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Original Article

EVALUATION OF SERUM INTERLEUKIN-34, AS A MARKER OF DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Aim of the work: To evaluate the role of IL-34 in the pathogenesis of SLE and RA and to assess its role as a biomarker of disease activity.
Subjects and methods: This study was carried out on 29 patients with SLE, 29 patients with RA, and 29 healthy control subjects. SLE disease activity was measured by systemic lupus erythematosus disease activity index (SLEDAI). RA disease activity was measured by 28-joint disease activity score (DAS-28). Serum IL-34 was measured by enzyme-linked immunosorbent assay (ELISA) .
Results: There was highly significant elevation in IL-34 level in SLE and RA when compared to control group ($p<0.001$). IL34 level did not differ significantly between SLE and RA groups ($p> 0.05$). There was a significant positive correlation between IL-34 level and SLEDAI in SLE patients as well DAS 28 score in RA patients. The highest level was detected in patients with high disease activity. There was statistically significant correlation between IL-34 levels and ESR, CRP, and anti-ds DNA antibodies but inversely correlated with C3 in SLE patients. There was also statistically significant correlation between IL-34 levels and ESR, CRP RF, and anti CCP antibodies in RA patients.
Conclusion: IL-34 could be useful marker for disease activity in SLE and RA.

Keywords: IL-34; Systemic lupus erythematosus; SLEDAI; Rheumatoid arthritis; DAS-28.

INTRODUCTION

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are autoimmune rheumatic diseases characterized by dysregulation of innate and adaptive immunity, breaking of tolerance, production of autoantibodies, and generation of autoreactive T and B cells, leading to necrosis, scaring, and organ dysfunction and account for many clinical manifestations [1]. Cytokines play an important role in the processes that cause inflammation, articular destruction and extra-articular manifestations [2]. Interleukin 34 (IL-34) is a hematopoietic cytokine that acts as a key regulator of survival, proliferation, and differentiation of myeloid lineage cells including monocytes, macrophages, and osteoclasts [3]. IL-34 was identified in 2008 as a second ligand of colony stimulating factor receptor 1 (CSF-1R), in addition to the previously well-known ligand, macrophage colony stimulating factor (M-CSF). IL-34 shares functional similarities with M-CSF, but also shows different characteristics and unique signaling patterns [4]. IL-34 binds to CSF-1R and promotes the differentiation and survival of monocytes and macrophages which are the predominant infiltrating cell types in the inflamed synovium and produce inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6). IL-34 also was shown to induce pro-inflammatory cytokines and chemokines like interleukin-8 (IL-8) [5]. In this study, we aimed to estimate IL-34 level in the sera of SLE and RA patients in comparison to the healthy controls and to determine its relation with disease activity.

SUBJECTS AND METHODS

This study was carried out in Rheumatology and Rehabilitation and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University Hospitals. Three groups were included in the study: group I included 29 SLE patients, they were 28 females and 1 male, their ages ranged between 21-55 years, and all patients were fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) revision of the American College of Rheumatology (ACR) classification criteria for SLE [6]. Group II included 29 RA patients they were 26 females and 3 males, and they were diagnosed according to 2010 American college of Rheumatology/ European League Against Rheumatism (ACR/EULAR) classification criteria for RA[7]. Group III included 29 healthy subjects they were 25 females and 4 males taken as a control group matched with patients groups regarding age and sex. Informed consent was signed by the SLE, RA patients and healthy controls.

This study was approved by the institutional review board (IRB) of the faculty. Written informed consent was obtained from all participants. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. Patients with other inflammatory autoimmune diseases and any associated chronic systemic disease (diabetes, hypertension, cardiac, renal or hepatic diseases) were excluded from the study. Systemic lupus erythematosus disease activity was evaluated by SLEDAI [8]. The SLEDAI in patients with SLE were categorized according to **Cook et al., 2000** [9]. Rheumatoid arthritis disease activity was evaluated by DAS-28 [10]. All patients were subjected to full history taking, thorough clinical examination, and laboratory investigations including complete blood count, erythrocyte sedimentation rate (ESR) [11], CRP was detected by immunohistochemistry. ANA was done by indirect immunofluorescent assay using kallestad HEP2 cell live substrate (Bio-rad laborations, Remond, USA). Anti-dsDNA antibodies titer was done by indirect immunofluorescent antibody using kallested crathidia leucilia substrate (Bio-rad laborations, Remond, USA). Complement 3 level (C3) was detected by use of immunonephlometry using BN prospec (DADE BEHRING, New York, USA). Complement 4 level (C4) was done by use of immunonephlometry using BN prospec (DADE BEHRING, New York, USA). Rheumatoid factor (RF) was measured by immunoturbidimetric technique (Done by kits for RF supplied by Roche Diagnostics GmbH, Mannheim). Anti-cyclic citrullinated peptide (Anti CCP) was measured using the Immunoscan CCPlus® test kit which is an enzyme-linked immunosorbent assay (ELISA) for qualitative and semiquantitative detection of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human sera [12]. Serum samples were collected from patients on the same day of examination. Measurement of serum interleukin-34 level was performed by a commercial enzyme-linked immune sorbent assay (ELISA) kit for SLE, RA patients, and controls according to the instruction of the manufacturer by Chongqing Biospes international trade company, Erlang Chuangye, China [13].

Statistical analysis:

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version **20.0. Armonk**.

RESULTS

Demographic data of SLE and RA patients and healthy controls included in the study:

SLE patients: Their ages ranged between 21–55 years, with a mean 39.2 ± 9.6 years. Disease durations ranged between 1 - 18 years with a mean 6 ± 5.2 years. RA patients: Their ages ranged between 19- 65 years with a mean 42.4 ± 13.8 years, disease durations ranged between 1 – 19 years with a mean 6.6 ± 4 years. Healthy control subjects: Their ages ranged between 19-55 with a mean 39.97±13.1. There were no significant differences found between all three studied groups according to age and sex and there was no significance difference in disease

duration between the studied SLE and RA groups ($p > 0.05$) (Table 1).

The mean value of IL-34 serum level showed a statistically significant difference in both SLE and RA patients when compared to controls but, IL34 level did not differ significantly between SLE and RA groups. It was (66.8± 21.6) pg/ml in SLE patients, (69.6± 22.2) pg/ml in RA patients, and (11.5± 3.9) pg /ml in the healthy controls (Table 2).

There was significant positive correlation between IL34 level and SLE activity score SLEDAI (Table 3). The highest level was detected in patients with high disease activity (Fig 1).

Also, there was significant positive correlation between IL-34 level and RA disease activity score DAS-28. The highest level was detected in patients with high disease activity (Table 4).

IL34 concentration showed highly significant positive correlation with SLEDAI and anti-ds DNA and significant positive correlation with ESR but, inversely correlated to C3 level ($p<0.05$). No significant correlation between Il-34 level and proteinuria ($p>0.05$) (Table 5).

IL34 concentration showed highly statistically significant positive correlation with DAS-28, NTJ, RF titre, Anti CCP titre and significant positive correlation with NSJ, CRP, and ESR (Table 6).

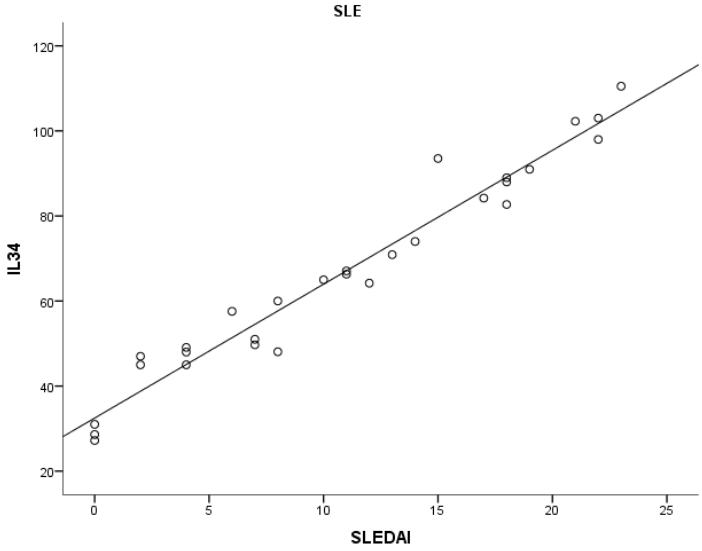

Figure 1 Correlation of IL34 with SLEDAI in SLE cases.

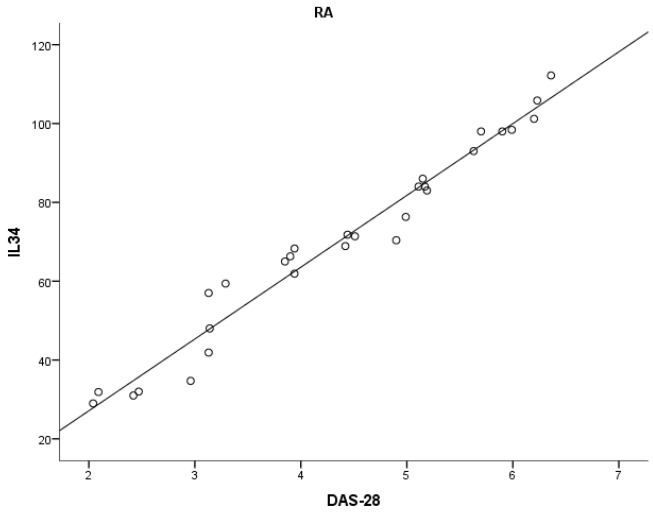

Figure 2 Correlation of IL34 with DAS 28 in RA cases.

Table 1 Comparison of demographic data between all studied groups.

	SLE N=29		RA N=29		Control N=29		*P1*	*P2*	*P3*
	Mean	**±SD**	**mean**	**±SD**	**Mean**	**±SD**			
Age (years)	39.2	9.6	42.4	13.8	39.97	13.1	0.166	0.480	0.380
	N	**%**	**N**	**%**	**N**	**%**			
Males	1	3.4	3	10.3	4	13.8	0.352	0.687	0.611
Females	28	96.6	26	89.7	25	86.2			
Disease duration (years)	6	5.2	6.6	4	-	-	-	-	0.100

P1, comparison between SLE and control; p2, comparison between RA and control; p3, comparison between SLE and RA.

Table 2. Comparison of IL34 level between all studied groups.

		SLE N=29	RA N=29	Control N=29	*P**	*P¹*	*P²*	*P³*
IL34 pg/ml	**Range**	27.2-110.5	31-112.2	0.6-25.7	<0.001	<0.001	<0.001	0.554
	Mean	66.8	69.9	11.5				
	±SD	±21.6	±22.5	±3.9				

Numerical data are expressed as mean and SD, compared by t test; p*, comparison between all studied groups by ANOVA; p1, comparison between control and SLE; p2, comparison between control and RA; p3, comparison between SLE and RA.

Table 3. Serum level IL-34 as regards SLEDAI categories in SLE patients

		IL34 level		
	N	**Mean**	**±SD**	***P***
No activity	3	28.9	1.9	<0.001
Mild	8	46.8	1.8	
Moderate	11	55.2	6.7	
High	4	79.2	10.9	
Very high	3	103.4	5.2	

Table 4. Serum level IL-34 as regards DAS-28 categories in RA patients

		IL34 level		
	N	**Mean**	**±SD**	***P***
Remission	5	30.9	1.4	<0.001
Low	13	48.9	7.6	
Moderate	7	64.9	11.1	
High	4	94.9	9.8	

Table 5. Correlation of IL34 with the clinical and laboratory parameters in SLE patients.

	IL-34 level	
	R	***P***
SLEDAI	0.976	<0.001
ESR (mg/h)	0.610	<0.01
24h proteinuria	0.105	0.586
Positive anti-ds DNA	0.734	<0.001
C3 level	0.693	<0.01

*R Correlation coefficient

Table 6. Correlation of IL34 with the clinical and laboratory parameters in RA patients.

	IL-34 level	
	R	***P***
NTJ	0.926	<0.001
NSJ	0.685	<0.01
DAS-28	0.972	<0.001
CRP (mg/L)	0.693	<0.01
ESR (mm/h)	0.709	<0.01
RF (IU/ml)	0.898	<0.001
Anti CCP (IU/ml)	0.746	<0.001

DISCUSSION

This study was conducted to determine the role of IL-34 in SLE and RA as a marker of disease activity in both disorders. Our results showed that the level of serum IL-34 was significant high in SLE and RA patients compared to healthy controls but, IL34 level did not differ significantly between SLE and RA patients. That was agreed by previous studies in SLE patients [14, 15]. Also, these studies reported elevation in IL-34 level in RA patients [16- 22].

In the current study, we found a highly significant positive correlation between serum IL-34 levels and SLEDAI score in SLE patients. Thus, higher serum IL-34 levels indicate higher disease activity in patients with SLE. Our results was in agreement with other studies [14, 15]. Also one of the main findings of our study is the significant correlation between serum IL-34 levels and different grades of DAS-28. Thus, higher serum IL-34 levels indicate higher disease activity in patients with RA. Our results was in agreement with previous studies [16, 18, 20, 22]. In contrast with our results, a study by **Chang and colleagues** [17]. revealed no significant correlation between IL-34 and disease activity measured by DAS-28, also **Moon et al** [19] found no significant difference in serum IL-34 level in relation to grades of DAS-28 score. However, these contradictory results may be related to differences in mean age and disease durations compared to ours.

In this study, IL34 concentration showed highly significant positive correlation with anti-ds DNA and significant positive correlation with ESR but inversely correlated with C3 level ($p<0.05$). That was agreed with those studies [14, 15]. On the other hand, **Wang et al.** [15] found no significant correlation between serum IL-34 levels and ESR.

Also, serum levels of IL-34 were significantly correlated with ESR, CRP, RF titre and anti-CCP antibodies in RA patients. In agreement with our results these studies [16, 17, 18, 20, 22] mentioned that there were significant correlations between IL-34 and ESR, CRP and RF titer. In contrast, a study done by **Moon et al.** [19] detected that ESR, CRP level did not correlate with serum IL-34 levels in patients with RA.

Conclusion: IL-34 levels were significantly increased in patients with SLE and RA compared with healthy controls. There was no significant difference in IL-34 level between RA and SLE patients. Its significant correlation with SLEDAI in SLE patients and DAS-28 in RA patients suggested that serum IL-34 level could be a useful marker of disease activity in SLE and RA. Direct IL-34 blockade may be an important key in controlling SLE and RA disease activity.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
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