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ORIGINAL ARTICLE

Serum and synovial fluid levels of interleukin-17 in correlation with disease activity in patients with RA

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Abstract The aim of this study was to evaluate serum and synovial levels of IL-17A by ELISA in rheumatoid arthritis (RA) and find out the correlations between IL-17A levels and various clinical, laboratory parameters and RA disease activity and severity indices. Group I consists of 30 adult active RA patients fulfilling the ARA 1987 revised criteria, with knee effusion and receiving basic therapy, and with a mean age of 41.47±11.49 years and mean disease duration of 9.5±4.16 years. Group II consisted of 13 healthy volunteers, age- and sex-matched, with a mean age of 39.08±14.19 years. RA patients showed significantly higher mean serum IL-17A levels than controls (11.25± 9.67 vs. 0.6±1.4 pg/mL, respectively, p=0.0002). Synovial IL-17A levels showed a significant positive correlation with serum IL-17A levels (r=0.5 and p=0.005). RA patients with negative rheumatoid factor (RF) had non-significantly higher mean serum IL-17A levels (12±9.86 pg/mL) compared to those with positive RF (10.82±9.81 pg/mL); however, the mean synovial IL-17A levels were nearly the same. Significant positive correlations were found between both serum and synovial IL-17A levels and DAS-28 scores (r=0.556, 0.392 and p=0.001, 0.032, respectively). RA patients with class III functional status showed significantly higher mean serum IL-17A levels (17.53±13.43 pg/mL) than classes I and II (8.97±6.97 pg/mL, p=0.009). These led us to conclude that the elevated serum and synovial IL-17A levels in RA patients parallel the degree of disease activity and severity. This may highlight the usefulness of IL-17 (especially serum level) as a possible marker for more aggressive joint involvement and damage.

Keywords Cytokines · DAS-28 scoring and functional status assessment · Rheumatoid arthritis · Serum and synovial interleukin-17

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Introduction

Rheumatoid arthritis is a chronic systemic autoimmune inflammatory disease that primarily affects the synovial diarthrodial joints in a symmetric pattern and leads to irreversible joint destruction. Extra-articular involvement of organs such as the skin, heart, lungs, and eyes can be significant [1].

Rheumatoid arthritis (RA) is considered an autoimmune disease marked by joint inflammation, T cell infiltration of the synovium, synovial hyperplasia, neoangiogenesis, involvement of many catabolic cytokines, and progressive destruction of articular cartilage and bone [2].

Cytokines play an important role in the pathogenesis of RA; they regulate a broad range of inflammatory processes that are implicated in the pathogenesis of RA. In rheumatoid joints, it is well known that an imbalance between pro- and anti-inflammatory activities favors the induction of autoimmunity, chronic inflam-

mation, and joint damage; therefore, inhibiting the action of pro-inflammatory cytokines by using specific cytokine inhibitors or anti-inflammatory cytokines is the basis for new therapies [3, 4].

IL-17A is a novel pro-inflammatory T cell cytokine expressed in the synovium and synovial fluid of patients with RA. It is produced by activated memory $CD4^+$ T cells (new T helper subset termed Th17 cells). It is a potent inducer of various cytokines such TNF -α and IL-1, it shares properties with IL-1 and TNF-α, and it may induce joint inflammation and bone and cartilage destruction. It increases IL-6 production, induces collagen degradation, and decreases collagen synthesis by synovium and cartilage and proteoglycan synthesis in cartilage. IL-17 is also able to increase bone destruction and reduce its formation [5].

Interestingly, IL-17 appears to stimulate predominantly the production of cytokines that either specifically attract neutrophils to the site of inflammation (IL-8, GROα, and GCP-2) or stimulate granulopoiesis in bone marrow (IL-6, G-CSF, and GM-CSF). Moreover, IL-17 was found to induce IL-1 βand TNF-α in macrophages, and these cytokines can further synergize with IL-17 to amplify the synthesis of neutrophil-specific chemokines and GM-CSF [6].

IL-23 promotes the production of IL-17, and a strong correlation between IL-15 and IL-17 levels in synovial fluid has been observed. IL-17 has the capacity to induce joint destruction in an IL-1-independent manner and can bypass TNF-dependent arthritis [2].

Neutralization of inflammatory mediators to reduce the progression of RA has been used successfully for several cytokines, particularly TNFα. IL-17 is also an important mediator of RA pathology as blockade of IL-17 in arthritis models reduces joint inflammation and bone erosion. Therefore, anti-IL-17 cytokine therapy is of interest as an additional new anti-rheumatic strategy for RA [7].

This study was performed to evaluate the serum and synovial levels of IL-17A in RA and find out the correlations between IL-17A levels and various clinical, laboratory parameters and RA disease activity and severity indices.

Materials and methods

Subjects

The present study comprised two groups: *Group I* is composed of 30 adult active RA patients with knee effusion and receiving basic therapy (22 women and 8 men). All fulfilled the ARA 1987 revised criteria for the classification of RA [8], with age ranging from 22 to 64 years, with a mean of 41.47±11.49 years, and disease duration ranging from 3 to 20 years, with a mean of 9.5±4.16 years. *Group II* comprised 13 healthy subjects, age- and sex-matched, with a mean age of 39.08±14.19 years, serving as a control group. All patients and controls were recruited from the Rheumatology and Rehabilitation Department, Kasr Al-Aini Hospitals, Cairo University.

RA patients were subjected to:

1. Full history taking including assessment of functional capacity according to the criteria for the classification of functional status in RA [9].
2. Thorough general, cardiopulmonary, abdominal, and neurological system examinations.
3. Articular examination (including the modified Ritchie articular index) [10] and knee synovial fluid aspiration.
4. Assessment of disease activity using DAS-28 scoring system [11].
5. Radiological examination: plain X-ray hands (and wrists) A-P views.
6. Routine laboratory investigations (CBC, liver and kidney functions, and urine analysis) and rheumatoid factor (RF) assay.
   - Blood samples (8 mL) were withdrawn from each patient. The samples were aliquoted into three aliquots: the first aliquot on EDTA tube for CBC assay on CellDyn 1300 System, the second aliquot on citrate for erythrocyte sedimentation rate (ESR), and third aliquot was put in a plain tube allowed to clot. Serum was separated and stored frozen at −20°C until assay of:
     (a) RF: assessed whether positive or negative using Latex agglutination slide test manually.
     (b) Liver function tests:
       - AST and ALT (U/L) were assayed by a kinetic UV test and serum albumin (gm/dL) by BCG method on Hitachi 911.
     (c) Kidney function tests:
       - Urea (mg/dL) was measured by a kinetic UV assay and creatinine (mg/dL) was assayed by Jaffe's kinetic UV colorimetric method on Hitachi 911. All kits done on Hitachi were purchased from Roche Diagnostics.
7. Measurement of serum and synovial IL-17A levels [12]
   - After taking the patients' consents, aspiration of knee joint under aseptic conditions was done to all patients; samples were put in a sterile plain tube and stored frozen at −20°C until assay of IL-17A (pg/mL) using ELISA technique.

Principle of IL-17A immunoassay

IL-17A levels were measured in serum of both patients and controls (pg/mL) using ELISA technique. IL-17A assay is a solid phase enzyme amplified sensitivity immunoassay performed on a microtiter plate. The kit was purchased from Biosource* (BioSource Europe S.A. Rue de l' Industrie, 8 B-1400 Nivelles, Belgium).

Standards or samples containing IL-17A react with capture monoclonal antibody (Mab1) coated on the microtiter well and with a biotinylated monoclonal antibody (Mab2) specific for IL-17A. After an incubation period allowing the formation of a sandwich, coated Mab1–IL-17A–Mab2 biotin, the microtiter plate was washed to remove unbound biotinylated antibodies.

Sterptavidin peroxidase was added and binds to the biotinylated antibody. After incubation, the unbound enzyme was removed by washing and a substrate solution was added. The reaction was stopped with the addition of a stop solution, and the microtiter plate was then read at the appropriate wavelength. The amount of substrate turnover was determined colorimetrically by measuring the absorbance which is proportional to the IL-17A concentration. A standard curve was plotted and IL-17A concentration in a sample was determined by interpolation from the standard curve.

- Concerning the administered basic therapy, 25 (83.3%) patients received MTX with a dose ranging from 12.5 to 25 mg/week, 7 (23.3%) patients received Leflunomide (20 mg/day), and 4 (13.3%) patients received combined MTX and Leflunomide. In addition, 21 (70%) patients received anti-malarials (250 mg/day), 1 (3.3%) patient received sulphasalazine (2 g/day), and 12 (40%) patients received oral corticosteroids with a dose ranging from 2.5 to 10 mg/day.
- In RA patients, plain X-ray hands examination revealed juxta-articular osteopenia in 28 (93.34%), joint space narrowing in 23 (76.67%), marginal erosions in 18 (60%), subchondral cysts in 15 (50%), and deformities in 4 (13.3%) patients.

Statistical analysis

The data were collected, tabulated, and analyzed by SPSS, version 14 (SPSS Corporation, USA). Qualitative data were presented in the form of number and percentage and quantitative data presented in the form of mean and standard deviation. Student's t test was used to compare between two means. Comparison among more than two groups was done by ANOVA test. Correlation between variables was performed using Pearson's correlation test. Values of $p<0.05$ were considered significant.

Results

Baseline clinical and laboratory features of the study RA group are shown in Tables 1 and 2.

The various parameters used for the assessment of disease activity of RA patients are presented in Table 3. Two patients (6.67%) had low (<3.2), 23 patients (76.67%) had moderate (3.2–5.1), and 5 patients (16.67%) had high disease activity (>5.1) according to the DAS-28 scoring system.

RA patients showed significantly higher mean serum IL-17A levels than healthy controls (11.25±9.67 vs. 0.6± 1.4 pg/mL, respectively, $p=0.0002$). The measured serum IL-17A levels in RA patients and controls ranged from 6.2 to 40 and from 0 to 4.8 pg/mL, respectively, as shown in Fig. 1.

In RA patients, synovial IL-17A level ranged from 6.4 to 13 pg/mL, with a mean of 8.41±1.55 pg/mL. In addition, synovial IL-17A levels showed a significant positive correlation with serum IL-17A levels ($r=0.501$ and $p=0.005$), as shown in Fig. 2.

No significant correlations were found between both serum and synovial IL-17A levels and various demographic features of patients, including patients' ages ($r=-0.289$, 0.046 and $p=0.122$, 0.811, respectively), age at disease onset ($r=-0.226$, 0.06 and $p=0.229$, 0.755, respectively), and disease duration ($r=0.019$, −0.063 and $p=0.92$, 0.741, respectively).

As shown in Table 4, synovial IL-17A levels showed significantly positive correlations with WBC count and serum albumin ($r=0.427$, 0.409 and $p=0.019$, 0.025, respectively).

RA patients with negative RF had non-significantly higher mean serum IL-17A levels (12±9.86 pg/mL) compared to those with positive RF (10.82±9.81 pg/mL

Table 1 Baseline clinical manifestations of RA patients

Clinical manifestation	No. of patients (%)
History of constitutional manifestations	3 (10%)
2 ry Sjogren's syndrome	3 (10%)
Associated cardiac manifestations	2 (6.7%)
Associated IPF	2 (6.7%)
Drug-induced nausea and vomiting	2 (6.7%)
Articular involvement	
Flexion deformities of knees	4 (13.3%)
Flexion deformities of elbows	6 (20%)
Swan neck deformity	3(10%)
Boutonniere deformity	2 (6.7%)
Ulnar deviation of MCPs	4 (13.3%)
Sublaxation of MCPs	2 (6.7%)
Rheumatoid subcutaneous nodules	5 (16.7%)

Table 2 Main laboratory parameters of RA patients

Laboratory parameter	Range	Mean ± SD
ESR mm/1st hour	25–120	73.83±25.39
Platelet count (/cm3)	206–772	405.7±135.69
Hb (gm/dL)	8.9–13.7	11.03±1.23
WBCs count (/cm3)	3.4–23.2	6.89±3.91
Albumin (gm/dL)	3.4–4.5	3.89±0.35
AST (U/L)	10–34	18.97±5.07
ALT (U/L)	5–40	15.37±7.64
Urea (mg/dL)	15–50	23.8±7.26
Creatinine (mg/dL)	0.5–1	0.73±0.13

Ninteen patients had positive RF (63.3%) and 11 patients (36.7%) had negative RF

and p=0.839). However, the mean synovial IL-17A levels were nearly the same in RF-positive (8.63±1.69) and RF-negative patients (8.02±1.26 pg/mL).

RA patients were classified into group (1): <1 h, 17 (56.67%) patients, and group (2): ≥1 h, 13 (43.33%) patients, according to the duration of morning stiffness. Group (2) showed statistically significantly higher mean synovial IL-17A levels (9±1.73 pg/mL) than group (1) (7.95±1.72 pg/mL and p=0.03), as shown in Fig. 3.

Significant positive correlations were found between both serum and synovial IL-17A levels and DAS-28 scores (r=0.556, 0.392 and p=0.001, 0.032, respectively), as shown in Fig. 4. Serum and synovial IL-17A levels had significant positive correlations with tender joint count (TJC; r=0.495, r=0.403 and p=0.005, 0.027, respectively), as shown in Fig. 5.

Furthermore, serum IL-17A levels had significant positive correlation with swollen joint count (SJC; r=0.573 and p=0.001). On the contrary, synovial IL-17A levels showed no significant correlation with SJC (r=0.223 and p=0.236). Non-significant correlations were found between both serum and synovial IL-17A levels and general health (GH) based on visual analogue scale (VAS; r=0.238, 0.317 and p=0.206, 0.08, respectively) and modified

Table 3 Assessment of disease activity of RA patients

Disease activity parameter	Range	Mean ± S.D
DAS-28 scoring system	3.14–6.67	4.8±0.87
SJC	1–26	10.87±6.08
TJC	2–28	11.9±6.99
GH (VAS in mm)	40–70	54±8.14
Modified Ritchie articular score	1–24	11.5±6.54
Morning stiffness (min)	0–180	48.5±47.18

SJC swollen joint count, *TJC* tender joint count, *GH* general health based on VAS

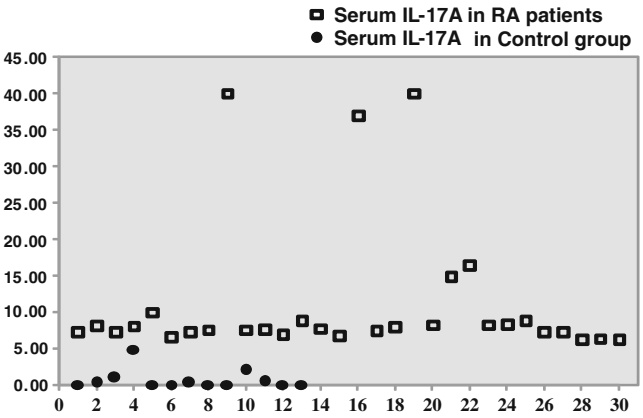

Fig. 1 Serum IL-17A levels in RA patients and controls

Ritchie articular index (r=0.308, 0.157 and p=0.098, 0.408, respectively).

According to the values of the DAS-28 scoring system, RA patients were divided into patients with low, moderate, and high disease activity. There was a significant difference in the mean serum IL-17A levels between the three groups (p=0.017), as shown in Table 5.

According to the assessment of the functional capacity, one RA patient (3.33%) was classified as class I, 21patients (70%) as class II, and 8 patients (26.67%) as class III. Group (II), class III showed significantly higher mean serum IL-17A levels (17.53±13.43 pg/mL) than group (I), classes I and II, (8.97±6.97 pg/mL, p=0.009). However, there was no significant difference between the two groups regarding the mean synovial IL-17A levels (8.17±1.64 vs. 9.05±1.12 pg/mL, respectively, p=0.659), as shown in Fig. 6.

Discussion

Rheumatoid arthritis is a chronic inflammatory autoimmune disease of unknown etiology. It causes persistent synovitis,

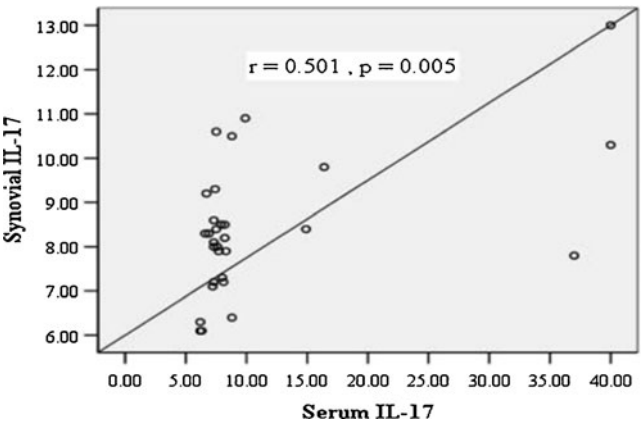

Fig. 2 Correlation between serum and synovial IL-17A levels in RA patients

Table 4 Correlations between serum and synovial IL-17A levels and RA patients' laboratory parameters

Laboratory parameter	Serum IL-17A level	Synovial IL-17 A level
ESR	r=0.122	0.117
	p=NS	NS
Platelet count	r=0.222	0.068
	p=NS	NS
Hb	r=−0.238	0.006
	p=NS	NS
WBC count	r=0.061	0.427
	p=NS	0.019
Serum albumin	r=0.156	0.409
	p=NS	0.025

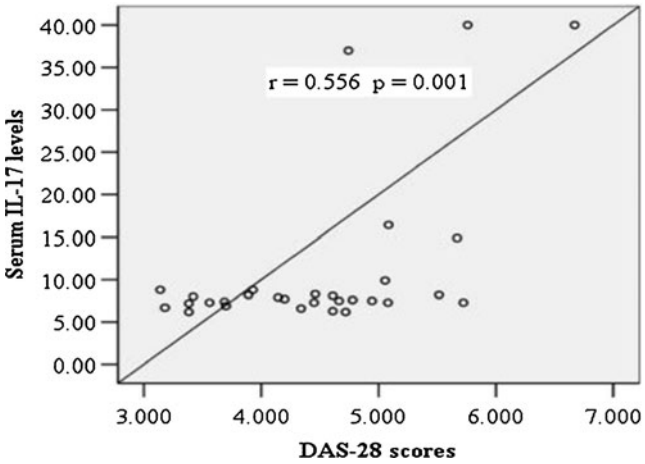

Fig. 4 Correlation between serum IL-17A levels and DAS-28 scores

pain, joint destruction, and functional disability. Irreversible joint destruction can be prevented by early diagnosis and treatment [1].

Cytokines play an important role in the pathogenesis of RA. Many cytokines are activated in the synovium by various cell populations including TNF-α and IL-1. They constitute the therapeutic targets of several compounds for RA and licensed over the past few years that directly inhibit these inflammatory mediators or interfere with their receptor binding, or both. Another pro-inflammatory cytokine, IL-6, which can be induced by both TNF-α and IL-1 has been implicated in the pathogenesis of RA [13].

IL-17A is a novel pro-inflammatory cytokine expressed in the synovium and synovial fluid of patients with RA, it is produced by activated $CD4^+$ T lymphocytes, and is a potent inducer of other cytokines such as TNF-α, IL-1-β, IL-6, IL-8, and G-CSF in a variety of epithelial, endothelial, and fibroblastic cell types [14].

IL-17 plays an important role in the pathogenesis of RA and was found to be important in both the early initiation phase and late progression phase; it induces collagen degradation and decreases collagen synthesis by synovium and cartilage and is also able to increase bone destruction and reduce its formation. Levels of IL-17 are elevated in the serum and the synovium of patients with RA, and synovial cultures from patients with RA spontaneously secrete IL-17 [15].

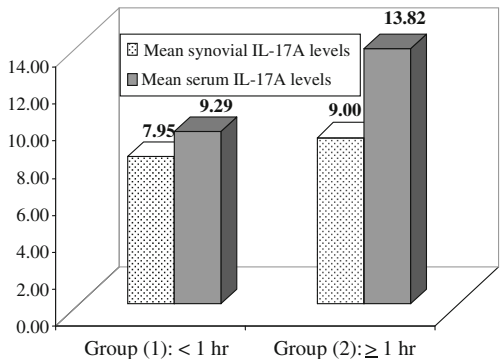

Fig. 3 Graph representing the comparison between the mean serum and synovial IL-17A levels in the two RA groups according to MS duration

The current study demonstrated significantly higher mean serum IL-17A levels in RA patients than controls. The data demonstrated by several authors in the literature were in agreement with our results. Melis et al. [16] reported a high serum IL-17 level in 22 RA patients. Furthermore, Mika et al. [17] showed that the mean level of IL-17 gene expression in peripheral blood mononuclear cells (PBMC) from 52 RA patients was significantly higher than 34 controls (0.044±0.111 vs. 0.013±0.003, respectively, p=0.011). Serum IL-17 level was elevated in 41 RA patients compared to 21 healthy controls [18].

In addition, Ziolkowska et al. [19] found a non-significantly higher mean serum IL-17 level in 15 RA patients compared to eight osteoarthritis (OA) patients (300 and 5 pg/mL, respectively). On the contrary, no significant

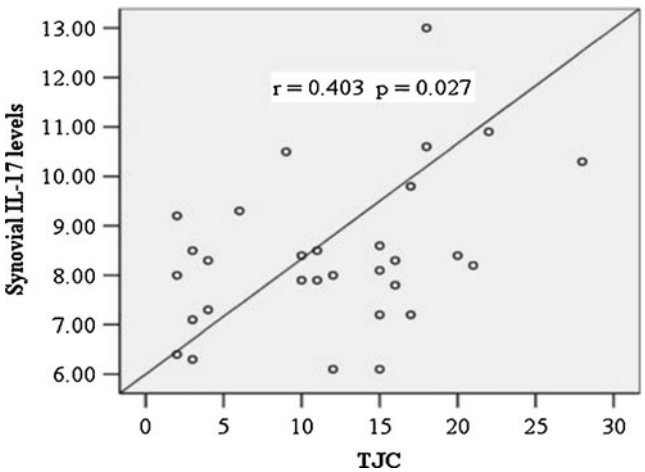

Fig. 5 Correlation between synovial IL-17A levels and TJC

Table 5 Comparisons between the mean serum and synovial IL-17A levels in RA patients' groups regarding DAS-28 scores

DAS-28 score No. of RA patients	Synovial IL-17A A levels (mean ± SD, pg/mL)	Serum IL-17A levels (mean ± SD, pg/mL)
Low (<3.2), n=2	7.8±1.98	7.75±1.48
Moderate (3.2–5.1), n=23	8.24±1.32	9.2±6.39
High (>5.1), n=5	9.42±2.29	22.08±16.62
p value	NS	0.017

difference was found in the frequency of peripheral blood TH-17 cells between RA patients and controls [20, 21].

In the present work, synovial IL-17A level in RA patients ranged from 6.4 to 13 pg/mL, with a mean of 8.41±1.55 pg/mL. A high synovial IL-17 level was reported in 22 RA patients [17]. Moreover, higher mean synovial IL-7 levels were observed in RA patients compared to OA patients [22, 23].

A significant difference was found in the mean IL-17 level between ten RA patients, nine OA patients, and six controls [20]. In addition, a high synovial IL-17 level in ten RA patients in comparison to undetectable synovial IL-17 level in five OA patients was demonstrated by de Jager et al. [24]

In support of our data, Jovanovic et al. [25] found high detectable amounts of IL-17 in synovial fluid of RA patients; however, neither synovial fluid of OA patients nor controls (patients with peri-articular traumatic injuries of the knee joint with no evidence of intra-articular abnormality) contained measurable amounts of IL-17.

No significant correlations were found between serum and synovial IL-17A levels of our patients and their disease durations. The disease duration of our RA patients ranged from 3 to 20 years with a mean of 9.5±4.16 years. In contrast to our results, Raza et al. [22] reported significant negative correlations between synovial IL-17 levels and disease duration of RA patients (r=0.007, p=0.002). This

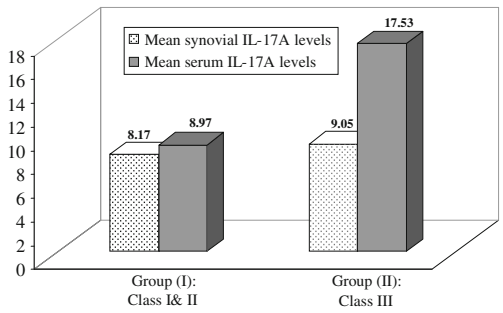

Fig. 6 Graph showing the comparison between the mean serum and synovial IL-17A levels in RA patients regarding the functional status

discrepancy can be justified as their study included patients with early active RA (<12 months) before receiving any treatment.

The present study revealed no significant difference in serum and synovial IL-17A levels in relation to the presence of RF, in accordance with the findings of Raza and colleagues. They also observed a non-significant correlation between the RF titer and synovial IL-17 level in RA patients [22].

In the present study, serum and synovial IL-17A levels of RA patients had non-significant correlations with ESR. In agreement, Hitchon et al. [18] found that the serum IL-17 level in RA patients did not correlate significantly with ESR. Furthermore, a non-significant correlation between the level of IL-17 gene expression in PBMC of RA patients and ESR was reported [17].

Only, synovial IL-17A levels showed a significant positive correlation with the total lymphocyte counts (TLCs) of our RA patients. In accordance, a non-significant correlation was demonstrated between the level of IL-17 gene expression in PBMC of RA patients and TLCs [17].

The current work revealed an increase in both serum and synovial IL-17A levels with higher DAS-28 scores and TJC and increased serum IL-17A levels with higher SJC. Patients with class III functional status showed significantly higher mean serum IL-17A levels than classes I and II. Similarly, Melis et al. [16] reported elevated serum and synovial IL-17 levels in 22 RA patients that correlated significantly with local and systemic disease activity parameters.

On the contrary, Yamada et al. [21] reported non-significant correlations between serum and synovial IL-17 levels of 69 RA patients and DAS-28 scores, TJCs, and SJCs. Non-significant correlations were revealed between serum IL-17 levels of 41 RA patients and SJC and functional status assessed by modified HAQ [18].

Non-significant correlations were observed between both serum and synovial IL-17A levels of our RA patients and GH based on VAS. In agreement, Hitchon et al. [18] observed non-significant correlation between the serum IL-17 levels of their RA patients and GH based on VAS.

In the present work, synovial IL-17A levels were significantly increased in accordance with serum IL-17A levels. On the other hand, Ziolkowska et al. [19] reported a non-significant correlation between synovial and serum IL-17 levels in 15 RA patients. This difference can be explained as the latter study included many patients with early active RA (with disease duration of 4 months).

We can conclude that the elevated serum and synovial IL-17A levels in RA patients parallel the degree of disease activity and severity. This may highlight the usefulness of IL-17 (especially serum level) as a possible marker for more aggressive joint involvement and damage. The main

limitation of this study is the small number of included patients, so we recommend conducting studies on a larger scale of RA patients, especially those with early onset, in order to consider utilizing IL-17 as a useful marker for the early identification of RA patients who are liable to more aggressive disease course to provide earlier intervention.

Disclosures None.

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