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Serum IL17 and IL6 levels in a Sample of Iraqi Patients with Rheumatoid Arthritis: A Case Control Study

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Abstract
Objectives: To determine serum IL17 and IL6 levels in a sample of Iraqi patients with rheumatoid arthritis (RA) and to evaluate the correlation between them if present.
Methods: A case control single center study was conducted for 11 months. A total of 50 patients with RA diagnosed according to the 1987 American College of Rheumatology (ACR) and 30 individuals matched in age and sex as control group were included. Serum IL17 and IL6 levels were measured in both groups by enzyme-linked immunosorbent assay (ELISA). The cut off value was assessed by receiver operating characteristics (ROC) test and correlation by spearman's Rho linear correlation coefficient.
Results: Frequency of females was more than males in patients and controls (88% and 76.7% respectively).Ages of patients range between 20-70 years with a median 43.76 years while in controls, ages range between 20-60 years with a median 37.67 years.Serum IL17 concentration was significantly higher in patients compared to controls (median 154.4 pg/ml versus 111.1 pg/ml respectively, p=0.02). Also serum IL6 concentration was higher and highly significant in patients compared to controls (median 150 versus 49 pg/ml respectively, p=0.001). There was a direct strong highly statistical significant correlation between serum IL17 and serum IL6 concentration (r=0.771, p<0.001).The optimum cut off value for IL17 was ≥ 71.8 pg/ml and IL6 concentration≥8.1pg/ml.
Conclusions: serum IL17 and IL6 concentrations were significantly higher in Iraqi sample of RA patients compared to controls with direct strong highly significant correlation between these cytokines. This may help in diagnosis of RA and suggest potentially an effective treatment.
Key words: Rheumatoid arthritis, IL17, IL6, rheumatoid arthritis and cytokines.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by infiltration of macrophages and T cells into the joints, synovial hyperplasia, cartilage degradation and bone erosions; and mediated by the production of several cytokines [Christodoulou,2006].T helper-type17 (Th17) cells, a novel and distinct subset of Th cell, can secrete interleukin (IL)-17 in humans [Bettelli et al, 2007; Harrington et al , 2005].

Interleukin-17 is a pleiotropic cytokine that participates in tissue inflammation and destruction by inducing the expression of proinflammatory cytokines and matrix metalloproteases [Agarwal et al, 2008].An enhanced expression of IL-17 has been observed in the rheumatoid synovium and synovial fluids of patients with early RA [Raza et al, 2005]. Moreover, IL-17 has become a new therapeutic target for animal models with collagen-induced arthritis and human RA [Hueber et al,2010; Genovese et al, 2010]. Recent studies have reported that IL17 has an important role in RA pathogenesis [Hueber et al,2010] and combined inhibition of IL-17A and -F can control RA inflammation and joint destruction[Genovese et al 2010. In addition to significantly increased level in RA synovial tissue and readily detected in RA SFs[Shahrara et al,2008].

Another important cytokine is IL6. It promotes synovitis by inducing neovascularization, infiltration of inflammatory cells, and synovial hyperplasia. Also it causes bone resorption by inducing osteoclast formation via the induction of RANKL in synovial cells, and cartilage degeneration by producing matrix metalloproteinases (MMPs) in synovial cells and chondrocytes. Moreover, IL-6 is involved in autoimmunity by altering the balance between T(h)17 cells and T(reg). Furthermore, it acts on changing lipid concentrations in blood and on inducing the production of hepcidin which causes iron-deficient anemia [Hashizume and Mihra, 2011].Recent studies have demonstrated that IL-6 plays a crucial role in RA pathogenesis. In fact, treatment of RA patients with the humanized anti-interleukin-6receptor (IL-6R) antibody, tocilizumab(TCZ), is highly effective [Kremer et al ,2011; Garneroet al, 2010].

This study was designed to determine serum IL17 and IL6 Levels in Iraqi patients with RA and to evaluate possible correlation between them if present.

2. Methods

2.1 Study design

This was a case control single center study conducted at Rheumatology Unit, Baghdad Teaching Hospital,

Baghdad, Iraq carried out from October 2011 till August 2012. Serum IL17 and IL6 concentrations were measured in patients with RA and compared to healthy individuals served as a control group with age and sex matched. Informed consent was obtained from all participants and this study was approved by the ethical committee of Baghdad University, College of Medicine- Medical Department.

2.2 **Sample selection**

A total of 50 eligible patients had confirmed RA by a rheumatologist according to the Revised 1987 American College of Rheumatology (ACR) criteria **(**Arnett et al,1988) were included in the study. Patients were excluded from the study if they had comorbid diseases, overlapped with other connective tissue diseases or inflammatory arthritis, and vasculitis. Additionally, a 30- healthy age and sex matched individuals were considered as a control group.

2.3 **Data collection and laboratory measurements**

We used paper clinical research form through interview and questionnaires. We asked the patients about age, sex, disease duration, and disease activity. Full history was taken and complete clinical exam of participants was done. Then Serum levels of IL-6 and IL-17 were determined in both RA and healthy controls using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Abcam- UK).

2.4 **Statistical analysis**

Statistical analysis was done using Statistical Package for Social Sciences software (SPSS version 20). Frequency distribution for selected variables was done first. Kolmogorov-Smirnov test was used to assess the normal distribution of continuous variables. It was shown that serum IL17 and serums IL6 were non-normally distributed. These variables were described by median and interquartile range. In addition they were tested for statistical significance using non-parametric Mann-Whitney test. An association between 2 categorical variables was assessed by Chi-square (χ^2) test of homogeneity.

ROC analysis was used to assess validity parameters and set optimum cut-off values for quantitative variables when used to predict a diagnosis of RA differentiating it from healthy controls.

The statistical significance, direction and strength of linear correlation between 2 quantitative variables, one of which being non-normally distributed was measured by Spearman's Rho linear correlation coefficient. P value < 0.05 was considered statistically significant.

3. Results

Frequency of females was more than males in patients and controls (88% and 76.7% respectively).Ages of patients range between 20-70 years with a median 43.76 years while in controls, ages range between 20-60 years with a median 37.67 years. The age group 30 -49 years was highest age range for patients (46%) and controls (53.3%) compared to other age groups. No significant difference was seen between patients and control group ($p>0.05$, Table1)

In Table2, serum concentration of IL17 and IL16 was significantly more in patients than those in controls ($p=0.02$, $p=0.001$ respectively).

The cutoff value of serum IL17 was measured by ROC curve between sensitivity and specificity in patients and controls. We found that area under the curve (AUC) at value 0.678 was statistically significant ($p=0.02$) and had intermediate accuracy (Figure1). IL17 concentration ≥71.8 pg/ml was the optimum cutoff value that can differentiate between RA and healthy controls with accuracy 71.2% as shown in Table3.

Also, the ROC curve between sensitivity and specificity for IL6 was shown in Fig2. The AUC was statistically highly significant(AUC= 0.743, $p=0.001$) .It showed that IL6 concentration≥8.1pg/ml was the optimum cut-off value and the highest sensitivity at the same time. The accuracy was 77.9% and sensitivity100% as in Table4. This means the cut-off value of IL6 ≥8.1pg/ml was the best value to differentiate RA and healthy controls on base of serum IL6.

Finally we found a direct strong highly statistical significant correlation between serum IL17 and serum IL6 concentration as in Fig 3.

Table 1: Age and sex distribution in patients and controls

	Controls		Cases (RA)		
	N	**%**	**N**	**%**	**P**
Gender					
Female	23	76.7	44	88.0	0.18[NS]
Male	7	23.3	6	12.0	
Total	30	100.0	50	100.0	
Age, years	20-70	20-60			
Range	43.76	37.67			
Median	12.64	11.35			
SD	1.79	2.07			
SE					
Age group (years)					
<30	8	26.7	10	20.0	0.4[NS]
30-49	16	53.3	23	46.0	
50+	6	20.0	17	34.0	
Total	30	100.0	50	100.0	

NS, not significant

Table 2: Comparison of serum IL17and IL6 between patients and controls

	Study group		**P (Mann-Whitney)**
	Controls	**Cases (RA)**	
Serum IL17 conc., pg/ml			
Range	(2.5 - 191.8)	(37.5 - 1604.9)	0.02*
Median	111.1	154.4	
Interquartile range	(70.9 - 143.4)	(91.7 - 216.3)	
N	21	45	
Mean rank	25.43	37.27	
Serum IL6 conc., pg/ml			
Range	(1.1 - 193.2)	(9.1 - 1813.1)	0.001**
Median	49	150	
Interquartile range	(7.1 - 155.1)	(66.6 - 254.2)	
N	23	45	
Mean rank	23.59	40.08	

*p-value is significant; **p-value highly significant.

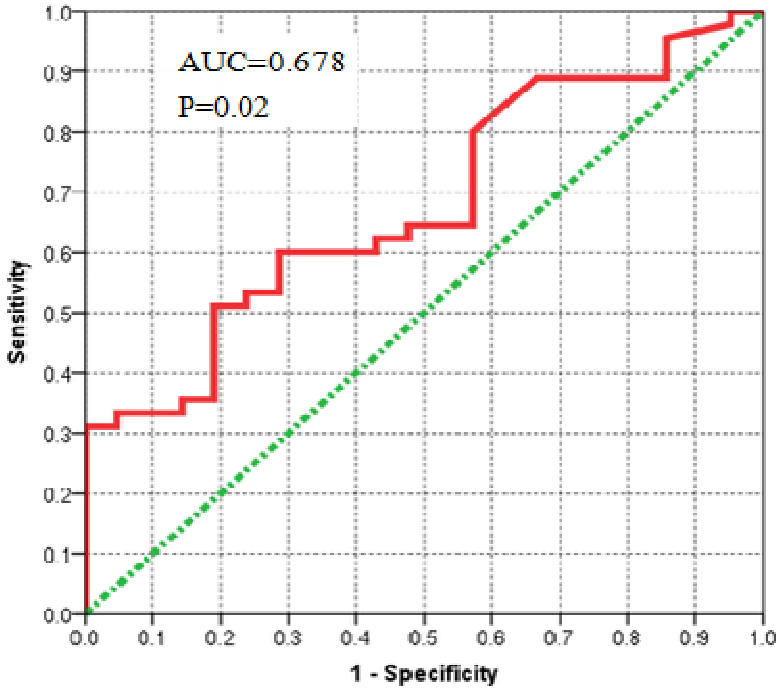

Figure1. ROC curve showing the trade-off between sensitivity and 1-specificity for serum IL17 when used in the context of differentiation between RA cases and healthy controls.

Table 3: Validity parameters for serum IL17 when used as a test to predict a diagnosis of RA differentiating it from healthy controls.

Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy
Serum IL17 concentration, pg/ml			
20.0 (Highest sensitivity)	100.0	4.8	69.7
71.8 (Optimum cut-off)	88.9	33.3	71.2
196.5 (Highest specificity)	31.1	100.0	53.0

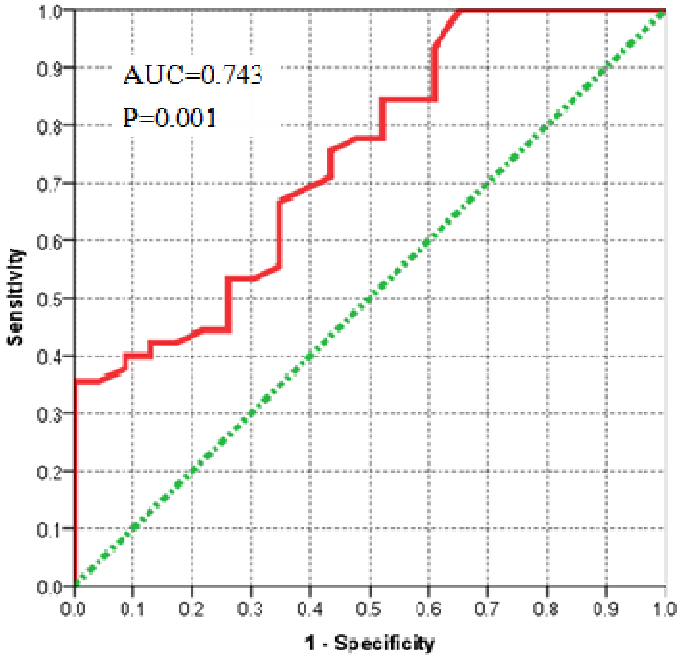

Figure2: ROC curve showing the trade-off between sensitivity and 1-specificity for serum IL6 when used in the context of differentiation between RA cases and healthy controls**.**

Table 4: Validity parameters for serum IL6 when used as a test to predict a diagnosis of RA differentiating it from healthy controls.

Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy
Serum IL6 concentration, pg/m			
8.1 (Highest sensitivity and Optimum cut-off)	100.0	34.8	77.9
196.1 (Highest specificity)	35.6	100.0	57.4

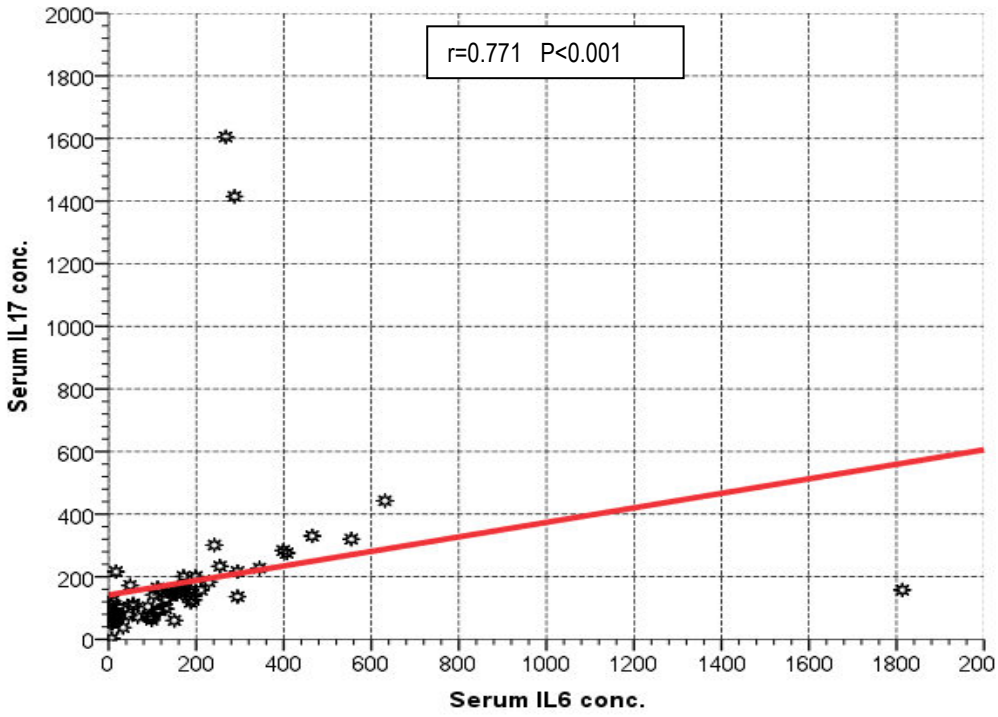

Figure 3: Scatter diagram showing the linear correlation between serum IL6 and serum IL17 conc. among cases with RA.

4. Discussion

IL-6 has a very important role in regulating the balance between IL-17-producing Th17 cells and regulatory T cells (Treg). The two T-cell subsets play a prominent role in immune functions: Th17 cell is a key player in the pathogenesis of autoimmune diseases while T reg functions to restrain excessive effector T-cell responses. Th17 cells are considered to be the primary cause of RA pathology. The critical role of IL-6 in altering the balance between T reg and Th17 cells may have a clinical therapeutic importance in various autoimmune and inflammatory diseases (Bettelli et al 2006, Nishimoto et al 2008) .

This study evaluated serum IL17 and serum IL6 in a sample of Iraqi patients with RA and showed significant increase compared to controls. Similar findings were reported by other studies. Chen et al (2011) investigated the effects of tumor necrosis factor (TNF)-a inhibitors on circulating Thelper-type17(Th17)cellsandTh17 related cytokines (RA) in 48 RA patients both before(baseline) and six months after anti-TNF-a therapy and found significantly higher baseline frequencies of serum IL17 and IL6. Roso et al (2012) assessed IL-17 patterns in synovium, serum, and synovial fluid from 30 treatment-naïve, early rheumatoid arthritis patients and compared it to 29 control osteoarthritis patients and found in early RA patients, strong correlations of serum and SF IL-17A levels compared to controls. Liu et al (2012) evaluated the role of interleukin IL-17 in anxiety and depression of 18 patients with rheumatoid arthritis compared to 18 healthy controls and showed that serum IL-6 and IL-17 levels were significantly higher in RA patients than those of healthy subjects. Rico et al (2008) found IL6 was significantly more in RA than controls.

Validity parameters for IL17 and IL6 to predict the diagnosis of RA were assessed by ROC test. This study showed that the optimum cut-off value of IL17 was 71.8 pg/ml and IL6 was 8.1 pg/ml which may play a role in diagnosing Iraqi patients with RA. Other published data were variable. Stoy et al(2013) studied the levels of circulating IL-22- and IL-17A-producing T helper cells and plasma cytokines in patients with alcoholic hepatitis

from Denmark and found the optimum cut-off value that can differentiate healthy controls from patients was 2.7pg/ml. Nogueira et al (2009) measured serum levels of IL-17A and associated upstream cytokines and the frequency of IL-17-producing autoantigen specific T cells in a sample of British patients with ANCA associated vasculitis compared to controls and reported that the optimum cut off for normal level of IL17 was 133pg/ml. Knudsen et al(2009) determined changes in plasma IL-6 in 25 Danish active rheumatoid arthritis (RA) patients during treatment with etanercept alone or in combination with methotrexate in a prospective, randomized, international study and found that the optimum cut-off value of IL6 was≤ 3.3 ng/l. Hergenroeder et al (2010) evaluated serum IL-6 as a candidate biomarker for intracranial pressure elevation following isolated traumatic brain injury in a sample from united states and showed the cut off value for serum IL6 was 5 pg/ml.
The differences of cut off value of IL17 and IL 6 from previous studies may be related to different technical methods of measurement, environmental and racial factors and type of disease studied.
Interestingly, we found a direct strong significant correlation between serum IL17 and IL6 concentrations in RA patients. This may suggest that controlling IL-6 activities is potentially an effective approach in the treatment of RA that can reduce serum IL17 and subsequently improve patients with RA. A study by Kimura and kishimoto (2010) reviewed the role of IL-6 in regulating Th17/Treg balance and described the critical functions of IL-6 and Th17 in immunity and immune-pathology.
The main limitations of the present study were the small size of the studied sample and short period of the study and these can be solved by larger prospective studies with longer period of follow up to support the reported data. Yet, in spite of that, this study has points of strength like strict inclusion and exclusion criteria, and defined data measurement and collection.

5.Conclusions

serum IL17 and IL6 levels were significantly higher in RA patients compared to controls. Also there was a direct strong significant correlation between both interleukins. This may indicate that targeting IL17 and IL6 is potentially an effective treatment for RA.

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