The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 483-490

Sirtuin-1 in Rheumatoid Arthritis Patients, Relation to Disease Activity and Interleukin-17A

Rasha I Noreldin[a], Heba A Esaily[b], Samar M Kamal Eldin[a]
Departments of [a] Clinical Pathology and [b] Physical Medicine, Rheumatology and Rehabilitation, Faculty of Medicine, Menoufia University, Menofia Governorate, Egypt
Corresponding author: Heba A. Esaily, MD, **Mobile:** +20 109 272 2125, **ORCID:** 0000-0002-8660-6817,
E-mail: hebaesaily@yahoo.com

ABSTRACT

Background: A systemic condition with a very complex pathophysiology is rheumatoid arthritis. It has been discovered that IL-17A has strong pro-inflammatory characteristics. Sirtuin-1 contributes to chronic inflammation, and its levels in chronic inflammatory illnesses are still under investigations. Up to our Knowledge, the relation between such two markers not yet studied in rheumatoid arthritis patients.

Objectives: The aim of the current study is to evaluate sirtuin-1 in rheumatoid arthritis patients and analyze the potential association between sirtuin-1 level and serum IL-17A levels and their relations to disease activity.

Patients and methods: A cross-sectional study was conducted on 60 Rheumatoid Arthritis (RA) patients and 30 healthy volunteers. Every participant was subjected to history taking, clinical examination, and laboratory evaluation.

Results: both IL-17A and sirtuin-1 were substantially more in RA patients and significantly correlated to most of measured variables. IL-17A and sirtuin-1can be diagnostic indicator of RA with sensitivity (96.67% and 96.67%, respectively) and specificity (96.67% and 86.67%, respectively) and can be indicators of high disease activity with sensitivity (90% and 80%, respectively) and specificity (94% and 94%, respectively), while combined sirtuin-1 and IL-17A diagnostic performance adds much to specificity to discriminate those with high active disease; Sirtuin-1 (Coefficient B= 0.249, P<0.006) and IL-17A (Coefficient B= 0.043, P<0.012) were independent predictors of disease activity.

Conclusion: Compared to healthy individuals, RA patients had considerably greater levels of IL-17A and sirtuin-1. IL-17A has better sensitivity and specificity than situin-1 in discriminating patients, remission and high disease activity states. Sirtuin-1 combined with IL-17 is beneficial in improving the discriminative power for high disease activity, therefore, both might be thought of as potential biomarkers for RA disease activity.

Keywords: IL-17A; Sirtuin-1; disease activity; rheumatoid arthritis, case control study.

INTRODUCTION

One of the most prevalent autoimmune illnesses, rheumatoid arthritis (RA), affects about 1% of people worldwide. Systemic loss of self-tolerance and immune-mediated inflammation are its defining characteristics [(1)]. Inflammatory proliferation of fibroblast-like synoviocytes (FLSs) is the pathogenic aspect of RA; they form invasive synovial pannus, emit a number of pro-inflammatory cytokines, and make proteases that damage bone and cartilage [(2)].

Macrophages, mast cells, dendritic cells (DCs), T cells, B cells, and fibroblast-like synoviocytes (FLSs) are only a few of the cell types implicated with RA (FLSs) [(3)]. Helper T cells are the most crucial immune cells in adaptive immunity and are often referred to as the "master of immunity" since they are essential for practically all immunological responses, including those that control the actions of other lymphocytes and a large portion of the innate immune response [(4)].

T cells are the primary source of IL-17A. IL-17, IL-22, and other cytokines are released by TH17. When TGF-, IL-6, and IL-l are present, antigen stimulates their differentiation from TH0, which is then blocked by IFN- or IL-4 [(5)]. A novel subclass of cytokines with strong pro-inflammatory characteristics was founded by IL-17 [(6)]. Retinoic acid receptor-related orphan receptor t (RORγt), a key transcription factor, is required for Th17 cell development [(7)].

A histone deacetylase known as Sirtuin1 is involved in a variety of physiological processes, including oxidative stress, glucose metabolism, DNA integrity, aging, and cancer. Sirtuin1 is nicotinamide-adenine dinucleotide (NAD)+-dependent [(8)]. The sirtuin family of proteins includes sirtuin-1, which has the greatest molecular mass (120 kDa). SIRT-1, which has 8 introns and 11 exons, is found on chromosome 10q21.3 [(9)].

Sirtuin-1 suppression lowers the expansion of Th1, Th17, and dendritic cells as well as the production of inflammatory cytokines, matrix metalloproteinase, and RORγt [(10)]. Sirtuin-1 contributes to chronic inflammation, and its protein levels in numerous chronic inflammatory illnesses including RA are still under investigations. Therefore, the aim of the current study is to evaluate sirtuin-1 in rheumatoid arthritis patients and analyze the potential association between sirtuin-1 level and serum IL-17A levels and their relations to disease activity.

PATIENTS AND METHODS

The 60 rheumatoid arthritis patients who addressed the outpatient clinics of the Physical Medicine,

Received: 01/10/2022
Accepted: 12/12/2022

Rheumatology and Rehabilitation department, Faculty of Medicine, Menoufia University between November 2020 and October 2021 were the patients of this case-control research.

The case group included 60 RA patients, diagnosed according to the 2010 American College of Rheumatology (ACR) classification criteria [(11)]. Patients below age of 18 and those over 80 were not included in this study. Additionally, patients with extraarticular symptoms, autoimmune illnesses, cancer, infections, or any other conditions that can impact sirtuin levels were eliminated. The control group included 30 healthy participants of similar age and sex.

The following procedures were applied to all patients:

Clinical evaluation: All patients had clinical and physical evaluations, including morning stiffness, tender joint count (TJC), swollen joint count (SJC), and pain visual analogue scale (VAS) over the past week. The disease activity score (DAS) 28-erythrocyte sedimentation rate was used to determine the state of the disease in the RA group (ESR). Patients with a DAS-28 score of less than or equal to 2.6 were deemed to be in remission, and those with a score of equal to or higher than 2.7 were deemed to have active disease. The disease activity was divided into low activity (DAS-28 range: 2.7-3.2), moderate activity (DAS-28 range: 3.3-5.1), and high activity (DAS-28 range: >5.1) [(12)].

Laboratory evaluation: All patients underwent standard laboratory examinations, which included measuring complete blood counts using a Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan) hematology autoanalyzer and ESR using a Westergren pipette [(13)], detecting anti-cyclic citrullinated peptide (anti-CCP) antibodies using the immunoscan CCP test kit [(14)], rheumatoid factor (RF) (QUANTA ELISA Lit TM RF IgM) (15) and latex agglutination test for C-reactive protein (CRP) [(16)]. The identification of sirtuin-1 and IL-17A was done using an enzyme-linked immunosorbent assay (ELISA). In order to conduct laboratory tests, 6 ml of peripheral venous blood from each patient was separated into: 2 ml on thylenediaminetetraacetic acid (EDTA) tubes for CBC, 1.6 ml blood into sodium citrate tubes for ESR and the remaining blood on plain vacutainer which were allowed to clot and separated serum was used for Anti-CCP, RF, CRP assays and the remaining serum was stored in -25 C for further assessment of sirtuin-1 and IL-17A by ELISA.

For Sirtuin-1 and IL-17A analysis: IL-17A ELISA kits (Invitrogen by thermofisher scientific Waltham, MA USA) and sirtuin-1 (FineTest, Wuhan Fine Biotech Co, Hubei, China) were used as directed by manufacturer. A microplate has been precoated with a monoclonal antibody that is specific for Sirtuin-1 and IL-17A. An enzyme linked monoclonal antibody specific for sirtuin-1 and IL-17A is introduced to the wells after any unbound compounds have been removed by washing. When a substrate solution is introduced to the wells, color begins to develop in direct proportion to how much sirtuin-1 and IL-17A were bound in the first stage. Color development is halted and color intensity is measured.

Ethical approval:

The work received ethical approval from Menoufia University Ethics Committee on Human Research. Each participant acknowledged receipt of written informed consent. The conduct of this study was governed by the Declaration of 5 Helsinki, the World Medical Association's rule of ethics for human subjects' research.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) and inter quartile range for non-parametric data, and mean and standard deviation (SD) for parametric data after testing normality using Kolmogrov-Smirnov test.

Chi-Square test was used for comparison of 2 or more groups. Student's t-test was used to compare 2 independent groups. Mann-Whitney U test was used to compare 2 independent groups. Kruskal Wallis test was used to compare more than 2 independent groups with Mann Whitney U test to detect pair-wise comparison. Spearman's correlation: The Spearman's rank-order correlation is used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and / or ordinal variables. Receiver Operating Characteristic (ROC) curve analysis: The diagnostic performance of a test or the accuracy of a test to discriminate diseased cases from non-diseased cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and Specificity were detected from the curve and PPV, NPV and accuracy were calculated through cross tabulation. P value <0.05 was considered significant.

RESULTS

Demographic and laboratory characteristics of the studied population are summarized in table 1. There is no statistical significant difference between the two groups in terms of age, gender, HB or WBCs. When compared to the control group, rheumatoid patients showed significantly higher levels of ESR, CRP, RF, platelets, sirtuin-1, and IL-17A (**Table 1**).

Table 1: Comparison between the two studied groups according to different parameters.

Variable	Cases (n = 60)	Control (n = 30)	Test of Sig.	P-value
Sex				
Female	46 (76.7%)	19 (63.3%)	□□□	0.183
Male	14 (23.3%)	11 (36.7%)	□□□□□	
Age (years)				
Mean ± SD.	44.3 ± 13.16	49.73 ± 13	t= 1.854	0.067
Sirtuin-1 (ng/mL)				
Median (Min. – Max.)	6.95 (3.5 – 8.3)	2.1 (0.8 – 4)	U= 11.0*	<0.001*
IL-17A (pg/mL)				
Median (Min. – Max.)	19.20 (4.1 – 31.8)	3.80 (3.2 – 5.7)	U= 13.50*	<0.001*
ESR (mm/h)				
Median (Min. – Max.)	23 (10 – 95)	10 (7 – 13)	U= 70.0*	<0.001*
CRP (mg/L)				
Median (Min. – Max.)	11 (4 – 27)	4 (3 – 5)	U= 67.0*	<0.001*
RF (U/mL)				
Median (Min. – Max.)	40.5 (7 – 128)	5 (3 – 14)	U= 43.0*	<0.001*
Anti-CCP (U/mL)				
Median (Min. – Max.)	157 (14 – 472)	– –	–	–
HB (g/dL)				
Mean ± SD.	11.98 ± 1.44	11.66 ± 0.77	t= 1.368	0.175
WBCs ($\times 10^3$/uL)				
Median (Min. – Max.)	6.50 (3.70 – 13.70)	6 (4.40 –11)	U= 809.50	0.438
Platelets ($\times 10^3$/uL)				
Mean ± SD.	294.6 ± 63.29	267.23 ± 51.10	t= 2.053*	0.043*

HB: hemoglobin, WBCs: white blood cells, SD: Standard deviation, t: Student t-test, U: Mann Whitney test used for nonparametric data, χ^2: Chi square test, P: p value for comparing between the two studied groups. *: Statistically significant at $p \leq 0.05$

In RA patients, illness duration ranged between 6 and 300 months with a mean of 99.40 (SD 58.06). SJC range was 1and 4 with a mean of 1.98 (SD 0.81), TJC ranged between 1 and 6 with a mean of 3.50 (SD 1.31). Pain VAS ranged between 1 and 9 with a mean of 5.43 (SD 1.90). Lastly, DAS-28 ranged between 1.10 and 5.8 with a mean of 3.65 (SD 1.25). RA patients were categorized according to DAS-28 into remission (20%), low activity (26.7%), moderate activity (36.7%) and high disease activity (16.7%) subgroups.

IL-17A and sirtuin-1 levels in different patients' subgroups demonstrated in ***Table 2 and Figure 1***. Statistically significant difference was noted in IL-17A and Sirtuin-1 levels among the studied subgroups (P value <0.001).

Table 2: Relation between DAS 28 with Sirtuin-1, IL-17A in RA patients (n = 60).

Variable	DAS 28				H	P-value
	Remission (≤2.6) (n = 12)	Low activity (2.7 – 3.2) (n = 16)	Moderate activity (3.3 – 5.1) (n = 22)	High activity (>5.1) (n = 10)		
Sirtuin-1 (ng/mL)						
Median (Min. – Max.)	5.5 (3.5 – 6.8)	6.9 (5.8 – 7)	7.1 (4.2 – 7.8)	8 (6.2 – 8.3)	36.753*	<0.001*
IL-17A (pg/mL)						
Median (Min. – Max.)	6.3 (4.1 – 6.9)	17 (5.9 – 19.2)	22.25 (12 – 26.3)	25.85 (25 – 31.8)	47.848*	<0.001*

IL-17A: Interleukin-17A, H: H for Kruskal Wallis test *: Statistically significant at $p \leq 0.05$
p: p value for Relation between DAS 28 with Sirtuin1, IL-17A.

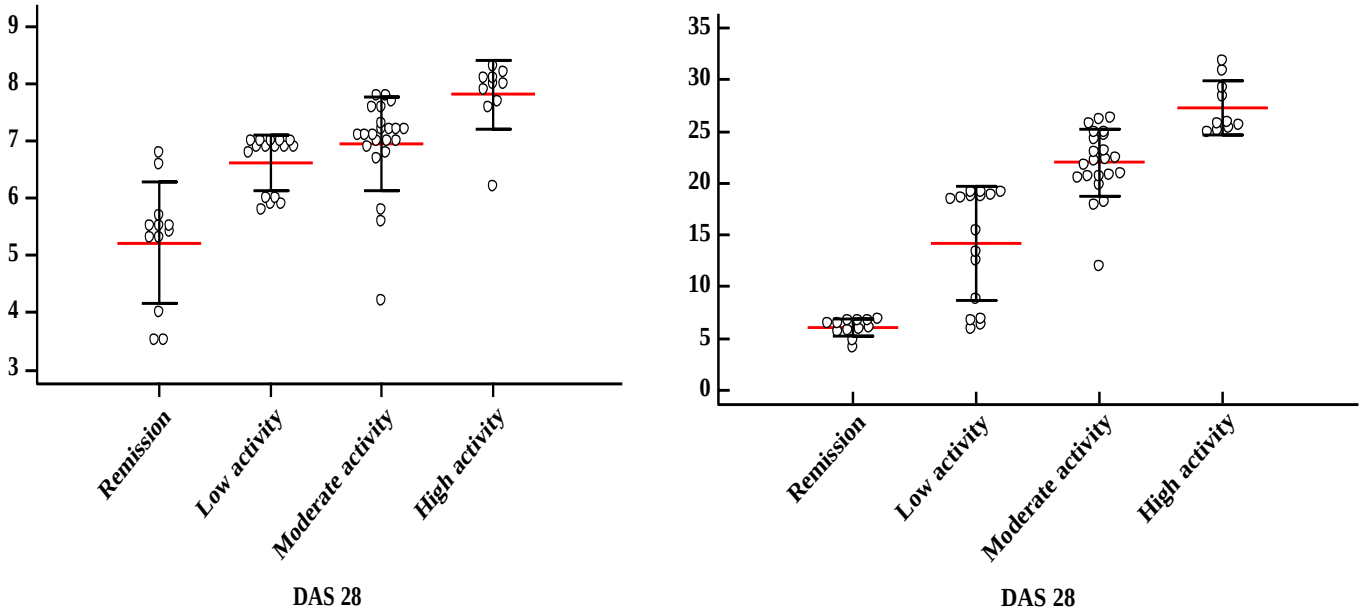

Figure 1: Sirtuin-1 & IL-17 concentrations in different RA patients' subgroups.

ESR, CRP, RF, Anti-CCP, SJC, TJC, pain VAS, and DAS 28 all had positive correlations with serum IL-17A levels (P <0.001). Sirtuin-1 showed similar correlation to such variables except for RF. Both were not correlated with disease duration (**Table 3**). Regarding correlation of IL-17A with sirtuin-1, it was significantly high (r= 0.766; P <0.001).

Table 3: Correlations between Sirtuin-1 and IL-17A in RA patients and other parameters.

Variable	Sirtuin1 (ng/mL)		IL-17A (pg/mL)	
	r_s	p	r_s	p
DAS 28	0.747	<0.001*	0.880	<0.001*
ESR (mm/h)	0.628	<0.001*	0.799	<0.001*
CRP (mg/L)	0.454	<0.001*	0.659	<0.001*
RF (U/mL)	0.252	0.052	0.408	0.001*
Anti-CCP (U/mL)	0.295	0.022*	0.419	0.001*
Duration (months)	-0.013	0.924	0.036	0.786
SJC	0.540	<0.001*	0.632	<0.001*
TJC	0.626	<0.001*	0.832	<0.001*
Pain VAS	0.578	<0.001*	0.685	<0.001*

In order to distinguish patients from controls, patients in remission from patients with disease activity, and patients with highly active from the remainder of the patients, ROC curve analysis was performed for IL-17A and sirtuin-1. Sirtuin-1 has a cutoff value of >3.5 ng/mL, which can be used to diagnose RA with sensitivity of 96.6% and specificity of 86.67%. At a cutoff value of ≤ 6 ng/mL, which can be utilized to determine which patients have experienced remission, sensitivity and specificity are both 83.3%, while a cutoff value of >7.6 ng/mL can be used to identify patients with high disease activity. IL-17A at a cut off value >5.5 pg/mL can be diagnostic indicator of RA with sensitivity 96.67%and specificity 96.67%, at cut off value ≤6.8 pg/mL can recognize patients who achieved remission with sensitivity 91.67% and specificity 93.75%, whereas at cut off value of >25 pg/mL can be indicator of high disease activity with sensitivity 90.0%, and specificity 94.0%. Of note, Diagnostic performance of IL-17A is superior to sirtuin-1 in the above studied comparisons, while combined sirtuin-1 and IL-17A diagnostic performance adds much to specificity to discriminate those with high active disease (**Table 4 and Figure 2**).

Table 4: Diagnostic performance for Sirtuin-1, IL-17A and their combined measurements to discriminate different groups.

To discriminate		AUC	P-value	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
Patients (n = 60 vs. 30)	Sirtuin-1 (ng/mL)	0.994	<0.001*	0.984 – 1.000	>3.5	96.67	86.67	93.5	92.9
	IL-17A (pg/mL)	0.993	<0.001*	0.981 – 1.000	>5.5	96.67	96.67	98.3	93.5
	Combination	0.996	<0.001*	0.989 – 1.000		96.67	96.67	98.31	93.55
Remission (n = 12 vs. 48)	Sirtuin-1 (ng/mL)	0.951	<0.001*	0.896 – 1.000	≤6	83.3	83.3	55.6	95.2
	IL-17A (pg/mL)	0.972	<0.001*	0.936 – 1.000	≤6.8	91.67	93.75	78.6	97.8
	Combination	0.976	<0.001*	0.943 – 1.000		91.67	91.67	73.33	97.78
High disease activity (n = 10 vs. 50)	Sirtuin-1 (ng/mL)	0.923	<0.001*	0.803 – 1.000	>7.6	80.0	94.0	72.7	95.9
	IL-17A (pg/mL)	0.966	<0.001*	0.924 – 1.000	>25	90.0	94.0	75.0	97.9
	Combination	0.998	<0.001*	0.991 – 1.000		90.0	98.0	90.0	98.0

AUC: Area Under a Curve. P value: Probability value. CI: Confidence Intervals. NPV: Negative predictive value. PPV: Positive predictive value. *: Statistically significant at $p \leq 0.05$

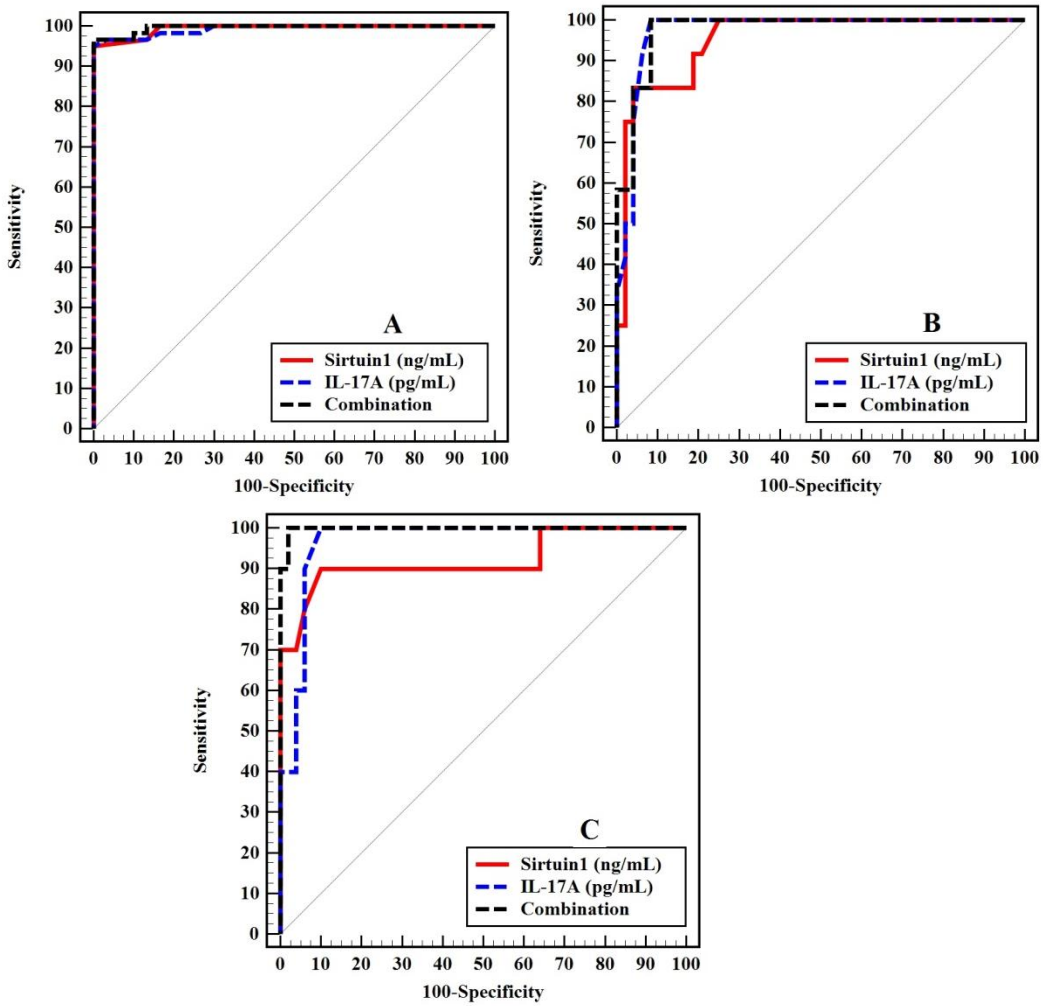

Figure 2: ROC curve for Sirtuin-1, IL-17A and combined to discriminate:
A- Patients from controls (n = 60 vs. 30);
B- Patients in Remission (n = 12 vs. 48);
C- Patients with high disease activity (n = 10 vs. 50).

Univariate logistic regression analyses for parameters affecting RA disease revealed that both IL-17A & Sirtuin-1 were predictors of the disease with IL-17A shows more significance than Sirtuin-1 (**Table 5**), whereas, in multivariate regression analyses ESR (Coefficient B= 0.015, P<0.001), Sirtuin-1 (Coefficient B= 0.249, P<0.006) and IL-17A (Coefficient B= 0.043, P<0.012) were independent predictors of disease activity in decreasing order after controlling other variables (**Table 6**).

Table 5: Analysis of the variables impacting RA disease patients using univariate logistic regression (n = 60 vs. 30)

Variable	Univariate	
	P-value	OR (95%CI LL – UL)
Female	0.186	1.902 (0.733 – 4.936)
Male	0.186	0.526 (0.203 – 1.364)
Age (years)	0.070	0.969 (0.937 – 1.003)
ESR (mm/h)	<0.001*	2.675 (1.582 – 4.525)
CRP (mg/L)	0.001*	20.591 (3.655 – 115.991)
RF (U/L)	0.001*	2.775 (1.500 – 5.134)
HB (g/dL)	0.263	1.227 (0.858 – 1.754)
WBCs ($\times10^3$/uL)	0.412	1.119 (0.855 – 1.466)
Platelets ($\times10^3$/uL)	0.047*	1.008 (1.000 – 1.016)
Sirtuin-1 (ng/mL)	0.005*	15.291 (2.244 – 104.210)
IL-17A (pg/mL)	<0.001*	11.235 (2.971 – 42.483)

OR: Odd`s ratio. CI: Confidence interval. LL: Lower limit. UL: Upper Limit. *: Statistically significant at P ≤ 0.05

Table 6: Analysis of linear regression, both univariate and multivariate, for the factors influencing DAS-28 measurement of RA disease activity.

Variable	Univariate		#Multivariate	
	P	B (95%CI LL – UL)	p	B (95%CI LL – UL)
Female	0.023*	-0.861 (-1.598 – -0.125)	0.551	-0.109 (-0.472 – 0.255)
Male	0.023*	0.861 (0.125 – 1.598)	0.551	0.109 (-0.255 – 0.472)
Age (years)	<0.001*	0.048 (0.027 – 0.070)	0.420	0.005 (-0.007 – 0.017)
ESR (mm/h)	<0.001*	0.034 (0.027 – 0.040)	<0.001*	0.015 (0.008 – 0.023)
CRP (mg/L)	<0.001*	0.115 (0.083 – 0.148)	0.539	0.009 (-0.020 – 0.037)
RF (U/L)	<0.001*	0.016 (0.009 – 0.024)	0.051	0.004 (-0.00002 – 0.009)
Anti-CCP (U/mL)	0.003*	0.004 (0.001 – 0.007)	0.076	0.001 (-0.0001 – 0.002)
HB (g/dL)	0.307	0.117 (-0.110 – 0.344)	---	
WBCs ($\times10^3$/uL)	0.878	0.014 (-0.166 – 0.193)		
Platelets ($\times10^3$/uL)	0.526	-0.002 (-0.007 – 0.004)		
Sirtuin-1 (ng/mL)	<0.001*	0.757 (0.540 – 0.975)	0.006*	0.249 (0.076 – 0.422)
IL-17A (pg/mL)	<0.001*	0.131 (0.110 – 0.152)	0.012*	0.043 (0.010 – 0.076)

B: Unstandardized Coefficients. CI: Confidence interval. LL: Lower limit. UL: Upper Limit. #: All variables with P≤0.05 was included in the multivariate. *: Statistically significant at P≤ 0.05.

DISCUSSION

As RA therapies have evolved, achieving remission as soon as feasible has become the aim of therapy. In order to achieve tight control using treat-to-target techniques, RA disease activity must be quantitatively monitored on a frequent basis. Measures which based on physical examination and history are arbitrary and dependent on perspectives of observers. Therefore, objective measurements that are more sensitive to joint inflammation and better able to predict disease activity than current methods are still needed [17]. Biomarkers are indicators that can be used to improve diagnosis, track the progression of a disease, and gauge how well a treatment is working. The hunt for better biomarkers is still ongoing because none of the RA biomarkers currently in use appear to represent the gold standard [18]. Thus, in this study, sirtuin-1 levels were measured in rheumatoid arthritis patients, and potential relationships between sirtuin-1 levels, serum IL-17A levels, and disease activity were examined.

Sirtuin-1 regulates the activity of numerous transcription factors crucial for immunological function in mammals acting as an epigenetic regulator [19]. It positively affects the activity of Th17 cells by modifying

RORγt activity. In fact, it promotes the growth and functionality of Th17 cells by deacetylating RORγt, the unique transcription factor of Th17 cells, which promotes autoimmunity [7]. While other researchs on sirtuin-1-deficient mice revealed that sirtuin-1 predominantly functions as an anti-inflammatory agent. [20].

The Th17 lineage is associated with IL-17A, and the IL-17 cytokine family as a whole promotes inflammation. An innate-type inflammatory effector gene expression program that induces robust inflammation and is essential for host defense is the overall result of IL-17A signaling [21].

In our investigation, Sirtuin-1, IL-17A, ESR, CRP, RF, and Anti-CCP levels were considerably higher in the RA group than in the control group, which was consistent with Li et al findings in 2021 [22]. Moreover, statistically significant difference was noted for IL-17A and Sirtuin-1 levels among patients' subgroups (p value <0.001) with higher levels noted in high active disease. Previous studies concluded similar results [23,24,25].

This study demonstrated significant relationships between IL-17A and various disease activity indicators including DAS-28, which was consistent with earlier investigations [26,27,28]. In a meta-analysis by Lee and Bae, which comprised 14 studies totaling 3118 patients with RA and 2725 controls, it was found that circulating IL-17A levels were considerably higher in patients with RA, and that there was a connection between the genetic variations for IL-17A and the etiology of RA [29].

Dissanayake et al. (2021), conducted a study on 29 RA patients with aim to quantify peripheral blood mononuclear cells (PBMCs) secreting different cytokines by enzyme-linked immunosorbent spot (ELISPOT) that is much more accurate than ELISA. In their work IL-17A was significantly correlated with DAS-28, clinical disease activity index (CDAI), SJC, TJC and pain VAS and it was superior to other measured cytokines namely TNF-α, IL-1β and IL-10 [18].

Our results for ROC curve showed that the AUC of IL-17A for diagnosing RA was 0.993, with a sensitivity of 96.67% and a specificity of 96.67%, while it marks patients who achieved remission with sensitivity 91.67% and specificity 93.75%. Regarding IL-17A discriminative power for high disease activity, it showed sensitivity of 90.0%, and specificity of 94.0%.

A ROC curve was employed in a Tunisian study to assess the quantification of IL17 plasma levels in 115 RA patients and 91 normal controls and to determine its capacity to detect RA. This study's cut-off value of 18.25 pg/ml showed 100% specificity and 61.7% sensitivity [30].

In a study done by QU et al, ROC curve of IL-17 for diagnosing RA was 0.856, with a sensitivity of 69.90% and a specificity of 87.90% **[31].**

Sirtuin-1 at a cut off value of >3.5 ng/mL and AUC 0.994 can be diagnostic marker of RA with sensitivity 96.6% and specificity 86.67%. Li et al, found in their study that specificity of sirtuin-1 for diagnosis of RA was 97%, sensitivity 71%, with AUC 0.87 and these findings were improved by combination with anti-CCP. Thus, they concluded that both significantly improve the diagnostic accuracy of RA [22]. Our results of combined diagnostic performance of sirtuin-1 and IL-17A raised the specificity of RA diagnosis up to 96.67 %.

The link between sirtuin-1 and IL-17A was highly significant (P<0.001) according to this study. The sirtuin inhibitory effects of nicotinamide during the ex vivo induction of Th17 were validated by Lim et al. They observed a dose-dependent suppression of IL-17A and IL-17F production in response to nicotinamide [20]. In a different study, sirtuin-1 directly boosts the generation of pro-inflammatory mediators and prolongs the survival of RA-fibroblast-like synoviocytes [32]. Numerous relationships between RA and sirtuin-1 have been found in earlier investigations. For instance, Yang et al study has shown that sirtuin-1-mediated reduction of anti-inflammatory IL-27 production in DCs promotes Th17 differentiation and the onset of inflammatory illness [33].

Univariate logistic regression analyses revealed that both IL-17A & Sirtuin-1 are disease predictors & can serve as potential diagnostic biomarkers for rheumatoid arthritis with superiority for IL-17A which came in accordance to similar studies [30,31]. Sirtuin-1 and RF levels were also demonstrated by Li et al. to be substantial, independent predictors of RA [22].

To evaluate the DAS 28 score's predictability, multiple linear regression models were built. According to Dissanayake et al. who discovered a substantial connection between IL-17A and DAS-28 & CDAI scores that supports its potential use as a biomarker of disease severity, ESR, Sirtuin-1, and IL-17A were demonstrated in our work to be independent predictors of disease activity in descending order [18].

In conclusion, RA patients had significantly higher serum levels of IL-17A and sirtuin-1 than healthy people. Both demonstrated correlations with multiple disease related variables. IL-17A has better sensitivity and specificity than situin-1 in discriminating patients, remission and high disease activity states. Sirtuin-1 combined with IL-17A is beneficial in improving the discriminative power for high disease activity, therefore, both can be considered as potential biomarkers for disease activity in RA.

LIMITATIONS OF THE STUDY

As we run a cross-sectional study, the exact relationship between the two studied biomarkers (1L-17A, Sirtuin-1) not yet clarified either its causation or association. Also, the clear discrimination of high disease activity by such 2 markers raises the question whether they are related to destructive aspect of the disease with

subsequent disability, or they may be contributing factors in pathogenesis of extra-articular manifestations. Consequently, more researches in these areas are required.

DECLARATIONS

- **Availability of information and resources:** The information will be made available upon request.
- **Conflicts of interest:** The authors state that they have no interests in conflict.
- **Funding:** For their research, writing, and/or publication of this work, the authors got no financial funding.

We certify that all authors have given their consent for the work to be submitted.

REFERENCES

1. **Picerno V, Ferro F, Adinolfi A *et al.* (2015):** One year in review: the pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol., 33:551-558.
2. **Jutley G, Raza K, Buckley C (2015):** New pathogenic insights into rheumatoid arthritis. Curr Opin Rheumatol., (27):249-255.
3. **McInnes I, Schett G (2011):** The pathogenesis of rheumatoid arthritis, N Engl J Med., 365:2205-2219.
4. **Steinman L (2007):** brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nature Med., 13:139-145.
5. **Umemura M, Yahagi A, Hamada S *et al.* (2007):** IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J Immunol., 7(178):3786-3796.
6. **Yoshida Y, Umemura M, Yahagi A *et al.* (2010):** Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. J Immunol., 184:4414-4422
7. **Ivanov, I, McKenzie B, Zhou L *et al.* (2006):** The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell, 126:1121-1133.
8. **Li T, Zhang J, Feng J *et al.* (2013):** Resveratrol reduces acute lung injury in a LPS-induced sepsis mouse model via activation of Sirt1. Mol Med Rep., 7:1889-1895.
9. **Fang Y, Nicholl M (2011):** Sirtuin 1 in Malignant Transformation: Friend or Foe? Cancer Lett., 306(1):10-14.
10. **Hyung W, Kang S, Ryu J *et al.* (2015):** SIRT1 deacetylates RORγt and enhances Th17 cell generation. J Exp Med., 212(5):607-617.
11. **Aletaha D, Neogi T, Silman A *et al.* (2010):** rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis., 69:1580-1588.
12. **Barczyńska T, Dura M, Blumfield E *et al.* (2015):** DAS28 score vs. ultrasound examination for assessment of rheumatoid arthritis disease activity: comparison and discussion of pros and cons. Reumatologia/Rheumatology, 53(4):213-218.
13. **Westergren A (1957):** Diagnostic tests: the erythrocyte sedimentation rate range and limitations of the technique. Triangle, 3(1):20-25.
14. **Van Gaalen F, Visser H and Huizinga T (2005):** A comparison of the diagnostic accuR.A.cy, prognostic value of the first, second anti-cyclic citrullinated peptides (C.C.P.1, C.C.P.2) autoantibody tests for rheumatoid arthritis. Ann Rheum Dis., 64:1510-1512.
15. **Aho K., Heliövaara M, Maatela J *et al.* (1991):** Rheumatoid factors antedating clinical rheumatoid arthritis. J Rheumatol., 18(9):1282-1284.
16. **Barnes E, Narain S ,Naranjo A (2005):** High sensitivity C-reactive protein in systemic lupus erythematosus: Relation to disease activity, clinical presentation and implication for cardiovascular risk. Lupus, 14(8):576-582.
17. **Jurgens M, Safy-Khan M, de Hair M *et al.* (2020):** The multi-biomarker disease activity test for assessing response to treatment strategies using methotrexate with or without prednisone in the CAMERA-II trial. Arthritis Res Ther., 22(1):205.
18. **Dissanayake K, Jayasinghe C, Wanigasekara P *et al.* (2021):** Potential applicability of cytokines as biomarkers of disease activity in rheumatoid arthritis: Enzyme-linked immunosorbent spot assay-based evaluation of TNF-α, IL-1β, IL-10 and IL-17A. PLoS One, 16(1):e0246111.
19. **Kwon H, Brent M, Getachew R *et al.* (2008):** Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation. Cell Host Microbe, 3:158-167.
20. **Lim H, Kang S, Ryu J *et al.* (2015):** SIRT1 deacetylates RORγt and enhances Th17 cell generation. J Exp Med., 212(5):607-617.
21. **Gaffen S (2009**): Structure and signalling in the IL-17 receptor superfamily Nat Rev Immunol., 9(8):556.
22. **Li X, Li X, Zeng T (2021):** The clinical value of serum sirtuin-1 in the diagnosis of rheumatoid arthritis: a pilot study. British Journal of Biomedical Sci., 78(4):191-194.
23. **Kim J, Kang S, Kim J *et al.* (2013):** Elevated levels of T helper 17 cells are associated with disease activity in patients with rheumatoid arthritis. Ann Lab Med., 33:52-59.
24. **Chen D, Chen Y, Chen H *et al.* (2011):** Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF-α therapy. Arthritis Res Ther., 13:R126.
25. **Zivojinovic S, Pejnovic N, Sefik-Bukilica M *et al.* (2012):** Tumor necrosis factor blockade innate inflammatory and Th17 cytokines in rheumatoid arthritis. J Rheumatol., 39:18-21.
26. **Farag M, El Debaky F, Abd El-Rahman S *et al.* (2022):** Serum and synovial fluid interleukin-17 concentrations in rheumatoid arthritis patients: Relation to disease activity, radiographic severity and power Doppler ultrasound. The Egyptian Rheumatologist, 42(3):171-175.
27. **Robert M, Miossec P (2019):** IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. Front Med (Lausanne), 14(5):364.
28. **El-Maghraby H, Rabie R, Makram W (2019):** Correlation between Relative Expression of IL 17 and PERP in Rheumatoid Arthritis Patients and Disease Activity. Egypt J Immunol., 26(2):9-29.
29. **Lee Y, Bae S (2017):** Associations between circulating IL-17 levels, rheumatoid arthritis & between IL-17 gene polymorphisms, disease susceptibility: a meta-analysis. Postgrad Med J., 93(1102):465-471.
30. **Dhaouadi T, Chahbi M, Haouami Y *et al.* (2018):** IL-17A, IL-17RC polymorphisms and IL17 plasma levels in Tunisian patients with rheumatoid arthritis. PLoS One, 13(3):e0194883.
31. **Qu C, Hou Y, Bi Y *et al.* (2019):** Diagnostic values of serum IL-10 and IL-17 in rheumatoid arthritis and their correlation with serum 14-3-3η protein. Eur Rev Med Pharmacol Sci., 23(5):1899-1906.
32. **Niederer F, Ospelt C, Brentano F *et al.* (2011):** SIRT1 overexpression in the rheumatoid arthritis synovium contributes to proinflammatory cytokine production and apoptosis resistance. Ann Rheum Dis., 70:1866-1873.
33. **Yang H, Lee S, Gao B *et al.* (2013):** The histone deacetylase Sirtuin 1 deacetylates IRF1 and programs dendritic cells to control Th17 differentiation during autoimmune inflammation. J Biol Chem., 288:37256-37266.