Zagazig University Medical Journal
www.zumj.journals.ekb.eg

https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 1.6, September 2024, Supplement Issue
Manuscript ID:ZUMJ-2312-3060 (R1) DOI:10.21608/ZUMJ.2024.256785.3060.

ORIGINAL ARTICLE

Serum interleukin 29 in Rheumatoid Arthritis and Systemic Lupus Erythematosus patients and its association with disease activity.

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Submit Date 22-12-2023
Revise Date 06-01-2024
Accept Date 11-01-2024

ABSTRACT

Background: Interleukin-29 (IL-29) is a recently identified cytokine that has sparked considerable academic interest due to its importance in inflammation, and autoimmune diseases and hence as an avenue for potential therapy creation and disease monitoring. This research sought to determine the role of IL29 in (RA and SLE) and its relationship to disease activity.**Methods:**A Case-control study including 144 participants, 48 in each group (RA, SLE, and control. Levels of serum IL-29 in both patients and controls were measured by Human Interleukin 29 (ELISA)**.Results:**IL-29 serum levels in patients with RA and SLE were significantly higher than the control group. Moreover, there is a highly significant value of IL29 in RA patients compared to SLE patients $p<0.001$, there was no significant difference in serum IL29 value according to activity grade of DAS 28 in the RA group $p>0.05$, while in SLE there was a significant difference in serum IL29 value regarding SLEDAI grading scores $p<0.001$.**Conclusion:**This research provides data on the relationship between IL-29 and SLE and RA, demonstrating higher IL-29 serum levels in all patients compared to controls. IL-29 may act as a pro-inflammatory cytokine and contribute to the development of RA and SLE. It was also linked to lupus activity.

Keywords:IL29, RA, SLE, Disease activity.

INTRODUCTION

Interferon (IFN) 1, commonly known as Interleukin (IL)-29, is a novel type III IFN or IFN family member. IFN-1 (IL-29), IFN-2 (IL-28A), and IFN-3 (IL-28B) are members of the Type III IFN family. By methods comparable to type I interferons, IL 29 plays a vital role in the immune response to pathogens, particularly viruses[1]. Several studies have shown that IL-29 is involved in the development of several autoimmune and inflammatory illnesses, including rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome, and psoriasis[2-4].In the lining layers of the RA synovium, IL29 has been detected in CD68+ macrophage and FGF2+ fibroblast. Also, IL29 expression was high in serum, peripheral blood mononuclear cells (PBMCs), and synovial tissue in RA patients. IL29 levels in synovial fluid (SF) were elevated in RA patients. Increased levels of granzyme M (GrM) in RA synovial fluid could cause the release of IL29[5].

Many studies found that IL-29 inhibits human Th2 responses and modifies the Th1/Th2 response by reducing IL-13 production [6]. Moreover, IL-29 can boost the synthesis of IL-6, IL-8, and IL-10 by monocytes, the production of tumor necrosis factor (TNF) by macrophages, and the IL-2-dependent multiplication of CD4+CD25+Foxp3+ T cells generated by dendritic cells. In peripheral blood mononuclear cells, the release of chemokines IFN-inducible protein-10 (IP-10), Monokine

induced by gamma (MIG), and IL-8 play a significant role in the occurrence of systemic lupus erythematosus (SLE)[7, 8].The manufacture of cytokines can be influenced by IL29 via activating the cytokine-activated Janus kinase/signal transducer and activator of transcription (JAK/STAT), protein kinase B (Akt/PKB), and mitogen-activated protein kinase (MAPK) signaling pathways[9]. The IL-28 receptor (IL-28R) and IL-10 receptor (IL-10R2) complexes mediate IL-29 signalling[10].

The aim of our study was to investigate the role of IL-29 in RA and SLE patients because of its possible involvement as a regulator in inflammatory autoimmune diseases and how it could be employed in clinical therapy. In this study, we additionally examined the relationship between IL29 and disease activity.

METHODS

This study was a Case-control study, including 144 participants, 48 in each group (RA, SLE, and control).

Inclusion criteria:Using open epi program with a test power of 80%, and a CI of 95%, Patients with RA and SLE fulfilling the revised 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis[11] and the Systemic Lupus International Collaborating Clinics (SLICC) revision of the American College of Rheumatology (ACR) classification criteria for SLE respectively [12] were chosen at random from a database of registered patients.

Exclusion criteria:Patients with cancer, cardiovascular disease, liver disease, infection, or other autoimmune conditions were not eligible.

Sample size:144 participants, 48 in each group (RA, SLE, and control).

Data Collection and Procedures:

1-Patient's history and clinical examinations, which included general, musculoskeletal, and various systems evaluation, yielded basic demographics and clinical information,laboratory tests and current medications were provided.

2-Evalution of disease activity by measurement of DAS-28**[**13**]**, for RA patients and by SLEDAI-2K scores [14] for SLE patients.

3-Levels of serum IL-29 in both patients and control were measured by Human Interleukin 29(IL-29) double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) Kit (Cat number: 201-12-0041) (SunRed, Shanghai)according to the manufacturer ‘s instructions. The IL29 levels were calculated by a standard curve. The results were interpreted as ng/ml. All determinations were performed by laboratory technicians blinded to all clinical data.

Ethical and administrative considerations: Everyone involved provided written given consent. The research protocol authorization code was obtained from the Institutional Review Board (IRB) at Zagazig University, Egypt ZU-IRP#6636, following the World Medical Association's Code of Ethics (Declaration of Helsinki 1964) for human studies.

STATISTICAL ANALYSIS

SPSS is used to manage data. IBM Corporation, 2015. Version 23.0 of IBM SPSS Statistics for Windows. IBM Corp., Armonk, New York. The mean, standard deviation (SD), and median (range) were used to describe quantitative data, while absolute frequencies (number) and relative frequencies (%) were used to convey qualitative data. The Kruskall-Wallis test was developed to compare two groups of non-normally distributed variables. Spearman's correlation coefficient was determined to analyze the relationship between various study variables, with (+) indicating direct correlation and (-) indicating inverse correlation, and values close to 1 indicating strong correlation and values close to 0 indicating weak correlation. All of the tests were two-sided. P-values less

than 0.05 were considered statistically significant (S), whereas p-values more than 0.05 were considered statistically insignificant (NS). To create the Receiver Operating Characteristic (ROC) curve, plot the true positive rate (Sensitivity) on the (y) axis and the false positive rate (100-Specificity) on the (x) axis.

RESULTS

As demonstrated in Table 1, IL-29 serum levels in patients with RA and SLE were significantly higher than those in the control group, at 475.15(64-906.9) ng/mL and 50(18.9-150) ng/mL, respectively. Moreover, there is a highly significant value of IL29 in RA patients compared to SLE patients p<0.001.

The commonest clinical manifestations of RA were subcutaneous nodules (22.9%), followed by dry mouth (20.8%).Other demographic, clinical characteristics and laboratory measures are demonstrated in (Table 2).

Table (3) shows; that there was no significant difference in serum IL29 value according to activity grade of DAS 28 in the RA group p>0.05, while in SLE there was a significant difference of serum IL29 value regarding SLEDAI grading scores p<0.001(Table 4).

There is a significant direct correlation of serum IL29 with SLEDAI grading r=0.733, P=0.0001. Otherwise, there is nosignificant correlation of serum IL29 with age, BMI, clinical and laboratory findings of RA & SLE patients p>0.05.

At cut-off ≥64.1 (ng/ml), serum IL29 in RA patients had 95.8% sensitivity, 85.4% specificity, and accuracy was 90.6% so, IL29 is a good marker for the detection of rheumatoid arthritis patients, P=0.0001. Otherwise, at cut-off ≥35 ng/ml in SLE patients fail to detect SLE patients p=0.052, with sensitivity and specificity 89.6% and 39.6% respectively (Figure 1-a, b).

Moreover, IL29 can distinguish inactive patients from moderate activity in SLE patients with 80% sensitivity, 75% specificity, and accuracy was 79.2% at a cut-off value ≥40 ng/ml, p=0.014, while in RA patients has no role (Figure 2).

Table (1): Comparison of the studied groups regarding IL29 serum value in ng/mL.

	RA group n=48	SLE group n=48	Control group n=48	KW	p-value
IL29 Median(range)	475.15(64-906.9)	50(18.9-150)	42.79(10.94-69.9)	91.4	0.0001 **
	P1=0.0001** (RA&SLE)	P2=0.015* (SLE& control)	P2=0.0001** (RA& control)		

RA: Rheumatoid arthritis, SLE: Systemic lupus patients,KW: Kruskall-Wallis, *P≤0.05 is statistically significant **P≤0.001 is statistically highly significant.

Table (2): Demographic, clinical characteristics, and laboratory measures of RA patients.

Variables	RA(n=48)	SLE(n=48)
Age (mean± SD) **Sex** N (%) • Males • Females **BMI** (mean± SD) **Disease duration** (year) Median (range) **Family history** N (%)	37.8±5.2 8(16.7%) 40(83.3%) 29.7±4.2 5(1-18) 7(14.6%)	35.9±4.8 2(4.2%) 46(95.8%) 30.2±4.6 7(2-16) 2(4.2%)
Clinical characteristics of RA: ***Morning stiffness***(min) Median (range) ***S.C nodule*** N (%) ***DAS28 score*** Median (range) ***DAS28 grade*** N (%) • inactive • mild • moderate • severe	 12.5 (0-120) 11(22.9) 3.64(1.35-6.21) • 9(18.8) • 10(20.8) • 19(39.6) • 10(20.8)	
Clinical characteristics of SLE: ***Articular*** N (%)***: Arthritis,Arthralgia*** ***Visual disturbance*** N (%) ***Pericarditis*** N (%) ***Mucocutaneus manifestations:*** N (%) ***Neuropsychiatric, and Renal*** ***SLEDAI score*** Median (range) ***SLEDAI grade*** N% • inactive • mild • moderate • severe • very severe	 	 8 (16.7), 25 (52.1) 2 (4.2) 1(2.1) 38(79.1) 0 5(0-18) • 4(8.3%) • 18(37.5%) • 15(31.3%) • 9(18.7) • 2(4.2)
Laboratory measures: **WBCs** Median (range) **HGB** Mean ±SD **Platelet** Mean ±SD **ESR** Median (range) **CRP** Median (range) **Albumin** mean± SD **24h protein in urine** Median (range) **RF** Median (range) **Anti-CCP** Median (range) **Anti-ds DNA Abs N (%)**	 7.5(3.3-15) 12.2±1.06 268.5±74.5 24(6-61) 7.6(1-30) 4.3±0.31 - 40 (3.9-504.5) 37.4(5-640) -	 7.1(1.9-12) 11.7±1.2 257±71.3 23(6-96) 6(0.11-13) 4.1±0.11 168(46-3000) 21(43.7%)

Anti-CCP: cyclic citrullinated peptide, Anti-ds DNA Abs: Anti-double-stranded deoxyribonucleic acid antibodies, BMI:Body Mass Index, CRP: C-reactive protein, DAS: disease activity score, ESR: erythrocyte sedimentation rate, HGB: Hemoglobin, RF: rheumatoid factor, SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, WBC: white blood cell.

Table (3): Comparison of serum IL29 regarding to DAS28 grade of rheumatoid arthritis patients (n=48).

	DAS28 grade				KW	p
	Inactive n (9)	**Mild** n (10)	**moderate** n(19)	**severe** n(10)		
SerumIL29 Median(range)	401 (64-737)	627.5 (369-897.7)	364.7 (64-744.3)	581.3 (64-906.9)	5.6	0.13

KW=KruskallWallius test of sig, *P≤0.05 is statistically significant **P≤0.001 is statistically highly significant.

Table (4): Comparison of serum IL29 regarding SLEDAI grade of systemic lupus erythematosus patients (n=48).

	SLEDAI grade					KW	P
	Inactive n (4)	**Mild** n (18)	**moderate** n (15)	**severe** n (9)	**Very severe** n (2)		
Serum IL29 Median(range)	36.9 (33.8. -40)	40.5 (18.9-80)	54 (43.1-79)	70 (46.8-90)	150 (150-150)	25.4	0.0001 (HS)
	Reference	Inactive & mild p=0.23	Inactive& moderate p=0.003	Inactive & severe p=0.005	*		

KW= Kruskal Wallis test of sig, (NS) insignificant p>0.05, *P≤0.05 is statistically significant **P≤0.001 is statistically (HS) highly significant.*Excluded from analysis.

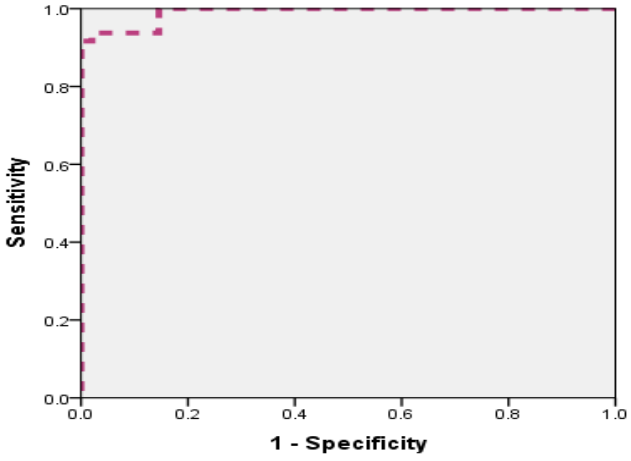


Fig 1 (a):ROC Curve to detect validity of IL29 ng/mL at cut-off at ≥ 64.1 for detection of RA with predictive value for positive (PVP) = (86.8%), predictive value for negative (PVN) = (95.3%), 95.8% sensitivity, 85.4% specificity, and

Fig 1 (b):ROC Curve to detect validity of IL29 ng/mL at cut-off at ≥ 35 for detection of SLE with predictive value for positive (PVP) = (59.7%), predictive value for negative (PVN) = (79.2%), 89.6% sensitivity, 39.6% specificity, and

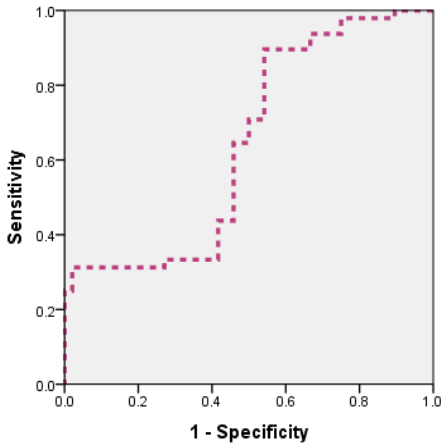
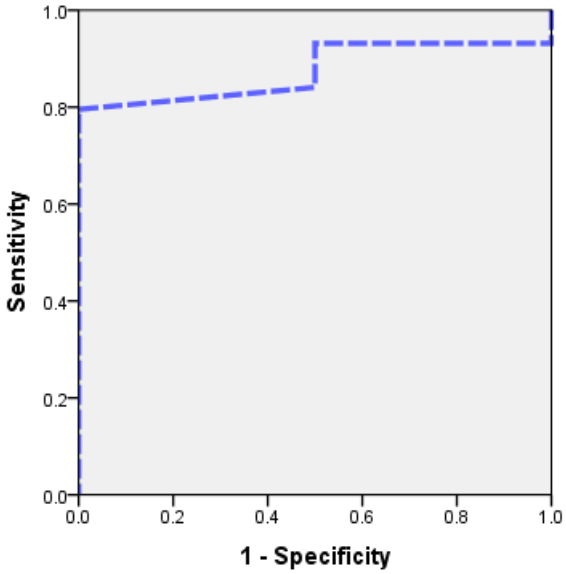


Figure 1: ROC Curve to detect the best cut-off of serumIL29 (ng/ml) to detect RA and SLE patients.



ROC Curve to detect validity of IL29 ng/mL at cut-off at ≥40ng/ml to discriminate between inactive disease and moderate activity in SLE with predictive value for positive (PVP) = (97.2%), predictive value for negative (PVN) = (25%), 80% sensitivity, 75% specificity, and (79.2%) accuracy.

Figure 2: ROC Curve to detect the best cut-off of serum IL29 (ng/ml) to discriminate activities in SLE patients.

DISCUSSION

As described above, IL-29, an important subtype of the type III interferon (IFN) protein family, is linked to the pathogenesis of numerous autoimmune and inflammatory illnesses [4]. In the current research, RA patients had significantly higher serum levels of IL-29, 475.15 pg/ml (IQR = 64-906.9) when compared to SLE patients and healthy controls, which agrees with Da Rocha *et al.* and Xu *et al.* who observed Serum median IL-29 levels were significantly higher in RA patients compared to healthy controls [15, 16].Moreover, serum IL29 at cut-off value ≥64.1 (ng/ml) in RA patients had 95.8% sensitivity, and 85.4% specificity

with AUC=0.97(CI 0.94-1) for detection of RA patients in this study, similarly Da Rocha *et al.*[15]who found that the AUC from ROC analysis 0.810 (CI 0.747–0.864) and the optimal cut-off value of IL-29 (61.11ng/ml) for discriminating patients with RA. IL-29 levels and Tfh cell frequencies were elevated in RA patients, and they were positively linked with anti-CCP antibodies, implying that IL-29 and Tfh cells may act as proinflammatory mediators in RA. Furthermore, IL-29 lowers the amount of Tfh cells via modulating the activity and expression of STAT3 and BCL6, which participate in RA's pathogenesis[16]. Wang *et al*. found that IL-29 increases TLR-induced proinflammatory cytokine production in RA-FLS via TLRs amplification [17].

The present study demonstrates non-statistical significance between IL29 and disease activity in RA patients, in accordance with Da Rocha *et al.*[15] who observed that there is no any association between IL-29 levels and activity indexes of disease (DAS28 and CDAI), on the contrary with Chang *et al.* colleagues discovered that serum levels of IL-29 were significantly higher in RA patients who tested positive for anti-CCP antibodies than in healthy individuals and that this rise had a positive correlation with disease activity[18].As regards SLE patients, the current research revealed that there is a significantly higher value of IL29 in SLE patients compared to the control group (P= 0.015) indicating that IL-29 probably participated in the pathogenesis of SLE[3, 19]. Gallagher *et al*. revealed that IL-29 appears to be a human Th2 response inhibitor whose activity is predominantly focused on IL-13 but may potentially affect Th2 responses in general and does not induce a complementary increase in IFN- γ production[7].

This research revealed a significant positive correlation of serum IL29 with SLEDAI grading p=0.0001 with a statistically significant difference of serum IL29 value to variable SLEDAI grades (p<0.001). For mild (p=0.23), moderate (p=0.003), and severe (p=0.005) Similarly, El Aggan*et al***.** found SLEDAI score was positively correlated with that IL-29 levels in peripheral blood (P < 0.001).Also,Adel and Sadeq reported that a higher disease activity SLEDAI > 6 was related to a high IL-29 level P-value ≤0.05[19, 20]. Multiple studies have revealed that IL-29 can be up-regulated in a variety of cell types and has immune-regulatory effects in both naive PBMCs and Dendritic Cells[21]. Meanwhile, SLE is an autoimmune illness characterized by an enormous aberrant immune cell response, resulting in autoimmune diseases and dysfunction. Furthermore, higher IL29 mRNA and protein levels in SLE patients were most likely associated with an excessive and inappropriate immune cell reaction. So, IFN-λ1 (IL29) is probably involved in the disease activity of SLE[3].Additionally, Zahan *et al.* found that serum IL-29 levels were found to be elevated in the serum of patients with cutaneous lupus erythematosus with active skin lesions[22].These discoveries will help to understand the pathophysiology and treatment of SLE and RA. This will shed information on immune system dysfunction in autoimmune disorders.

Limitations:

We realize that this study faces a few limitations. The research we performed had a limited sample size and was a single-center study; another disadvantage was the lack of follow-up studies; and the effects of drugs on IL29 were not explored.

CONCLUSION

This research provides data on the relationship between IL-29 and SLE and RA, demonstrating higher IL-29 serum levels in all patients compared to controls. IL-29 may act as a pro-inflammatory cytokine and contribute to the development of RA and SLE. It was also linked to lupus activity.

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Citation:

Abdelsattar, A., Hazem, M., Shabana, M., Kamal, D. Serum interleukin 29 in Rheumatoid Arthritis and Systemic Lupus Erythematosus patients and its association with disease activity.. *Zagazig University Medical Journal*, 2024; (2932-2940): -. doi: 10.21608/zumj.2024.256785.3060