Interleukin-17 level in rheumatoid arthritis patients and its relation to disease activity: a clinical and ultrasound study

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Objective
The aim of this study was to measure the level of interleukin-17A (IL-17A) in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and its relation to disease activity.

Patients and methods
A total of 100 patients suffering from RA were chosen from the outpatient clinic, Department of Physical Medicine and Rehabilitation, Menoufia University Hospital. The patient group was divided into three subgroups — mild, moderate, and severe — according to the disease activity score. All patients were subjected to clinical, laboratory, and ultrasound evaluation and to measurement of IL-17A in the serum and synovial fluid by means of the enzyme-linked immunosorbent assay technique. Fifty healthy individuals were evaluated for IL-17A level in the blood, and served as the control group.

Results
The present study revealed an increase in serum ($P = 3.1$) and synovial fluid ($P = 5.2$) IL-17A levels in RA patients with increased disease activity. The ultrasound study showed an increase in serum IL-17A levels with increased erosion of the knee ($P = 5.99$) and wrist ($P = 5.03$). There was an increase in serum IL-17A with increased effusion of the knee ($P = 22.6$) and wrist ($P = 33.3$). There was an increase in serum IL-17A with increased synovial hypertrophy of the knee ($P = 6.39$), wrist ($P = 12.23$), and second metacarpophalangeal (MCP) ($P = 53.34$). Finally, there was an increase in the blood IL-17A level, dryness of the eye ($P = 3.8$), dryness of the mouth ($P = 3.2$), and number of subcutaneous nodules ($P = 2.5$).

Conclusion
In our study; the mean serum and synovial IL-17A levels were found in high titers in patients with disease activity, and with extra-articular manifestations like dry eyes, dry mouth, and subcutaneous nodules. Also erosions, synovial hypertrophy, and effusions were found with significantly high titers of IL-17A, denoting its usefulness as a measurement tool for high disease activity and destruction. Also targeting IL-17A may be useful as a treatment option for aggressive disease and for rheumatoid patients with poor prognosis.

Keywords:
disease activity score, interleukin-17, rheumatoid arthritis, ultrasonography

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Introduction

Rheumatoid arthritis (RA) is a complex, chronic autoimmune disease characterized by an inflammatory infiltration of immune cells, in particular T cells, which represent ~40% of the synovial cellular infiltration and participate in a number of inflammatory and destructive events, such as synovial hyperplasia, pannus setting, cartilage and bone erosion, and joint destruction [1,2].

Various cells from the immune system and from the synovium are involved, and a panel of cytokines are produced, expressed, and become functionally active even in the early stages of RA [2]. Among the cytokines produced by T cells, interleukin-17A (IL-17A) (previously known as IL-17) and IL-17F constitute the signature cytokines of the newly described T-helper cell subset (Th17) [3].

IL-17 responds by stabilizing the mRNA of cytokines, enhancing the receptor expression, and increasing migration, chemokine gene expression, and invasiveness of synoviocytes, and contributes to disease chronicity by inhibiting synoviocyte apoptosis [3].

Finally, it enhances metalloprotease secretion, leading to cartilage damage. These properties support the combined inhibition of IL-17A and IL-17F to control RA inflammation and joint destruction [3].

Patients and methods

This study was performed at the Department of Rheumatology and Rehabilitation, Faculty of Medicine, Menoufia University Hospital, and was approved by its ethical committee.

After giving their informed consent, 100 patients suffering from RA [1] were enrolled in the study, with 50 healthy individuals serving as controls. Patients were divided into three groups — G1 (mild), G2 (moderate), and G3 (severe) — according to the disease activity score (DAS28), where a DAS28 score greater than 5.1 implies highly active disease, a DAS28 score less than 5.1 and greater than 3.2 implies moderately active disease, a DAS28 score less than 3.2 implies low active disease, and a DAS28 score less than 2.6 implies remissions [4].

All patients were subjected to clinical examination to determine the number of tender joints and the number of swollen joints and were also evaluated on the basis of the visual analogue scale. Laboratory measurements were taken of erythrocyte sedimentation rate, complete blood count, C-reactive protein, rheumatoid factor, anticyclic citrullinated peptide, and IL-17A in the serum and synovial fluid [5] by means of the enzyme-linked immunosorbent assay (ELISA) technique [6]. The control group was subjected to measurement of IL-17A in the serum.

The patients were subjected to ultrasound evaluation of knees, wrists, and second MCP, in accordance with European Society of Musculoskeletal Radiology techniques [7], to assess erosion, effusion, vascularity, and synovial thickness.

Cytokine detection

Venous blood samples of 3 ml each were collected in sterile plane tubes, allowed to stand for 30 min at room temperature, and then centrifuged at 300*g* for 5 min. Sera were separated immediately, and stored at −20°C until the time of analysis. The IL-17A assay kit, The RayBio Human IL-17 ELISA kit, is an in-vitro ELISA for the quantitative measurement of human IL-17A in serum, plasma, cell culture supernatants, and urine. This assay uses an antibody specific to human IL-17A coated on a 96-well plate. Standards and samples are pipetted into the wells, and IL-17A present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-17A antibody is added. After washing away unbound biotinylated antibody, horseradish peroxidase-conjugated streptavidin is pipetted into the wells. The wells are again washed, a TMB substrate solution is added, and the color develops in proportion to the amount of IL-17A bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm RayBiotech [6].

Statistical analysis: The data collected were tabulated and analyzed with SPSS (Statistical Package for the Social Science software), version 16, on an IBM compatible computer [8].

Descriptive statistics were derived as percentage (%), mean (*X*), and SD.

Analytic statistics were subjected to the χ^2-test, the Student *t*-test, and the *F*-test.

Results

Our study involved 100 patients suffering from RA, recruited from the outpatient rheumatology clinic of the Department of Rheumatology and Rehabilitation, Faculty of Medicine, Menoufia University Hospital. The study included both sexes. Seventy-eight patients were female and 22 were male. Their ages ranged between 20 and 69 years (mean age of G1, 42.7 ± 11.3; mean age of G2, 42.8 ± 11.6; mean age of G3, 46.5 ± 12.2). Their disease duration ranged from 2 to 23 years (mean disease duration in G1, 6.8 ± 4.1; mean disease duration in G2, 6.82 ± 4.9; mean disease duration in G3, 9.1 ± 6.4). The ages of controls ranged from 22 to 64 years (mean age, 41.8 ± 12.6).

The comparison of IL-17A level in the blood samples of the studied groups showed significant increase in IL-17A in the patient groups in comparison with the control group ($P < 0.05$) (Table 1).

The comparison of IL-17A level in the synovial fluid of the patient groups with that of the control group showed significant increase in IL-17A in the synovial fluid of the patient group ($P < 0.05$) (Table 2).

The comparison of IL-17A levels in the blood as well as erosions of the knee, wrist, and the second MCP in the patient groups with its corresponding values in

Table 1 Comparison between serum interleukin-17A in the studied groups in relation to disease activity score

	Patients groups (*X* ± SD)			Control group (*X* ± SD)	*F*-test	*P* value
	Mild (*n* = 32)	Moderate (*n* = 34)	Severe (*n* = 34)			
IL-17 blood	16.15 ± 7.5	16.7 ± 6.06	20.77 ± 10.6	9.1 ± 3.5	3.1	<0.05

IL-17, interleukin-17.

Table 2 Comparison between synovial interleukin-17A level in the studied group in relation to disease activity score

	Patients groups ($X \pm SD$)			Control group ($X \pm SD$)	*F*-test	*P* value
	Mild ($n = 32$)	Moderate ($n = 34$)	Severe ($n = 34$)			
IL-17 synovial fluid	46.5 ± 6.7	56.4 ± 10.2	70.8 ± 27.6	42.8 ± 3.7	5.2	<0.05

IL-17, interleukin-17.

the control group showed highly significant increase in IL-17A level in the blood, with increased erosion of the knee and wrist ($P < 0.001$) and insignificant increase as regards the second MCP in the patient groups ($P > 0.05$) (Table 3).

The comparison of IL-17A levels in the blood as well as effusion (as per the musculoskeletal US study — Figs. 1 and 2) of the knee, wrist, and second MCP in the patient group with the corresponding values in the control group showed highly significant increase in IL-17A in the blood, with increased effusion of the knee and wrist ($P < 0.001$) and insignificant increase as regards second MCP effusion ($P > 0.05$) in the patient group (Table 4).

The comparison of IL-17A level in the blood in the patient group as well as synovial hypertrophy (as per the musculoskeletal US study — Fig. 2) with the corresponding values in the control group showed highly significant increase in IL-17A level in the blood, with increased synovial hypertrophy of the knee, wrist, and second MCP ($P < 0.001$) (Table 5).

There were highly significant statistical differences between the clinical signs of severity as regards dry eye, dry mouth, and subcutaneous nodules but insignificant statistical differences as regards chest complain, Raynaud's phenomenon, and IL-17A level in the blood of the patient group ($P < 0.001$) (Table 6).

There were insignificant statistical differences between the clinical signs of severity and IL-17 level in the synovial fluid in the patient group ($P > 0.05$) (Table 7).

Table 3 Comparison between serum interleukin-17A in the patients group and erosion of the knee, wrist, and second MCP

Erosion	IL-17A blood ($X \pm SD$)			*F*-test	*P* value
	Mild ($n = 32$)	Moderate ($n = 34$)	Severe ($n = 34$)		
Knee					
G1	3.4 ± 0.81	4.96 ± 2.3	18.05 ± 2.8	5.99	<0.001
G2	8.8 ± 3.1	7.43 ± 6.4	12.3 ± 4.7		
G3	9.2 ± 5.21	3.2 ± 0.2	3.7 ± 0.9		
Wrist					
G1	3.2 ± 1.04	6.05 ± 3.02	28 ± 1.21	5.03	<0.001
G2	5.84 ± 5.75	7.58 ± 7.6	12.6 ± 11.4		
G3	4.7 ± 1.3	4.6 ± 1.7	9.6 ± 6.03		
Second MCP					
G1	3.9 ± 2.1	4.7 ± 2.07	21.3 ± 10.2	2.2	>0.05
G2	4.05 ± 1.4	10.5 ± 4.4	9.07 ± 3.9		
G3	5.81 ± 2.43	3.4 ± 2.4	6.3 ± 2.2		

Grade 1 = absence of erosion, grade 2 = more than two erosions, grade 3 = any large erosions; IL-17A, interleukin-17A.

Table 4 Comparison between serum interleukin-17A in the patient group and effusion of the knee, wrist, and second MCP

Effusion	IL-17A blood ($X \pm SD$)			*F*-test	*P* value
	Mild ($n = 32$)	Moderate ($n = 34$)	Severe ($n = 34$)		
Knee					
Minimal	10.7 ± 11.2	8.3 ± 8.9	7.8 ± 8.2	22.26	<0.001
Mild	6.6 ± 2.75	5.24 ± 2.3	8.3 ± 9.3		
Moderate	3.52 ± 0.89	5.4 ± 2.5	4.5 ± 2.1		
Marked	6.2 ± 1.12	3.2 ± 1.31	2.9 ± 1.12		
Wrist					
Minimal	3.7 ± 0.85	9.7 ± 11.5	5 ± 1.3	33.3	<0.001
Mild	5.3 ± 1.5	11.2 ± 3.24	6.23 ± 1.25		
Second MCP					
Minimal	3.7 ± 0.84	9.7 ± 11.5	5.34 ± 2.11	3.5	>0.05
Mild	5.3 ± 1.5	3.1 ± 1.42	2.4 ± 1.21		

IL-17A, interleukin-17A.

Discussion

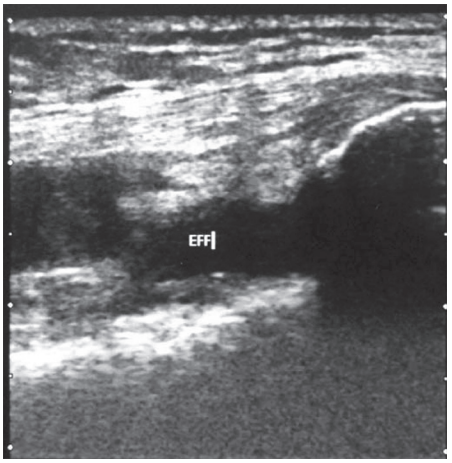
In this study we found a statistically significant increase in IL-17A level in the blood and synovial fluid in the patient group in comparison with the control group. Roşu *et al.* [2] reported that simultaneous IL-17A assessment in serum and synovial fluid was valuable for defining activity.

This study also reported that there was a highly significant increase in clinical signs of severity as regards dry eye, dry mouth, subcutaneous nodules, and IL-17A level in the blood of the patient group.

This is in agreement with the results of Metawi *et al.* [9], who reported that there was a direct relation between the serum level of IL-17A and disease activity and severity in RA patients.

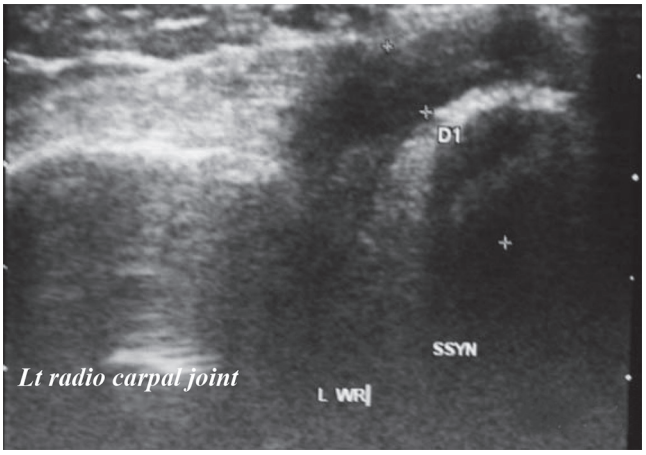
Also, Sarkar *et al.* [10] found that IL-17A increases during inflammatory arthritis and that neutralization of IL-17A reduces the severity of arthritis. Moreover, significantly higher levels of IL-17A were detected in

Figure 1



This figure shows moderate effusion of the knee joint at suprapatellar pouch (Grade 2).

Figure 2



This figure shows moderate synovial hypertrophy with synovial thickness (4.6) mm oflt the wrist.

Table 5 Comparison between serum interleukin-17A level in the studied groups and synovial hypertrophy

Synovial hypertrophy	IL-17A blood (X ± SD)			*F*-test	*P* value
	Mild (n = 32)	Moderate (n = 34)	Severe (n = 34)		
Knee					
Mild	4.3 ± 1.98	4.8 ± 1.7	6.77 ± 8.92	6.39	<0.001
Moderate	6.16 ± 5.32	6.6 ± 5.8	14.2 ± 13.02		
Wrist					
Mild	5.22 ± 5.9	5 ± 2.4	12.1 ± 11.1	12.23	<0.001
Moderate	5.95 ± 3.6	4.3 ± 1.2	13.5 ± 10.9		
Second MCP					
Mild	4.01 ± 1.4	5.7 ± 2.4	13.6 ± 11.03	53.34	<0.001
Moderate	6.44 ± 2.24	4.5 ± 2.4	11.21 ± 1.21		

IL-17A, interleukin-17A.

Table 6 Comparison between the clinical signs of severity and interleukin-17A in the blood of the patient group

Signs of severity	IL-17A blood (mean ± SD)		*t*-Test	*P* value
	Yes	No		
Dry eye	14.2 ± 9.9	6.5 ± 7.5	3.8	<0.01
Dry mouth	10.9 ± 10.1	5.7 ± 6.1	3.2	<0.01
Subcutaneous nodule	10.2 ± 10.5	6.8 ± 6.3	2.5	<0.05
Chest complain	5.3 ± 2.1	7.99 ± 8.5	0.3	>0.05
Raynaud's phenomenon	11.4 ± 9.6	7.3 ± 8.2	1.8	>0.05

IL-17A, interleukin-17A.

the peripheral blood and synovial fluid of patients with rheumatoid.

We also observed significant increase in IL-17A level in the blood with increased number of erosions of the knee and wrist in the RA patients in the studied groups, when evaluated on the basis of their musculoskeletal US measurement.

This is in agreement with the findings of Brentano F *et al.* [11], who suggested that IL-17A cytokine could be used as a parameter for prediction of pre-erosive and destructive changes and rapid disease progression in RA patients.

In our study, we found significant differences in IL-17A levels in the blood samples were associated with high level of serum and synovial IL-17A.

This is commensurate with the results of Kim *et al.* [12], who suggest that Th-17 cells and IL-17 play an important role in RA pathogenesis, and that the level of IL-17A in the peripheral blood and synovial fluid is associated with increased disease activity and articular destructive effects, such as joint erosion, synovitis, and effusion in RA patients.

Pavlovic *et al.* [13] found that the mean serum IL-17A levels in patients with early RA corresponded with disease activity and severity. This might highlight the usefulness of the serum IL-17A level in defining the activity and predictive patterns, to aid in aggressive disease therapy, and it might express specific therapeutic targets.

Guggino *et al.* [14] suggest that treatment with MTX and methyl prednisolone could ameliorate RA disease activity by normalizing the distribution/imbalance of Th-17/Treg and could indicate a new regulatory role of IL-17 cells in RA patients, suggesting the role of IL-17 in RA activity.

Conclusion

In our study, the mean serum and synovial IL-17A levels were found in high titers in patients with

Table 7 Comparison between the clinical signs of severity and interleukin-17A measured as pg/ml in the synovial fluid in the patient group

Signs of severity	IL-17A synovial (mean ± SD)		*t*-Test	*P* value
	Yes	No		
Dry eye	17.4 ± 5.6	33.4 ± 22.9	−0.96	>0.05
Dry mouth	25.3 ± 16.2	37.99 ± 26.9	−1.12	>0.05
Subcutaneous nodule	26.7 ± 21.2	33.5 ± 23.1	−0.55	>0.05
Chest complain	11.2 ± 2.41	31.2 ± 21.9	−0.3	>0.05
Raynaud's phenomenon	37.9 ± 30.2	27.9 ± 17.6	0.82	>0.05

IL-17A, interleukin-17A.

disease activity, and with extra-articular manifestations like dry eyes, dry mouth, and subcutaneous nodules. Also, erosions, synovial hypertrophy, and effusions were found with significantly high titers of IL-17A, denoting its usefulness as a measurement tool for high disease activity and destruction. Also, targeting IL-17 A may be a treatment option for aggressive disease and for rheumatoid patients with poor prognosis.

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Conflicts of interest

There are no conflicts of interest.

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