregulated in RA patients is potentially contradictory. This suggests either that IL-39 can have both pro- or anti-inflammatory functions based on the underlying disease or that the role of IL-39 in RA-related inflammation is being masked by the effects of treatment. Future studies should aim to corroborate the existence of IL-39 in humans as well as the reduced levels of this cytokine in the context of RA. Following this, potential avenues for future work would include taking a closer look at the exact function of IL-39 in this patient group as well as understanding whether it has any prognostic value and/or therapeutic value.

ETHICAL CLEARANCE

This study was approved by The Scientific Ethics Committee of the College of Medicine, University of Baghdad. It was also approved by Iraq's Ministry of Health, and The Ministry of Education and Scientific Research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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