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ORIGINAL ARTICLE

Serum Interleukin-17 Level in Patients with Rheumatoid Arthritis and its Relation to Disease Activity

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a disease of progressive inflammatory autoimmune nature with articular and systemic effects. Its definite cause is unknown, but environmental and genetic factors are contributory. The aim of this study was to estimate the serum level of IL-17 in cases with RA and investigate its relation with disease activity among Egyptian population. **Methods:** This study included 20 RA cases and 20 healthy control subjects. All cases were subjected to full history taking, thorough clinical examination, laboratory investigations including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-cyclic-citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF). Enzyme linked immunosorbent assay was used to measure serum IL-17 level. Disease activity score-28 (DAS-28) was assessed. **Results**: The mean disease duration of the cases was 5.95±2.52 years (2–10years). DAS-28 ranged between 2.4 and 6.1 with a mean of 3.87±1.32. Serum IL-17 level was higher in the RA group (226.6±215.6 pg/ml) than the control group (48.17±54.9pg/ml) with p-value of <0.001. Significant correlations were found between IL-17 levels and the DAS-28, CRP, and hemoglobin. For detection of disease activity, IL-17 at a cut off value of > 98.2 pg/ml had sensitivity and specificity of 81.2% and 75%, respectively. **Conclusions:** Serum IL-17 level was significantly increased in cases with RA compared to healthy controls. Its significant correlation with DAS-28 suggested that serum IL-17 level could be an important marker of disease activity in RA.

Keywords: Serum Interleukin-17, Rheumatoid Arthritis, Disease Activity

INTRODUCTION

In the pathophysiology of rheumatoid arthritis, it is known that an imbalance between pro-inflammatory, anti-inflammatory cytokine activities favors the induction of autoimmunity and chronic inflammation leading to joint damage (1). Many cytokines are expressed, functionally active in synovial tissues. TNF-α, Interleukin-1β, IL-6, IL-10, IL-12,IL-17 or IL-23 may serve as useful prognostic factors of RA (2). Interleukin17 (IL-17) was cloned in 1993, previously called cytotoxic T-lymphocyte-associated protein 8 (CTLA-8). In 1995, it was renamed as IL-17, its receptor was cloned and it was identified as a cytokine expressed by T cells that exerts

effects on epithelial, endothelial, and fibroblast cells (3).

DAS-28 is a statistically derived index consisting of number of tender joints, number of swollen joints, ESR and global disease activity (4).

Park et al. (5) found that IL-17A concentrations in serum and synovial fluid in cases with RA are higher than with osteoarthritis (OA) or in healthy subjects. Also, Dhaouadi et al. (6) reported a significant correlation between IL-17 plasma concentration and the RA activity using disease activity score 28 (DAS-28).

The aim of this study was to estimate the IL-17 serum level in rheumatoid arthritis cases and its relation to disease activity in Egyptian population.

METHODS

After review, approval by the Institutional Review Board (IRB) committee at Faculty of Medicine, Zagazig University, this case-control study was carried out over 13 months from April 2017 to May 2018 at Medical Microbiology & Immunology Department, Rheumatology & Rehabilitation Department, Faculty of Medicine, Zagazig University. This study included 40 subjects. They were classified into 2 groups:

Cases group: This group included 20 rheumatoid arthritis cases (15 females, 5males). Their ages ranged from 20-55years with a mean of 37.1±9.33 years, their disease duration ranged from 2 to 10years with a mean of 5.95±2.52years. They were diagnosed according to the 2010 American College of Rheumatology (ACR), European League against Rheumatism (EULAR) classification criteria for rheumatoid arthritis [(7)]. Cases suffered from liver diseases, coronary artery disease, kidney disease, other inflammatory conditions were excluded from the study. Rheumatoid arthritis disease activity was measured by disease activity score-28 (DAS-28)[(4)]. Control group: This group included 20 apparently healthy, age and sex-matched subjects serving as controls. They were 15 females, 5males whose ages ranged between 24-55years with a mean value of 42.3±8.14years.

- All cases were subjected to full history taking, thorough clinical examination, laboratory investigations including complete blood count (8), erythrocyte sedimentation rate (ESR) by using Westergren method recorded mm/hr. The reading of first hour is taken (9), C-reactive protein (CRP) by latex agglutination test (10), rheumatoid factor (RF) (QUANTA ELISA Lite TM RF IgM) (11), anti-cyclic citrullinated peptide (anti-CCP) antibodies)(12), using the immunoscan CCP test kit which is an enzyme-linked immunosorbent assay (ELISA).

Serum interleukin-17 level was measured for RA cases and control subjects using ELISA kit according to the instruction of the manufacturer **(**Human Interleukin17(IL-17) ELISA Kit, Lot no. 201708, Bioneovan Co., Ltd, Beijing, China). Serum samples were collected from cases on the same day of examination.

A written consent was taken from all subjects for ethical consideration. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving human.

Statistical Analysis:

All data were collected, tabulated and analyzed using SPSS version 19. The significance level was set at $P < 0.05$.

RESULTS

This case-control study was carried out on 40 individuals; they were divided into two groups; case (RA) group included 20 RA cases, control group included 20 apparently healthy individuals. Table (1) showed that in the patient group the ESR levels ranged between 17-90 with a mean of 40.80±25.14 mm/h, CRP ranged between 3.2-37.5 with a mean of 13.45±9.80 mg/L. Hemoglobin level ranged between 9.2-12.8 with a mean of 11.1±1.16g/dl. As regards rheumatoid factor, it ranged between 5-288 with a mean of 79.08±97.28µ/ml. Anti-CCP antibodies ranged between 7-492 with a mean of 136.4±139.7µ/ml. Lastly, DAS-28 ranged between 2.4–6.1 with a mean of 3.87±1.32.

Table (2) showed that there was a significant difference between the studied groups regarding the level of IL-17. IL-17 level was significantly higher in the RA group (226.6±215.6) than the control group (48.17±54.9) with p value <0.001. Table (3) showed that there was significant positive correlation between IL-17 level and CRP. Also, there was critical negative correlation between IL-17 and hemoglobin. Also, there was highly critical positive correlation between IL-17 level and DAS-28. Table (4), figure (1) showed that the IL-17 at a cut off value of > 98.2 (pg/ml) had a sensitivity of 81.2%, a specificity of 75% for detection of disease activity. Table (5) showed that there was significant difference between the studied RA subgroups as regards IL-17 and CRP levels which were of their highest levels among those who had severe disease when compared to the others. However, the difference was non-significant between the subgroups as regards RF, anti-CCP, ESR and hemoglobin levels.

Table 1. Laboratory and clinical characteristics of patients with RA

Variable	RA group
ESR (< 10 mm/1st h): Range Mean ± SD	 17 – 90 40.80 ± 25.14
CRP (< 5 mg/L): Range Mean ± SD	 3.2 – 37.5 13.45± 9.80
Hemoglobin (12-15.5 gm/dl): Range Mean ± SD	 9.2 – 12.8 11.1 ± 1.16
RF (< 14 IU/ml): Range Mean ± SD	 5 - 288 79.08 ± 97.28
Anti-CCP CCP (< 20 U/ml): Range Mean ± SD	 7 - 492 136.4 ± 139.7
DAS 28: Range Mean ± SD	 2.4 – 6.1 3.87 ± 1.32

Table 2. Comparison of IL-17 among the studied groups

IL-17(pg/ml)	RA group (N=20)	Control group (N=20)	Mann-whitney test	P value
Mean ± SD Median Range	226.6 ± 215.6 146.6 25.2 - 765	48.17 ± 54.9 30.60 8.3 - 216	60.00	<0.001 (HS)

Table 3. Correlation between IL-17 and different laboratory parameters and DAS28 among RA patients

Variable	IL-17	
	r	p
ESR	0.249	0.208
CRP	0.883	<0.001**
Hemoglobin	-0.494	0.02*
RF	-0.038	0.872
Anti-CCP	0.097	0.685
DAS 28	0.904	<0.001**

Table 4. Validity of IL-17 in evaluation of disease activity

Variable	Cutoff	AUC	CI	Sens.	Spec.	+PV	-PV	Accu.	p-value
DAS28	> 98.2	0.844	0.660– 1.000	81.2%	75.0%	92.8%	50.0%	80.0%	0.028*

AUC: Area under curve; CI: Confidence interval; Sens.: Sensitivity; Spec.: Specificity
+PV: Positive predictive value; -PV: Negative predictive value; Accu: Accuracy.

Table (5): Comparison of laboratory characteristics among RA patients regarding their disease activity

Variable	Remission subgroup (n=4)	Mild activity subgroup (n=5)	Moderate activity subgroup (n=7)	Severe disease subgroup (n=4)	test	p- value
IL-17 Mean ± SD Median (Range)	 33.5 ± 6.76 33.7 25.2-41.5	 87.3 ± 41.5 77.4 51.9-159	 241.9±136 189 101-489	 567 ± 133 517 469-765	16.37#	0.001 (S)
RF Mean ± SD Median (Range)	 78 ± 110.2 32.5 7 - 240	 91.2 ± 117 10 5 - 256	 85.8±113.8 32 6 - 288	 53.1± 48.5 52.8 11 - 96	0.285#	0.963 (NS)
Anti-CCP Mean ± SD Median (Range)	 115 ± 171 42.3 8 - 368	 95.6 ± 135 47.2 7 - 330	 154.8±92.6 187 8 - 277	 176 ± 215 103.5 7 - 492	2.268#	0.519 (NS)
ESR Mean ± SD Median (Range)	 23 ± 31.3 53.5 187 - 88	 26.8 ± 8.16 22 20 - 38	 40.5± 27.9 29 18 - 90	 46.5±29.8 39 19 - 89	1.800#	0.615 (NS)
CRP Mean ± SD Median (Range)	 6.92 ± 4.20 5.35 4 - 13	 9.60 ± 7.25 7.7 3.2 - 21	 11.9± 8.08 10.3 3.3 - 24	 27.3±6.75 24 24 – 37.5	8.768#	0.03 (S)
Hb Mean ± SD Range	 11.4 ± 0.79 10.4 – 12.2	 11.5 ± 0.87 10.3 – 12.7	 10.8 ± 1.53 9.2 – 12.8	 10.6 ± 1.13 9.2 – 11.8	0.646*	0.597 (NS)

*: One way ANOVA test. #: Kruskall-walis test.

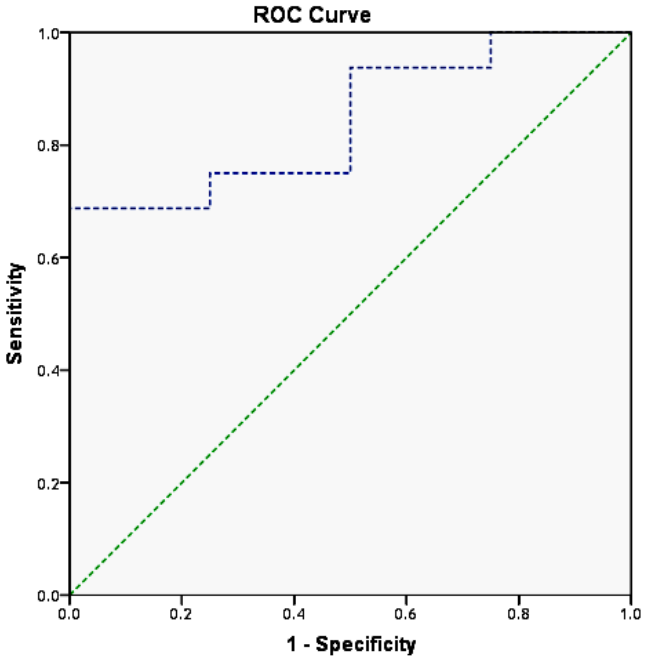

Figure 1. ROC curve for Validity of IL-17 in evaluation of disease activity.

DISCUSSION

The interleukin17 (IL-17) family is an important subgroup of cytokines. There are five receptors (IL-17 RA, IL-17RB/IL-25R, IL-17RC, IL-17RD, IL-17RE) and six ligands [IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), IL-17F] belonging to the IL-17 family (13).

In this study, the duration of the disease ranged between 2-10years with a mean of 5.95±2.52years. The ESR levels ranged between 17- 90 with a mean of 40.80±25.14mm/h, CRP ranged between 3.2-37.5 with a mean of 13.45±9.80 mg/L. Regarding rheumatoid factor, it ranged between 5-288 with a mean of 79.08±97.28 IU/ml, hemoglobin level ranged between 9.2-12.8 with a mean of 11.1±1.16 gm/dl. Anti-CCP ranged between 7-492 with a mean of 136.4±139.7 U/ml, lastly DAS28 mean was found to be 3.87±1.32.

These results were matched with the study of Metawi et al. (14) who found that among 30 studied RA cases, ESR (mm/1st hour) ranged between 25–120, with a mean of 73.83±25.39, Hb(gm/dL) ranged between 8.9–13.7 with a mean of 11.03±1.23 and DAS-28 mean was found to be 4.8±0.87. In the study of Kim et al.(15), the mean of tender joint (7.9±6.6), swollen joint (4.6±6.3), Visual analoge scale (VAS) (34.5±1.2) and DAS-28 (4.64±1.84).

The present study clarified that there was a significant difference between the studied groups regarding the level of IL-17. IL-17 level was significantly higher in the RA group (226.6±215.6 pg/ml) than the control group (48.17±54.9pg/ml). This was in agreement with Dhaouadi et al. (6) who reported that plasma IL-17A levels were significantly higher in Tunisian RA cases (55.07 pg/ml) than in healthy subjects (4.75pg/ml). Also, our findings coincide with those of Metawi et al. (14) who reported critically higher serum IL-17A levels in 30 RA cases (11.25±9.67) than 13 healthy controls(0.6±1.4pg/mL) and Melis et al. (16) who demonstrated that there were significant differences in the IL-17 serum levels of 22 RA cases and controls .

The meta-analysis of Lee and Bae (17) confirmed this association between RA and raised levels of circulating IL-17. This study

showed that there was highly significant positive correlation between IL-17level and CRP, significant negative correlation between IL-17 and hemoglobin (anemia of chronic diseases).

Anemia in rheumatic diseases most commonly reflects decreased production of red blood cells in the bone marrow caused by continued inflammation, with increased hepcidin production leading to disturbed iron metabolism. Anemia of chronic disease is commonly normocytic, normochromic; however, microcytic hypochromic anemia also can be associated with chronic disease.(18)

These results were matched with the study of Al-Saadany et al. (19) who documented that there was significant positive correlation between serum levels of IL-17, ESR, CRP and TNF-α. The results of this study were in agreement with those of Al-Saadany et al. (19) who reported that serum levels of IL-17 were significantly correlated with disease activity using DAS-28. DAS-28 is a statistically derived index consisting of number of tender joints, number of swollen joints, ESR and global disease activity (4). They demonstrated an important role for serum IL-17 in the pathogenesis of the destructive and inflammatory pattern characteristic of RA.

In this study, as regards to disease activity, 5 RA cases (25%) had mild disease activity, 7 RA cases (35%) had moderate disease activity,4 R.A. cases (20%) had severe disease activity, the remaining 4cases (20%) showed complete remission. There was critical difference between the studied subgroups regarding IL-17 levels which were of its highest levels among those who had severe disease when compared to the others. Also, the difference between the subgroups was significant as regards CRP which was also of its highest levels among those who had severe disease when compared to the others. However, the difference was non-significant between the subgroups as regards RF, anti-CCP, ESR, hemoglobin levels. Pavlovic et al. (20) also had similar finding, they reported that the mean serum IL-17A levels in cases with RA. corresponded significantly with disease activity and severity. This might demonstrate the usefulness of the IL-17A serum level in defining the activity.

CONCLUSION

The level of serum IL-17 was significantly increased in RA cases compared with healthy controls. Its significant correlation with DAS-28 suggested that IL-17 level could be an important marker of disease activity in RA.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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