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CORRESPONDENCE ARTICLE

Interleukin-35 (IL-35) and Interleukin-39 (IL-39) in Rheumatoid Arthritis: Relation to Disease Activity

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ABSTRACT

Background*: Imbalance of the cytokine networks is reported as attributing factor for rheumatoid arthritis. Interleukin 12 (IL-12) superfamily (IL-35, IL-39) has contributing effect in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis.* ***Objectives:*** *The objective of the current research is to perform an evaluation of the levels of interleukins-35 and 39 in RA and study their relation to disease activity.* ***Methodology****: The levels of interleukins (IL-35 & IL-39) were measured in blood samples of 60 RA cases, in addition to 20 healthy subjects who were involved in this study.* ***Results:*** *Estimated levels of the serum of IL-35& IL-39 had a higher significant elevation in RA cases compared to controls. IL-39 showed a positive significant correlation with ESR, CRP, RF and DAS-28 ($p< 0.05$) in RA patients. The levels of IL-35 revealed negatively significantly correlation with, DAS-28, ESR, CRP, and RF ($p < 0.05$). IL-35 and IL-39 were related to each other with statistical significance ($r = 0.405$; $p < 0.001$). IL-39 and IL-35 had diagnostic values for RA ($p < 0.05$)* ***according*** *to analysis of ROC curve.* ***Conclusions****: IL-35 and IL -39 had a vital immunoregulatory role in RA pathogenesis and they could be biomarkers for diagnosing and confirming RA activity. In the high-specificity range, however, IL-39 appears to be superior to IL-35.*

INTRODUCTION

Rheumatoid arthritis is considered one of the systemic illnesses of autoimmunity that has features of clinical symptoms of symmetric, chronic, multiple arthritis and extra-articular lesions[1,2]. Previous studies have revealed many cytokines that play significant roles in the pathogenesis of arthritis, such as IL-1, IL-17, and TNFα[3]. Some of them are categorized as members of the cytokine superfamily, such as the members of the superfamily of IL-12, which represent heterodimeric group, and while they share many structural similarities, their functional characteristics vary significantly between each others[4].

Cytokines belonging to the IL-12 members comprise the collection of cytokines that are distinct from each other. Thèse cytokines include IL-12, IL-23, IL-27, IL-35, and, finaly, IL-39[5]. Members of the IL-12 cytokine family take the form of an a/b heterodimer, which is composed of two different components: the first component includes IL-23p19, IL-27p28, and IL-12p35, and the second form Ebi3 and IL-12p40. Different heterodimers, p35/p40, p19/p40, p28/Ebi3, and p35/Ebi3, form IL-12, IL-23, IL-27, and IL-35, accordingly[6]. Some of those family members have important role in the regulation of immune reactions in various disorders, e.g., cancer, autoimmune disease, and infectious disease[7].

In 2007, two teams, one of Collison and colleagues and the other of Niedbala and colleagues, identified a novel kind of cytokine known as IL-35 at the same time[8,9]. The "A" chain includes (p35/IL-12a) and the other "B" chain is (EBI3/IL-27b) comprise IL-35[10].IL-35 is a cytokine featuring immunosuppressor and anti-inflammatory characteristics, mostly produced by Tregs[11]. Crohn's disease, systemic sclerosis, and rheumatoid arthritis all of them are examples of autoimmune disorders that are intimately associated with IL-35. Infections, inflammation, malignancies, and diseases of autoimmunity are also associated with IL-35[12].

In addition to inhibiting the developing Th17 cells, IL35 encourages developing the Tregs[13]. Synovial inflammation and joint deterioration are both attributed to the infiltration of a variety immune cells into the joints, including Tregs and Th17 cells, which contribute actively to this process [14]. In the degenerative phase of rheumatoid arthritis, IL-35 plays a significant role by ensuring a balance of Tregs and Th17 cells [15].

According to Wang et al.[5], IL-12family subunits p19 and Ebi3 form a novel cytokine member, IL-39 (p19/Ebi3) which is produced by B cells in systemic lupus erythematosus of a mouse model. Some research papers investigated the pathogenic role of IL-39. They reported that IL-39 performs one of the essential functions in developing SLE, by influencing the immune system through the promotion of the pro-

inflammation reaction in models of lupus-like mice[16,17,18] .

Several reports have demonstrated an association between circulating IL-12 family members and disease activity of RA [19,20].

This work aimed at evaluating the levels of IL-35 and IL-39 in patients with RA and studying their relation to the disease activity.

METHODOLOGY

Patients

Cases with rheumatoid arthritis visiting the Rheumatology and Rehabilitation Department's Outpatient Clinic at Sohag University Hospitals were the subjects of our case control study, which was carried out over six months from May 2024 to April 2025. Also, our study included 20 persons (female/male = 12/8) as a control group. They were recruited from a group of healthy matched sex and age with the patients, who attending for blood donation. The sixty patients were (40% men and 60 % women). They were selected according to the Classification Criteria of ACR/EULAR 2010 [21]. To be included in the control group, a subject should be aged ≥18 years old with no manifestations of any rheumatic, systemic, or metabolic diseases, malignancies, or acute inflammation. Our patients underwent full history taking and clinical examination. The DAS28 is assessed for all our patients including ESR, the number of swollen tender joints, and visual analogue scale of disease activity (VAS) [22,23].

Methods

Laboratory investigations included the following tests:

Complete blood picture, on an EDTA sample using the XN-1000 (Sysmex, Japan). ESR was measured using the Westergren method. The concentrations of serum CRP were evaluated using the immunonephelometry techniques on a Turboxnephelometer (Orion Diagnostica, Finland). The assay of rheumatoid factor IgM isotype was done by ELISA kit for RF (Orgentec Diagnostika GmbH, Germany) following guidelines of the manufacturers. The 20 IU/ml titer was considered positive for RF. Serum level Anti-CCP was evaluated by solid-phase ELISA (MyBioSource Inc., San Diego, California, USA). Anti-nuclear antibody (ANA) were measured by the Flurokit (DiaSorine), using indirect immunofluorescence for ANA screening and titration.

Serum level IL-39 testing was done by ELISA kit from (MyBioSource, Inc., USA**)**. The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). Standard Curve Range: 2-600 ng/L.

Determination of serum IL-35 level was measured using ELISA kits (provided by human Elabscience Biotechnology / USA) following the manufacturing company's guidelines. It is a sandwich-ELISA technique. IL-35 was found to have a measurable concentration of 0.08± 0.04 ng/ml at its lowest point. Detection Range 15.63-1000 pg/mL.

Ethical considerations:

Prior to carrying out this study approval for our protocol is obtained from the Research Ethics Committee of Faculty of Medicine in Sohag University (Approval Number and date: Soh-Med-25-4-IPD, at 13th April 2025). Every participant in our study whether patients or controls, assigned consent after discussing with them the objective of the study.

Statistics

Statistical analysis for our results was accomplished by utilizing SPSS (V. 19). The findings were presented in the form of standard deviations and means. Student's t-test is utilized for performance of differences of continuous variables between cases and control. The Chi-square test assessed the independence of categorical variables. In addition, Pearson's correlation helped to detect the relationship between any two quantitative parameters within a group. P-value ≤ 0.05 was set to have statistical significance. To evaluate the capacity of IL-39 and IL-35 to differentiate between RA and healthy participants in the control group, (ROC) curves were used. A p-value < 0.05 indicated a difference of statistical significance.

RESULTS

Clinical data, disease characteristics and laboratory data of patients: It was shown that there were no differences of statistical significance between rheumatoid arthritis subjects and control volunteers concerning sex, age, or BMI (p>0.05). Cases diagnosed with RA had disease activity (DAS28 <5.1). In Table 1, the demographics and clinical features of patients diagnosed to have RA, as well as control participants, are presented. The mean serum IL-35 in rheumatoid arthritis subjects (35.63±11.92pg/ml) was significantly higher (p=0.02) than control subjects (21.1±6.31mL). Also, the mean serum IL-39 (110.0155 32.19 ng/L) were significantly higher (p 0.0001) in rheumatoid arthritis subjects than the healthy subjects in the control group (58.015± 23.77) Table 1.

Table 1: Demographic, clinical and laboratory properties of rheumatoid arthritis subjects and the control group

Characteristics (mean ± SD)	Rheumatoid arthritis subjects (60)	Controls (20)	p-value
Age (years) Range	42.95 ± 11.40 -38-54	40.7 ± 9.8 27-42	0.532
Sex (F/M)	36/24	12/8	0.54
Age of onset of the disease	40.63±14.22		
Disease duration (years) Range	7.67± 4.57 1-15		
Morning stiffness (min) Range	31.78± 21.22 15-60		
VAS Range	2.98± 2.57 0-9.4		
Swollen joints	3.63± 1.1		
Tender joints	3.65± 0.51		
DAS28 score	3.379 ± 0.41 2.3-3.6		
HAQ score	11.48 ± 4.25		
BMI	13.15± 11.48	26.85±2.62	
ESR (mm/1st hr)	50.25± 24.48	8.433±3.99	< **0.0001***
Rheumatoid factor Range	62.8± 29.38 13-51	18.78±6.85 12-24	< **0.0001***
CRP (mg/dl) Mean ±SD	23.33± 7.66 10-89	3.78±1.43 5-15	< **0.0001***
Anti-CCP2 Mean ±SD Range (U/ml)	142.17±87.81 11-321	22.1±9.53 10-36	**<0.0001***
IL-35 Mean ±SD Range (pg/ml)	35.63±11.92 10-55.05	21.1±6.31 3-22.03	**0.024***
IL-39 Mean ±SD Range (ng/L)	110.015±32.19 11.2-152	58.015± 23.77	**<0.0001***

VAS: visual analog scale for pain; DAS28: disease activity of 28 joint score, HAQ: health assessment of questionnaire, BMI: body mass index, ESR: erythrocyte sedimentation rate RF: rheumatoid factor, CRP: C- reactive protein; Anti. -CCP; anti-cyclic citrullinated protein antibodies * P<0.05 is considered significant**.**

Table 2: Correlation between IL39 and IL35 and participants' properties and clinical indicators:

Parameters	IL39		IL35	
	R	p-value	r	p-value
Age	0.037	0. 770	0.067	0.49
Onset of rheumatoid arthritis	0.678	0.475	0.256	0.762
Disease duration	0.082	0.595	0.076	0.596
VAS	0.555	**0.0001***	- 0.234	0.077
DAS-28	0.359	**0.0048***	- 0. 41	**0.0009***
Number of tender joints	0.481	**0.0001***	0.489	**0.0001***
Number of Swollen joints	0.055	0.833	0.248	0.087
Morning stiffness	0.058	0.659	0.009	0.499
ESR	0.317	**0.0137***	-0.286	**0.0265***
RF	0.037	**.05***	-0.254	**0.05***
CRP	0.051	**.0003***	-0.0288	**0.0002**
ACCP	0.408	**0.0012***	0.384	**0.002***
ANA	0.334	**0.052***	0.652	0.232
IL35	0.405	**0.001***		

* P<0.05 is significant.

Correlation between serums IL-39, IL-35 and patients' characteristics:

The use of Spearman coefficient revealed insignificant positive correlations between IL-39 levels and age, RA onset, disease duration, number of swollen joints, in addition to morning stiffness ($p > 0.05$). In addition, a relationships with statistical significance were observed between IL-39 levels and the number of tender joints, ESR, CRP, Anti-CCP, RF, ANA, VAS and DAS-28 ($p<0.05$) in RA cases. IL-35 levels had insignificantly positive correlation with age, RA onset, disease duration, and number of swollen joints ($p < 0.05$). The level of IL-35 show significant negative correlation with VAS, DAS-28, ESR, CRP and RF ($p < 0.05$). IL-35 is positively correlated with tender joint & Anti-CCP ($p<0.05$). IL-35 and IL-39 correlated significantly to each other ($r = 0.405$ and $p < 0.001$) (**Table 2**).

***The curve analysis of ROC*:**

According to the ROC curve, the validity of IL-39 for rheumatoid arthritis diagnosis was evaluated to be 0.885, with a statistical significance of $p < 0.0001$, at an optimum cut-off value (> 67.5) with sensitivity (93.3%), specificity (80%), PPV (93.6), NPV (80%) and finally, accuracy (90%). For IL-35, at cut-off value (> 24.5), AUC (0.728, P=0.005), sensitivity (87.8 %), specificity (73.7%), (NPV 73.7%), (PPV 87.8%), (AUC=0.728, P=0.005.) and lastly, accuracy (83.3%), (Table 3, Figures 1 & 2).

Table 3: Validity parameters of IL-35 and IL-39 level to differentiate RA patients from healthy controls.

Variable	AUC	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
IL-39	0.885	93.3	80	93.6	80	90
IL-35	0.728	87.8	73.7	87.8	73.7	83.3

IL- 35: interleukin 35; IL-39:; PPV: Positive Predictive Value; NPV: Negative Predictive Value, AUC: Area Under Curve.

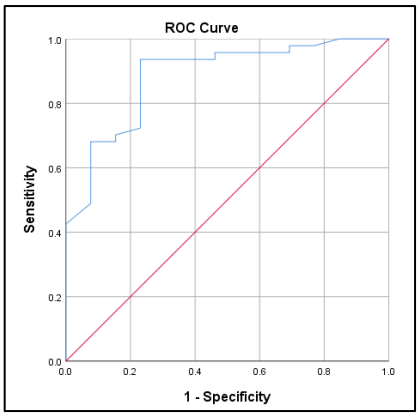

Fig. 1: ROC curve of IL-39 concerning differentiating RA patients from healthy subjects, AUC= 0.885 ($p < 0.0001$

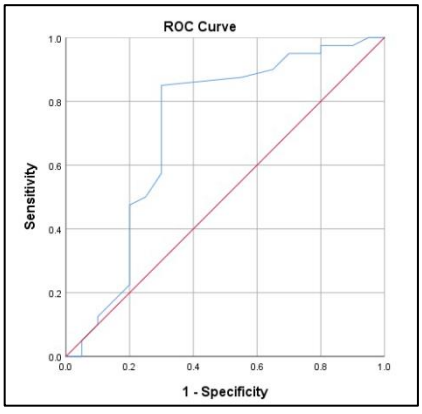

Fig. 2: ROC curve of IL-35 concentrations concerning the distinguishing RA from healthy subjects, AUC=0.728, P=0.005.

DISCUSSION

Rheumatoid arthritis is a chronic inflammatory disease characterized by imbalance between pro- and anti-inflammatory cytokines[24]. Pro-inflammatory cytokines are crucial in the processes that lead to inflammation, joint destruction, co-morbidities associated with RA, and many other disorders which are brought on by an uncontrolled self-directed immune response [25,26]. Therefore, therapy targeting these cytokines, or their receptors is considered an effective treatment for patients with RA. IL-12 family members play key roles in microbial infections, autoimmune diseases, and cancer [27].

In our work, we found, the expression of IL-39 was statistically and significantly elevated in rheumatoid arthritis subjects in comparisons with healthy subjects which is in consistent with Ying et al.[28], who found that IL-39 levels scored higher values of statistical significance among 46 cases diagnosed with rheumatoid arthritis in comparison to the healthy participants ($p < 0.0001$). Also, our result matches the results obtained by previous studies[29,30,31,32] who observed statistically significant elevated level of IL-39 in patients with RA compared to healthy control group. IL-39 exerts its immunomodulatory responses through its interactions with the IL-23R/gp130 receptor and by activating the signal transducer (STAT)1 and STAT3 [5].

On the other hand, Al Ghuraibawi et al[33] found that the levels of IL - 39, were significantly decreased in RA patients in comparison to healthy subjects ($p = 0.016$). Also, Ecoeur et al. and Bridgewood et al[34,35], did not

achieve successful results in detecting IL-39 in the cells of human beings.

In our present work, we could establish significant correlations between IL -39 titer and DAS-28, VAS, CRP, RF, ESR, and Anti-CCP ($p < 0.05$). This positive relationship between IL-39 has a significant value in the evaluation process of RA disease activity. Our finding matches the findings illustrated in the research of Ying et al[28] that reported that the levels of IL- 39 were considerably higher among RA group and positively correlated with RF and ESR. In addition, Similar reports[34,36] concluded that IL-39 elevates the levels of IFN-γ, TNF-α, and IL-17, hence eliciting a pro-inflammatory state and regulate the immune system. As a consequence of this, specifically targeting IL-39 might potentially be an effective therapy for the illnesses of autoimmunity. Interleukin-39 triggers proinflammatory responses by raising the levels of IL-17 A, interferon-γ, and TNF-α.[37]

In contrast, Al Ghuraibawi et al[33] reported that serum levels of IL–39 in RA patients correlated weakly and non-significantly with different patient parameters, including WBC count, Hb, ESR, disease duration, DAS-28, and age.

The marked elevation of IL-39 levels in the blood samples of our RA patients, together with its correlation with clinical signs, implies that IL-39 might function as a biomarker for diagnosing rheumatoid arthritis.

The present work revealed increased level of IL-35 in patients having RA, in comparison with the control subjects, which is in agreement with result obtained by Mahdi and Mohamed[38], who found that a very high significant rise ($P<0.0001$) in level of IL-35 in 30 female RA patients compared to control. In agreement with our findings, Xie et al[39], found that IL-35 levers were found to be higher in rheumatoid arthritis cases. In addition, IL-35 had a proper ability to diagnose and differentiate rheumatoid arthritis from the other rheumatic illnesses. Also, Li et al[40], conducted a study on 129 Chinese rheumatoid arthritis subjects and 83 healthy participants as the control group. They found that the levels of IL-35 were elevated in RA subjects, and the levels of serum IL-35 correlated statistically with low ESR and DAS28-ESR. Similarly, Jiang et al[41]. found that IL-35 level had elevated in subjects with early naïve RA treatment than the control subjects and significantly declined following the therapy.

On the other hand, studies done by some researchers[42,43,44,45] found a lower level of IL-35 in RA cases in comparisons with healthy subjects. This contradictory finding is interpreted due to the difference in sampling, disease duration, and type of medical treatment in the cases of rheumatoid arthritis, which suggests that IL-35 have many functions related to immunity regulation during the various stages of rheumatoid arthritis.

In our present study, IL35 level correlated negatively and significantly with VAS, DAS-28, ESR, CRP, and RF ($p < 0.05$). This finding is in agreement with that of the research of Li et al[40], in which they reported that elevated levels of IL-35 had correlations with DAS28-ESR and low ESR. Similarly, Akla et al[45]. found that with increased DAS-28 grading, serum IL-35 was significantly low. Also, Xie et al[39], found that the RA patients having active disease showed high IL-35 levels compared to those patients with less active disease. Also, our result is in accordance with the result obtained by Nakano et al[42], who reported that the IL-35 levels and Treg cells were considerably low in patients with active rheumatoid arthritis. There was a significant negative correlation between serum IL-35 and the 28-joint DAS in patients with active RA ($P < 0.01$, $R = -0.794$). Consistent with our findings, Li et al[37]. reported that IL-35 might have a role in the regulation of RA pathogenesis, especially with disease activity. IL-35 is an inhibitory cytokine secreted by Tregs have important role in the occurrence and development of RA regulating the immune functions by inhibiting the inflammatory response. [15]

IL-35 is mostly regarded as a biomarker in the investigation of rheumatoid arthritis etiology [40,46]or predicting the efficacy of certain drugs[40]. Several authors have reported that IL-35 may influence rheumatoid arthritis by inhibiting the Th-17/IL-17 pathway. Also, it may influence the etiology and progression of rheumatoid arthritis through affecting immunological and pathological mechanisms. Consequently, the authors make a suggestion that we can utilize IL-35 as one of the potential targets for future treatments of RA.[40]

In addition, IL-35 enhances the development of human B cells into B regulatory cells, which secrets IL-10. Thus, it is suggesting that IL-35 modulates the immune response [47].

Recently, Nakano et al.[42]illustrated that compared with normal controls, the serum IL-35 levels of RA patients were significantly reduced.

In contrast, Ning et al[43], did not find any relationship between IL-35 and DAS28 in rheumatoid arthritis patients.

Moreover, our work proved that IL-39 had a significantly higher value for diagnosis the cases of rheumatoid arthritis compared to IL-35 by assessment of the receiver operating characteristic curve, moreover IL-39 appears to be superior to IL-35.

CONCLUSIONS AND RECOMMENDATIONS

The augmentation of the levels of IL-39 & IL-35 has a potential value in the diagnosis of RA and might help to confirm the RA activity. However, more researches should be carried out in order to illustrate the extent to

which IL-39 and IL-35 could be considered as a therapeutic target for RA.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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