Validity of the Ratio of Serum Concentration of Interleukin 6 to Transforming Growth Factor-Beta1 in a Sample of Patients With Rheumatoid Arthritis

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ABSTRACT:

BACKGROUND:

Interleukin 6 (IL-6), a pro-inflammatory cytokine, plays a key role in the development of rheumatoid arthritis (RA). Transforming growth factor-beta1 **(**TGF-β1) is an anti-inflammatory cytokine which is considered to be an important (down) regulator of inflammation in RA**.**

OBJECTIVE:

To evaluate serum concentration of IL6 to TGF-β1 ratio in a sample of Iraqi patients with RA.

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 concentration of IL-6 to TGF-β1 ratio and serum IL-17 were determined in both RA groups using enzyme-linked immunosorbent assay (ELISA). The cut off value was assessed by receiver operating characteristics (ROC) test and correlation by spearman's Rholinear 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 concentration of IL-6 toTGF-β1 ratio was significantly more in patients than those in controls (p=0.018). Area under the curve (AUC) at value 0.686 was statistically significant (p=0.018) and had intermediate accuracy. Serum IL-6 to TGF- β1 ratio≥ 2.4pg/ml was the optimum cutoff value that can differentiate between RA and healthy controls with accuracy 71.9%. There was a significant positive linear correlation between IL-6 to TGF-β1 ratio and IL-17(r=0.56, p<0.001) and simple linear regression analysis showed that for each 1unit increase in IL6 to TGF-B1 ration there was a significant increase of 0.7 pcg/ml in IL-17(R^2=0.467, P < 0.001)

CONCLUSION:

Serum concentration of IL6 to TGF-β1 ratio was significantly higher in Iraqi sample of RA patients compared to controls. This may help in early diagnosis of RA and suggest potentially an early effective treatment**.**

KEY WORDS: Rheumatoid arthritis**,** IL6, TGF-β1, IL6 to TGF- β1 ratio**,** cytokines.

INTRODUCTION:

Rheumatoid Arthritis (RA), a chronic disease affecting 0.5-1% of adults, is characterized by persistent synovitis, systemic inflammation and immunological abnormalities. Uncontrolled active RA causes joint damage, disability, diminished quality of life and cardiovascular and other co-morbidities. [1,2]Interleukin 6 (IL-6), a typical cytokine featuring redundancy and pleiotropic activity, plays a key role in the development of RA [3]. IL-6 promotes the imbalance between T17 cells and regulatory T cells (Treg) and the production of autoantibodies such as Rheumatoid Factor (RF) and Anti-Citrullinated Peptide Antibody (ACPA). It also promotes synovial inflammation and cartilage and bone destruction as well as systemic features including cardiovascular, psychological and skeletal disorders[4].

In contrast, transforming growth factor-beta1 (TGF-β1) is an anti-inflammatory cytokine which is considered to be an important (down) regulator of inflammation in RA [5]. Moreover and more important, in the presence of TGF-β, IL-6 is able to promote Th17 cell differentiation through STAT3-mediated up-regulation of retinoid

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orphan receptor (ROR) γ t, while it inhibits TGF-β-induced regulatory T cell (Treg) differentiation [6]. IL-6 thus promotes predominance of Th17 over Treg in the effector CD4+Tcell subsets, which is thought to play a major role in the development of RA and various other immune-mediated diseases(7). This study designed to evaluate serum concentration of IL6 toTGF-β1 ratio in a sample of Iraqi patients with RA.

MATERIALS AND METHODS:

Study design

This was a case control single center study conducted at Rheumatology Unit, Baghdad Teaching Hospital, Baghdad, Iraq. It was carried out from October 2011 till August 2012. Serum concentration of IL6 toTGF-β1 ratio was 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.

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 for the classification of RA **(**8) 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.

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 concentration of IL-6 toTGF-β1 ratio and serum IL-17 were determined in both RA and healthy controls using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Abcam- UK).

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 concentration of IL-6 toTGF-β1 ratio 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 value < 0.05 was considered statistically significant.

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 IL-6 to TGF-β1 ratio was significantly more in patients than those in controls (p=0.018).

The cutoff value of serum IL6 to TGF-β1 ratio was measured by receiver operating characteristics (ROC) curve between sensitivity and specificity in patients and controls. We found that area under the curve (AUC) at value 0.686 was statistically significant (p=0.018) and had intermediate accuracy (Figure1). Serum IL-6 to TGF- β1 ratio≥ 2.4pg/ml was the optimum cutoff value that can differentiate between RA and healthy controls with accuracy 71.9% as shown in Table3.

In addition, we found significant positive linear correlation between IL6 to TGF-β1 ratio and IL-17(r=0.56, p<0.001, figure2) and simple linear regression analysis showed that for each 1unit increase in IL-6 to TGF-β1 ration there was a significant increase of 0.7 pcg/ml in IL-17(R^2=0.467, P < 0.001, Table 4).

Table 1: Age and sex distribution in rheumatoid arthritis patients and controls.

	Controls		Patients (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 Range Median SD SE Age group (years)	 20-70 43.76 12.64 1.79	 20-60 37.67 11.35 2.07			
<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; RA, rheumatoid arthritis; SD, standard deviation; SE, standard error.

Table 2: Comparison of serum IL6 to TGF- β 1 ratio between patients and controls.

	Study group		P (Mann-Whitney)
	Controls	Patients (RA)	
IL-6 to TGF- β1 ratio			0.018
Range	(0.8 - 261.8)	(0.6 - 1437.5)	
Median	15.3	65.2	
Interquartile range	(2.8 - 32)	(8.6 - 206.9)	
No.	20	44	
Mean rank	24.3	36.2	

IL-6, interleukin 6; TGF- β1, transforming growth factor β1, RA, rheumatoid arthritis

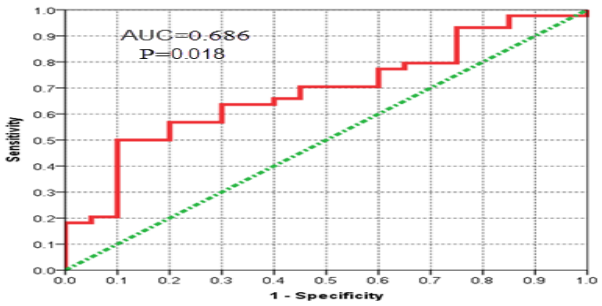

Figure 1: ROC curve showing the trade-off between sensitivity and 1-specificity for serum IL-6 to TGF-β1 ratio when used in the context of differentiation between rheumatoid arthritis patients and healthy controls. IL-6, interleukin 6; TGF- β1, transforming growth factor β1.

Table3: Validity parameters for serum IL-6 to TGF-β1 ratio when used as a test to predict a diagnosis of rheumatoid arthritis differentiating it from healthy controls.

Positive if ≥ cutoff value	Sensitivity	Specificity	Accuracy
Serum IL-6 to TGF-β1 ratio			
1.6 (Highest sensitivity)	97.7	15.0	71.9
2.4 (Optimum cut-off)	93.2	25.0	71.9
297.4 (Highest specificity)	18.2	100.0	43.8

IL-6, interleukin 6; TGF- β1, transforming growth factor β1

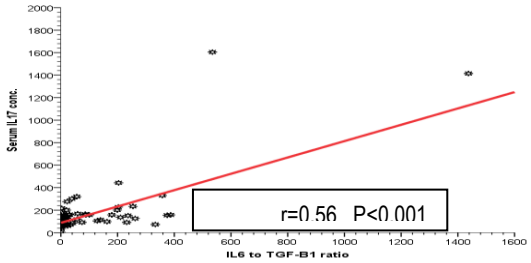

Figure 2: Figure 1: Scatter diagram showing the linear correlation between serum IL-6 to TGF-β1 ratio and serum IL17 conc. among patients with rheumatoid arthritis. IL-6, interleukin 6; TGF- β1, transforming growth factor β1, IL-17, interleukin 17.

Table 4: Simple linear model with IL-17 as the dependent(response) variable and the IL-6 to TGF-β1 ratio as the explanatory variable.

	Regression coefficient	P
(Constant)	87.3	0.002
IL-6 to TGF-β1 ratio	0.7	<0.001

P (model) < 0.001; R^2=0.467

IL-6, interleukin 6; TGF- β1, transforming growth factor β1, IL-17, interleukin 17.

DISCUSSION:

The crucial role of IL-6 in changing the equilibrium between T reg and Th17 cells may have a clinical therapeutic significance in various inflammatory and autoimmune diseases [9,10]. TGF-β1 promotes T reg differentiation, which in turn suppresses adaptive T-cell responses and prevent autoimmunity [11, 12]. IL6 inhibits TGF-β-induced Treg (iTreg) differentiation [13,14]. These important observations support the notion that IL-6 blockade may be an innovative treatment of RA patients that can improve their quality of life.

This study evaluated serum concentration of IL-6 to TGF-β1 ratio in a sample of Iraqi patients with rheumatoid arthritis (RA) and interestingly showed a significant increase in patients compared to controls. Also there was significant positive linear correlation between IL-6 to TGF-B1 ratio and IL-17.

Possible explanation is that for each increase in IL-6 there is a feedback inhibition in TGF-β1. This stimulation leads to stimulating TH17 and increase in IL-17.T helper cells that produce IL-17 (IL-17; 'T_H-17 cells') are a distinct subset of pro-inflammatory cells whose *in vivo* function requires IL-23 but whose *in vitro* differentiation requires only IL-6 and transforming growth factor-β (TGF-β). Zhou et al 2007 reported that IL-6 induced expression of IL-21 that amplified an autocrine loop to induce more IL-21 and IL-23 receptor in naive $CD4^+$ T cells. Both IL-21 and IL-23, along with TGF-β, induced IL-17 expression independently of IL-6. The effects of IL-6 and IL-21 depended on STAT3, a transcription factor required for the differentiation of T_H-17 cells *in vivo*. IL-21 and IL-23 induced the orphan nuclear receptor RORγt, which in synergy with STAT3 promoted IL-17 expression. IL-6 therefore orchestrates a series of 'downstream' cytokine-dependent signaling pathways that, in concert with TGF-β, amplify RORγt-dependent differentiation of T_H-17 cells [15].

Morishima et al [16] found that IL-6 up-regulated IL-23R mRNA expression, and IL-6 and IL-23 synergistically augmented its protein expression. The combination induced Th17 differentiation,

and TGF-β1 further enhanced it. IL-6 augmented endogenous TGF-β1 mRNA expression, whereas the amount of TGF-β produced was not enough to induce Th17 differentiation by IL-6 alone. However, unexpectedly, the up-regulation of IL-23R and induction of Th17 differentiation by IL-6 and IL-23 were almost completely inhibited by anti-TGF-β. These results suggested that the induction of IL-23R and Th17 differentiation by IL-6 and IL-23 is mediated through endogenously produced TGF-β.

Kimura and Kishimoto [17] 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. IL-6 induces the development of Th17 cells from naïve T cells together with TGF-β; in contrast, IL-6 inhibits TGF-β -induced Treg differentiation. Dysregulation or overproduction of IL-6 leads to autoimmune diseases such as RA, in which Th17 cells are considered to be the primary cause of pathology. Given the critical role of IL-6 in altering the balance between Treg and Th17 cells, controlling IL-6 activities is potentially an effective approach in the treatment of various autoimmune and inflammatory diseases.

Other Studies have shown that TGF-β and IL-6 are required for the lineage commitment of pathogenic IL-17-producing T helper cells (T(H)-17 cells) [18].

Recently, Abdullah et al [19] determined serum IL17 and IL6 levels in a sample of Iraqi patients with RA in a case control study and found serum IL-17 and IL-6 concentrations were significantly higher in Iraqi sample of RA patients compared to controls with direct strong highly significant correlation between these cytokines.

Validity parameters for IL-6/TGF-β1 ratio to predict the diagnosis of RA were assessed by ROC test with highest accuracy (71.9%) at optimum cut off value of≥ 2.4pg/ml that can differentiate between RA and healthy controls.

Up to our knowledge this is the first study in Iraq that assessed IL6/TGF-β1 ratio with strict inclusion and exclusion criteria, defined data measurement, and data collection. However the main limitations 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.

CONCLUSION:

Serum concentration of IL-6 toTGF-β1 ratio was significantly higher in RA patients with significant positive linear correlation with IL-17. This may indicate that targeting IL-6 is potentially an effective treatment for RA.

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