Role of interleukin-35 in rheumatoid arthritis pathogenesis and its relation to disease activity and joint damage

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Aim
This study aimed to discuss the role of interleukin-35 (IL-35) in the pathogenesis of rheumatoid arthritis (RA) and its relation to disease activity and radiological severity.
Patients and methods
Thirty patients diagnosed with RA were selected from the outpatient clinic and inpatient unit of Physical Medicine, Rheumatology and Rehabilitation Department, Tanta University Hospitals fulfilling the American College of Rheumatology/European League Against Rheumatism 2010 criteria for the diagnosis of RA, and 20 apparently healthy individuals who were matched in age and sex participated as controls. Patients with other autoimmune diseases, malignancy, or any current infections were excluded. Disease activity score in 28 joints was assessed for all patients. Rheumatoid factor, anticyclic citrullinated peptide, complete blood count, erythrocyte sedimentation rate and C-reactive protein and serum level of IL-35 measured by enzyme-linked immunosorbent assay were evaluated. The degree of joint destruction was assessed by Larsen score.
Results
Of the RA patients, 73.3%showed low serum levels of IL-35 with significant difference compared with controls, and its levels showed negative association with disease activity. IL-35 serum levels were significantly correlated with hemoglobin level, erythrocyte sedimentation rate, C-reactive protein, and rheumatoid factor and not correlated with anticyclic citrullinated peptide antibodies. Also IL-35 serum levels significantly correlated with radiological disease severity were assessed by Larsen score.
Conclusion
IL-35 had an immunoregulatory role in RA pathogenesis as its serum level is significantly low in RA patients and correlated with different parameters of disease activity and radiological severity.

Keywords:
disease activity and severity, interleukin-35, pathogenesis, rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that results in inflammatory cell infiltration of the synovium, with synovial hyperplasia, cartilage and bone erosion, and angiogenesis [1].

RA is a typical T-cell-mediated disease. The environment of the cytokines released from T-cells or other cells that infiltrate the joint may have an important role in the pathogenesis of RA and may have a strong effect on the outcome of the initial events that trigger autoimmune inflammation [2].

Cytokines are classified predominantly into proinflammatory and anti-inflammatory. These two types together enhance a cytokine environment that may change immune cell function and other effector responses in arthritis, thoroughly working in either a pathogenic, proinflammatory, or a protective, anti-inflammatory way [3].

Imbalance between proinflammatory and post-inflammatory cytokine activities favors induction of autoimmunity, chronic inflammation, and thereby joint damage; it is less clear how cytokines are organized within a regulatory network [4].

Several mediators and factors were reported to play a role in Egyptian RA. T-regulatory cells (Treg) and cytokines have also been implicated in pathogenic

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mechanisms of RA [5]. An imbalance between effector T-cell and Treg activities is known to contribute to RA pathogenesis [6].

Interleukin-35 (IL-35) is a cytokine related to the IL-12 family. IL-35 is a heterodimeric protein that consists of two subunits named IL-12 p35 and Epstein–Barr virus-induced gene 3 (EBI3). Among the different types of CD4+ T-cells, p35 and EBI3 are expressed mainly in the forkhead box protein 3 and Treg, but not in the effector CD4+T-cells, while only Treg cells constitutively release IL-35 protein as a p35/EBI3 dimer [7].

Treg cells play a major role in autoimmune control and protection from inflammation, so IL-35 may participate in the inflammatory process of RA pathogenesis [8].

The aim of this study was to discuss the role of IL-35 in RA and its relation to disease activity and severity.

Patients and methods

This study was carried out on 30 patients with RA (group 1) selected from the Outpatient Clinic of Physical Medicine and Rheumatology Department, Tanta University Hospitals. The patients fulfilled the American College of Rheumatology/European League Against Rheumatism 2010 criteria for the diagnosis of RA [9] and the control group (group 2) consisted of 20 apparently healthy individuals matched in age and sex. Patients with other autoimmune diseases, malignancy, or any current infections were excluded. The study was approved by the Local Research Ethical Committee of Tanta University Hospitals with a written consent from all participants in the study. All the patients were subjected to full history taking and thorough musculoskeletalexamination. Disease activity was assessed using disease activity score in 28 joints (DAS-28) [10] calculated using the formula:

$$\mathrm{DAS}\,28 = 0.56 \times \sqrt{\mathrm{TEN}\,28} + 0.28 \times \sqrt{\mathrm{SW}\,28} + 0.70 \times \log \mathrm{ESR} + 0.014 \times \mathrm{GH},$$

where TEN 28 is tender joint counts; SW 28 is swollen joint counts; Ln ESR is natural log of ESR, and GH=global health assessment using visual analog scale [7]. The final score is calculated and graded as follows:

(1) Low activity=DAS-28 scoring less than 3.2.
(2) Moderate activity=DAS-28 scoring 3.2–5.1.
(3) High activity=DAS-28 scoring more than 5.1.
(4) Remission=DAS-28 less than 2.6.

Laboratory investigations

(1) Routine laboratory investigation included: complete blood count, erythrocyte sedimentation rate (ESR) first hour by the Westergren method, C-reactive protein (CRP) by latex agglutination slide test, rheumatoid factor (RF) by latex agglutination slide test, and anti-cyclic citrullinated peptide (anti-CCP) antibodies by enzyme-linked immunosorbent assay (ELISA).
(2) Specific investigation:

Serum IL-35: by ELISA [11].

Serum interleukin-35 determination

Serum IL-35 was determined by human IL-35 ELISA kit manufactured by Cusabio Biotech Co. Ltd (Wuhan Huamei Biotech Co., Ltd, UK) (catalog number CSB-E13126h).

Calculation of the results

A standard curve was created by plotting the mean absorbance for each standard on the *y*-axis (vertical) against the concentration on the *x*-axis (horizontal) using a linear graph paper and the best fit curve was drawn through the points on the graph. Sample concentration was read directly from this curve (Fig. 1).

Radiological assessment

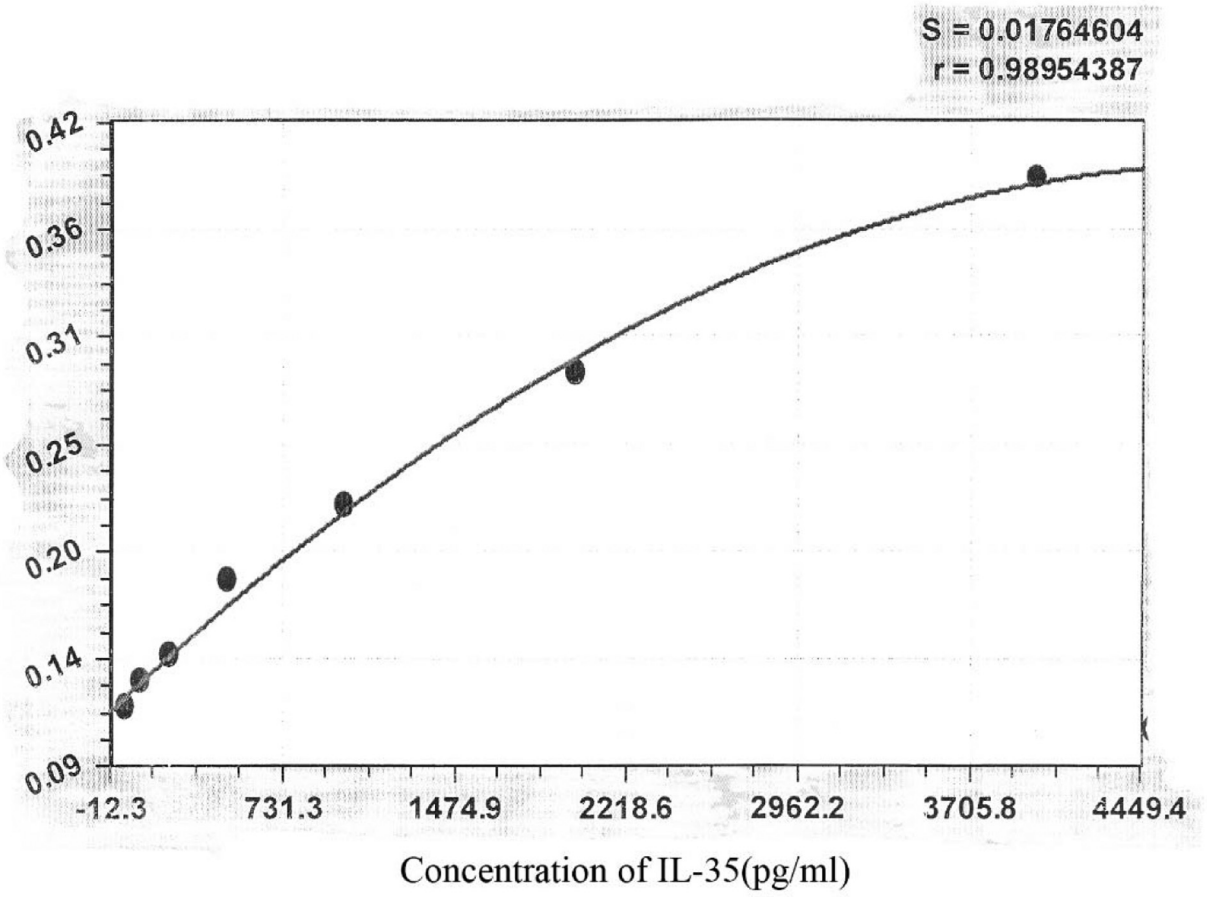
By Larsen score [12]

Plain radiograph of both hands and feet were done, the joints considered are proximal interphalangeal joints 2–5, metacarpophalangeal joints 2–5 in each hand, four quadrants in the wrist, and metatarsophalangeal 2–5 in each foot.

Intact bony outlines and normal joint space were scored 0, erosion less than 1 mm in diameter or joint space narrowing was scored 1, one or several small erosions, diameter more than 1 mm was scored 2, marked erosions was scored 3, while severe erosions, where there is usually no joint space left, and the original bony outlines that are partly preserved had the score of 4 and mutilating changes, where the original bony outlines have been destroyed had a score of 5.

Statistical analyses were applied by SPSS version 20 (IBM, Armonk, NY, United States of America) [13] using the mean value, SD, and standard error. Analytic statistics was done using Student's *t*-test,

Figure 1



Calculation of the results. IL, interleukin.

Table 1 Comparison between patients and control groups as regards serum interleukin-35 level

IL-35 cutoff value (62.5 pg/ml)	Groups [*N* (%)]			χ^2	*P* value
	Patients (*n*=30)	Control (*n*=20)	Total (*n*=50)		
Low level	22 (73.3)	0 (0.00)	22 (44.00)	26.190	<0.001*
High level	8 (26.7)	20 (100.00)	28 (56.00)		
Total	30 (100.00)	20 (100.00)	50 (100.00)		

*significant.

Mann–Whitney test, χ^2-test, sensitivity, and specificity test.

The cutoff value for IL-35 was calculated using the mean -2 SD method.

Power of significance ($P \geq 0.05$, nonsignificant; $P<0.05$, significant; $P<0.01$, highly significant).

Results

In RA patients group, 22 (73.3%) patients had low serum IL-35 levels (<62.5 pg/ml), while eight (26.7%) patients had high levels (>62.5 pg/ml), whereas, serum IL-35 levels were high in all control group with high statistically significant difference between two groups ($P<0.001$; Table 1).

In patients with clinical remission (*n*=3), serum IL-35 levels were high in all patients while serum IL-35 levels were high in 66.6% of patients with low disease activity; 82.3% of patients with moderate disease activity and all patients with high disease activity showed low serum IL-35 levels with statistically significant decrease of serum IL-35 levels with increased grading of DAS-28 (P=0.014; Table 2).

(1) Hemoglobin level showed a mean value of 9.4 ±0.9 in patients with low IL-35 level and 10.7 ±1.2 in patients with a high level, there was a

Table 2 Comparison of serum interleukin-35 levels and grading of disease activity score in 28 joints in rheumatoid arthritis patients

Disease activity score in 28 joints	Interleukin-35 [*N* (%)]			χ^2	*P* value
	Low level	High level	Total		
Clinical remission (>2.6)	0 (0.0)	3 (100.0)	3 (100)	10.548	0.014*
Low disease activity (2.6 to >3.2)	4 (66.6)	4 (66.6)	6 (100)		
Moderate disease activity (3.2–5.1)	14 (82.3)	3 (17.6)	17 (100)		
High disease activity (<5.1)	4 (100.)	0 (0.0)	4 (100)		
Total	22 (73.3)	8 (26.6)	30 (100)		

*significant.

Table 3 Comparison of serum interleukin-35 levels with laboratory and radiological data

	Interleukin-35		*t*	*P* value
	Low level	High level		
Hb (g/dl)				
Range	8–11.2	8.8–12.3	–2.985	0.006*
Mean±SD	9.4±0.9	10.7±1.2		
ESR first hour (mm/h)				
Range	35–110	18–53	2.177	0.038*
Mean±SD	49.9±15.5	36.8±10.6		
CRP (mg/dl)				
Range	16–48	0–24	2.841	0.009*
Mean±SD	29.17±12.94	14.2±7.2		
RF (IU/ml)				
Range	24–256	8–48	2.637	0.014*
Mean±SD	78.1±42.1	27.2±14.5		
Anti-CCP (IU/ml)				
Range	10–194	60–270	–1.209	0.237
Mean±SD	91.3±59.9	122.0±65.2		
Larsen score				
Range	3–5	0–3	–10.195	<0.001*
Mean±SD	4.05±0.65	1.63±0.34		

Anti-CCP, anticyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; RF, rheumatoid factor. *significant.

statistically significant decrease of serum IL-35 levels with decreasing hemoglobin level (*P*=0.006).

(2) ESR first hour showed a mean value of 49.9±15.5 in patients with low IL-35 level and 36.8±10.6 in patients with high level with a statistically significant decrease of IL-35 with increased ESR values (*P*=0.038).

(3) The CRP level showed a mean value of 29.17 ±12.94 in patients with low IL-35 level and 14.2 ±7.2 in patients with high level with statistically significant decrease of IL-35 with increased CRP values (*P*=0.009).

(4) The RF level showed a mean value of 78.1±42.1 in patients with low IL-35 level and 27.2±14.5 in patients with high level with a statistically significant decrease of IL-35 with increased RF level (*P*=0.014).

(5) Anti-CCP level had a mean of 91.3±59.9 with low serum IL-35 levels while its mean was 122.0±65.2 with a high level and no significant association between serum IL-35 and anti-CCP level (*P*=0.237).

(6) Radiological scoring of severity measured by Larsen score had a mean of 4.05±0.65 in patients with low IL-35 level, while its mean value in patients with high level was 1.63±0.34 with significant increase of IL-35 with decreased Larsen scores (Table 3).

(7) Specificity of serum IL-35 was 100% while its sensitivity was 73.3% in RA patients (Table 4).

Table 4 Sensitivity and specificity testing for serum interleukin-35

Sensitivity	Specificity	PPV	NPV	Accuracy
73.3	100.0	100.0	71.40	82.21

NPV, negative predictive value; PPV, positive predictive value.

Discussion

RA is a common autoimmune disease characterized by synovial inflammation and hyperplasia, autoantibody production (RF and anti-citrullinated protein antibody), cartilage, and bone destruction (deformity), and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders [14].

RA results from complex interactions of many mechanisms, most probably activation of T-cells which is mainly an antigen-dependent process. T-cells activation subsequently leads to multiple effects including activation and proliferation of synovial lining, recruitment, and activation of proinflammatory cells, secretion of cytokines and proteases, and autoantibodies production [15].

IL-35 is a member of IL-12 cytokine family [16]. It suppresses inflammatory responses of immune cells, and it has been reported that it induces proliferation of Treg cell, and reduces the activity of the Th17 cell populations [17].

In RA, it acts as a negative regulator of inflammation. IL-35, markedly reduces the incidence of clinical

symptoms of arthritis, and attenuates the severity of synovial hyperplasia and bony destruction [18].

Thirty RA patients and 20 apparently healthy volunteers were included in the study; serum levels of IL-35 were determined and the possible associations between IL-35 and disease activity and severity indicators for RA were analyzed.

The results showed that the serum IL-35 level was decreased significantly in the sera of RA patients than in the sera of healthy controls (Table 1), which is in accordance with Nakano *et al.* [19] and Ning *et al.* [20].

In the present study, serum IL-35 was significantly low with increased DAS-28 grading (Table 2), which is in accordance with Nakano *et al.* [14] and in contrast to Ning *et al.* [20] who found no significant correlation between them.

In accordance with Ning *et al.* [20], IL-35 was decreased with certain disease indicators such as increased ESR, CRP, and RF, indicating its participation in the inflammatory processes associated with RA development (Table 3).

On the same context, the present study revealed that radiological severity assessed by Larsen score for bone erosion and joint space narrowing were high in patients with low serum IL-35 levels drawing the attention for its impact on disease progression and radiological joint damage. However, more studies with a larger number of participants are needed in that field.

Nakano *et al.* [19] reported that treatment with IL-35 enhanced the regulatory function, suppressing the levels of inflammatory cytokines such as IL-17 and interferon-γ and the cellular growth of effector T-cells stimulated by conjugation with CD2, CD3, and CD28, suggesting that IL-35 might have multiple therapeutic targets in RA.

Wu *et al.* [21] reported that inhibition of the expression of mediators of angiogenesis and inflammation in fibroblast-like synoviocytes mediated by IL-35 provide a likely mechanism for antiangiogenetic effects seen in experimental models of RA. These data suggested that IL-35 may represent a therapeutic target for the treatment of RA and other angiogenesis-related diseases. Jiang *et al.* [22] also reported that IL-35 restrains RA angiogenesis and inflammation by downregulating basal and vascular endothelial growth factor and this supports the development of novel angiogenesis-targeting therapeutics for RA treatment.

Li *et al.* [23] reported that treatment with IL-35 inhibited the development of arthritis in CIA mice, accompanied by a decrease in the expression of IL-17 and receptor activator of the nuclear factor KB ligand and an increase in the expression of osteoprotegerin. Also IL-35 dose dependently inhibited the expression of receptor activator of the nuclear factor KB ligand and increase the expression of OPG in cultured fibroblast-like synoviocytes cells. In a further study by Li *et al.* [24] they found that treatment with IL-35 significantly alleviated arthritis symptoms and reduced synovial tissue inflammation.

Conclusion

The serum level of IL-35 may serve as a protective factor in RA pathogenesis and as a novel target for therapeutic interventions.

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Conflicts of interest

There are no conflicts of interest.

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