The Clinical Significance of Interleukin-15 and Interleukin -17 in Patients with Rheumatoid Arthritis

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ABSTRACT:

BACKGROUND:

Rheumatoid arthritis (RA) is a chronic, deforming arthritis that can lead to disabilities and poor quality of life. Cytokines produced by inflammatory cells play a pivotal role in synovial inflammation and joint destruction in rheumatoid arthritis; they are the final mediators and/or regulators of inflammatory process.

OBJECTIVE:

This study was designed to assess serum IL-15 and IL-17 levels in a sample of patients with RA and to evaluate the correlation between their levels and various clinical, laboratory parameters of RA disease activity and severity.

SUBJECTS AND METHODS:

A case – control study was carried out from January 2013 till October 2013. It was conducted on 80 RA patients who attended Basra General Hospital outpatient rheumatology clinic. Eighty apparently healthy adults, age and sex matched, were also included as control group. Peripheral blood samples were collected from both patients and controls and used for estimation of serum IL-15, IL-17 and anti-CCP antibodies by ELISA, RF and CRP by slide agglutination test and ESR by Westergren method. RA activity was measured using disease activity score28 (DAS-28).

RESULTS:

RA patients showed significantly higher serum IL-15 and IL-17 levels than controls ($P<0.01$). In addition, IL-15 and IL-17 were found to be significantly higher in RA patients with active disease compared to those at remission. No significant correlations were noted between IL-15 serum level and RF, CRP, ESR, anti-CCP but it was significantly correlated with DAS-28, TJC and SJC. Significant positive correlations of serum IL-17 level and ESR, CRP, anti-CCP, DAS28, TJC and SJC were found. Also there was a significant direct correlation between IL-15 and IL-17concentration.

CONCLUSION:

The current study demonstrated that serum IL-15 and IL-17 levels were significantly higher in RA patients than the controls confirming their important role in the pathogenesis of RA and possible target for future therapy .Elevated IL-17 levels could be considered as a possible indicator of more severe clinical course, This is underscored by relatively strong correlations between almost validated disease activity and severity markers including CRP, ESR, anti-CCP, DAS28, TJC and SJC.

KEY WORDS: rheumatoid arthritis, interleukine-15, interleukine-17, DAS-28, anti-CCP.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disorder that is characterized by polyarthritis with often progressive joint damage and disability, immunologic abnormalities, systemic inflammation, increased comorbidity and premature mortality. It is the most common inflammatory joint disease that affects 0.5- 1% of the world's population, with a female-to-male ratio of 3:1. The onset of disease can occur at any age but peak incidence occurs within the fourth and fifth decades of life[1, 2]

Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia (swelling), autoantibody production (rheumatoid factor and anti–citrullinated protein antibody [ACPA]), cartilage and bone destruction (deformity), In addition to systemic features including cardiovascular, pulmonary, psychological, and skeletal disorders [3].

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Cytokines that arise from numerous synovial cell populations are key mediators of inflammation and can be found in abundance both in the joint and blood of patients with active disease. They are responsible not only for the destructive synovitis but also for some of the systemic features [4].

Interleukine-15 has been identified as a pro-inflammatory cytokine of a potential importance in the pathogenesis of RA. In the synovial membrane of patients with RA, there is a substantial expression of IL-15, predominantly expressed in macrophages but also in fibroblast-like synoviocytes and endothelial cells [5,6]. It may contribute to an increased production of many pro-inflammatory cytokines like TNF-α, IFN-γ and IL-17 in T cells [7,8] .

These findings suggest that an IL-15-dependent pro-inflammatory pathways may be created in the inflamed synovium, in which IL-15 stimulates the production of TNF, IFN-γ and IL-17, which in turn stimulate the further production of IL-15, IL-8 and IL-6 in fibroblast-like synoviocytes[3,6].

Interleukine-17 which is the primary cytokine of Th17 cells exerts potent pro-inflammatory and joint-destructive activities. IL-17 stimulates the production of IL-1 and TNF-α from human macrophages [9], induces the secretion of IL-6 and IL-8 in synovial fibroblasts [10], induces up-regulation of receptor activator of nuclear factor kappa B ligand (RANKL), an important positive regulator of osteoclastogenesis[11], and also promotes cartilage degradation by inducing metalloproteinases and proteoglycan depletion [12].

It is already demonstrated that, in human RA, IL-17 plays a key role in the synergistic or additive effects expressed together with TNF-α and IL-1 [13].

Cytokines are often discussed optimistically in the context of diligent search for new biomarkers of RA, molecules that correlate with the level of disease activity &/or predict disease out come and severity [14] .

This study was performed to evaluate serum IL-15 and IL-17 levels in RA patients and to find out the correlations between their levels and various clinical and laboratory parameters of RA disease activity and severity indices.

SUBJECTS AND METHODS:

A total of 80 adults, RA patients satisfying the American College of Rheumatology (ACR) 1987 revised criteria for classification of RA[15], were included in the study. They comprise 66 females and 14 males with mean age of 46 $\pm$ 9.79 years.

Exclusion criteria included pregnancy, lactation, cancer patients, patients with comorbid diseases like diabetes mellitus, cardiovascular disease, renal and hepatic disease and patients with concomitant autoimmune diseases like SLE or Graves' disease.

Eighty apparently healthy adults, age and sex matched; with mean age of 43.38 ± 9.06 years were included as control group. Questionnaire form was filled with the information that obtained directly from the participants who were informed about the study and an agreement was taken , it included data on name , age , sex , residency , duration (since RA has been diagnosed) , morning stiffness , history of smoking , family history of RA, detailed medical history . Rheumatoid nodules were reported to be present if found on physical examination at time of interview for the study. Patients' assessment of morning stiffness was defined as the average duration in minutes during the past week.

Clinical evaluation was done by specialist doctor, which include examination of the 28 joints (Shoulders, elbows, wrists, MCPs, PIPs and knees) for swelling and tenderness, combining these with ESR and the patient's self-assessment of general health during the preceding 7 days on visual analogue scale between 0 and 100 mm for estimation of Disease Activity Score28 (DAS28). A DAS28 above 5.1 considered as high disease activity, 3.2-5.1 have moderate disease activity whereas DAS28 below 3.2 indicate low disease activity, Remission is achieved by DAS28 lower than 2.6 [16].

Laboratory evaluation including measurement of RF, CRP, ESR, anti-CCP, IL-15 and IL-17 levels was done. Venous blood samples were obtained from both patients and controls and used for estimation of ESR by westergren method[17], RF and CRP by latex agglutination slide test which were done using (RA and CRP latex test kits, PLASMATEC, UK) according to the manufacturer's instructions. Serum anti-CCP antibody was determined by ELISA using (AESKULISA-CCP assay kit, AESKU-DIAGNOSTICS, Germany), as described by the manufacture. Standard curve was established by plotting the optical density (OD) of each calibrator (provided with the kit) with respect to the corresponding concentration values in U/ml. Each serum sample >18 U/ml considered positive for anti-CCP. Serum concentrations of IL-15 and IL-17 were determined by ELISA using (Ray Bio Human IL-15 and IL-17 ELISA kits, Ray Biotech,

USA). Serum IL-15 and IL-17 levels were calculated from a standard curve constructed by plotting the absorbance value (OD) against concentration obtained from each reference standard in pg/ml. The cut-off value for the levels of IL-15 and IL-17 were derived from the mean +2SD of the controls which were equal to 67.8pg/ml for IL-15 and 91.32 pg/ml for IL-17.

Statistical analysis was performed using statistical package for social science (SPSS) version-15. The association between categorical variables was assessed by Chi-square ($\chi 2$) test. Independent samples t-test was used to compare two means. Comparison between more than two groups was done by one way ANOVA test. Correlation between variables was performed using Pearson's correlation test. A p-value <0.05 was considered as the lowest limit of significance.

RESULTS:

The general characteristic of patients and control groups are summarized in table1. The study population consisted of 80 RA patients with mean age of 46± 9.79 years and 80 apparently healthy adults with mean age of 43.38± 9.06 years represent the control group. The majority of patients (82.5%) were females while males represent 17.5%. There were no significant difference in age and sex distribution between the two groups (P>0.05). Most of the study population came from urban background. Most of them were non-smokers.

Table 1: The general characteristics of the study population.

Characteristics		Patients (N=80)	Control (N=80)	Significance
Age(years) mean ± SD		46±9.79	43.38±9.06	P >0.05
Sex N (%)	Females	66 (82.5)	61 (76.2)	χ^2=0.954,df=1 P >0.05
	Males	14 (17.5)	19 (23.8)	
Residency N (%)	Urban	49 (61.3)	56 (70)	χ^2=1.358,df=1 P >0.05
	Rural	31 (38.8)	24 (30)	
Smoking N (%)	Smoker	7 (8.8)	6 (7.5)	χ^2=0.084,df=1 P >0.05
	Non-smoker	73 (91.2)	74 (92.5)	

The characteristics of RA patients are presented in table 2. The age distribution of patient group showed that the majority of patients (61.3%) were within 30-50 years. Among patients 61.25% have disease duration ranged from 2-10 years, 23.7% less than 2 years and 15% greater than 10 years. Morning stiffness was observed in 85% of RA patients, 52.5% of them have morning stiffness lasting more than one hour. Rheumatoid nodule was found in 11.3% of RA cases. Family history of RA was reported in 10% of RA patients. According to Disease Activity Score 28 (DAS28) , 42.5% had a high disease activity, 20% had a moderate disease activity and 16.25% had low disease activity. There were 21.25% of RA patients on remission state.

Table 2: Basic characteristics of Rheumatoid arthritis patients.

Characteristics		N (%)
Age groups	< 30 yrs	5 (6.3)
	30-50 yrs	49 (61.3)
	>50 yrs	26 (32.5)
Sex	Male	14 (17.5)
	Female	66 (82.5)
Duration of disease	< 2 years	19 (23.75)
	2 – 10 years	49(61.25)
	>10 years	12 (15)
Morning stiffness	Present	68 (85)
	Absent	12 (15)
Duration of morning stiffness	< 1 hour	26 (32.5)
	≥ 1 hour	42 (52.5)
Rheumatoid nodule	Present	9(11.3)
	Absent	71 (88.8)
Family history of RA	Positive	8 (10)
	Negative	72 (90)
Disease Activity Score 28 (DAS28)	High disease activity	34 (42.5)
	Moderate disease activity	16 (20)
	Low disease activity	13 (16.25)
	Remission	17 (21.25)

The results of laboratory parameters measured in this study for both patients and control groups are shown in table 3. The prevalence of anti-CCP antibodies was 77.5% among RA group compared to 2.5% among the control. RF was positive in 75% of RA patients as compared to 5% among controls. CRP seropositivity was 82.5% and 7.5% for RA patients and controls respectively. Measurement of ESR by Westergren method revealed a mean ESR value of 46.53 $\pm$29.6 mm/hr for RA patients compared to 14.9 $\pm$7.45 mm/hr for the control. In all these parameters, there were significant differences between patients and controls.

Table 3: The laboratory parameters of rheumatoid arthritis patients and control.

Parameters		Patients N(%)	Controls N(%)	Significance
Anti-CCP	Positive	62 (77.5)	2 (2.5)	χ^2=93.75 df=1 P<0.01
	Negative	18 (22.5)	78(97.5)	
RF	Positive	60 (75)	4 (5)	χ^2=81.667 df=1 P<0.01
	Negative	20 (25)	76 (95)	
CRP	Positive	66 (82.5)	6 (7.5)	χ^2=90.9 df-1 P< 0.01
	Negative	14 (17.5)	74 (92.5)	
ESR , mm/hr (mean ± SD)		46.53 ± 29.6	14.9 ± 7.45	P < 0.01

Anti-CCP=anti-cyclic citrullinated peptide antibody, RF=rheumatoid factor, CRP=C-reactive protein, ESR= Erythrocyte sedimentation rate.

Result of mean serum IL-15 level of patients group was found to be 97.18±26.95 pg/ml which was significantly higher (p<0.01) than mean serum level for the control group (46.24±10.78pg/ml). Also a

statistically significant difference was detected between the estimated level of mean serum IL-17 for RA patients and that of the control group ($p < 0.01$).

The estimated level of mean serum IL-17 for RA patients was 134.79±39.37pg/ml while that for the control group was 59.7±15.81pg/ml (Table 4).

Table 4: Mean serum levels of IL-15 and IL-17 in RA patients and control.

Cytokines	Patients	controls	P value
Serum IL-15 (pg/ml)	97.18 ± 26.95	46.24±10.78	<0.01
Serum IL-17 (pg/ml)	134.79 ± 39.37	59.704±15.807	< 0.01

The mean serum IL-15 and IL-17 levels in relation to the duration of illness were presented in table 5. RA patients with disease duration less than 2 years showed lower IL-15 levels than those with long standing disease. This differences was statistically significant ($p<0.05$).On the other hand, there were no significant difference in serum IL-17 levels in relation to the duration of illness ($p>0.05$).

Table 5: Serum IL-15 and IL-17 levels in RA patients in relation to duration of illness.

Duration of illness	Patients N (%)	Serum IL-15 (pg/ml) Mean ± SD	Serum IL-17 (pg/ml) Mean± SD
< 2 years	19 (23.75)	79.78±26.37	130.45±37.75
2 – 10 years	49 (61.25)	100.61±24.39	136.26± 39.5
>10 years	12 (15)	102.6 ± 28.27	135.65 ±44.14

P<0.05 P>0.05

Serum IL-15 and IL-17 levels were significantly higher in RA patients with active disease as compared to their levels in patients at remission state (Table 6).

Table 6: Mean serum IL-15 and IL-17 levels in relation to disease activity in rheumatoid arthritis patients.

Disease state	Serum IL-15 level Mean ± S.D (pg/ml)	Serum IL-17 level Mean ± S.D (pg/ml)
Active disease N = 63	100.52±27.62	146.45±36.09
Remission N = 17	84.84±20.65	91.55±9.25

P < 0.05 P < 0.01

In addition, the results revealed a statistically significant differences in serum levels of IL-17 between low, moderate and high disease activity ($P<0.01$) in contrast to the levels of IL-15 which showed no significant differences between these three groups (Table 7).

Table 7: Comparisons of serum IL-15 and IL-17 levels between different groups of disease activity.

Groups	Patients N(%)	Serum IL-15 (pg/ml) mean± SD	Serum IL-17 (pg/ml) mean ±SD
High disease activity	34 (53.97)	104.49± 31.71	169.84±27.45[*,a]
Mod. disease activity	16 (25.39)	102.48 ±18.59	133.57±19.33[*]
Low disease activity	13 (20.63)	92.92±25.79	101.12 ± 13.78

[*] P<0.01 as compared to low disease activity group
[a] P<0.01 as compared to moderate disease activity

The correlations between serum IL-15 and IL-17 levels and different variables were analyzed using Pearson's correlation analysis. There were no significant correlation between serum IL-15 and laboratory parameters include CRP, ESR , RF and anti-CCP, while it was significantly correlate with DAS28, tender joint count (TJC) and swollen joint count (SJC) ($p<0.05$). There were direct significant correlations between serum IL-17 levels and inflammatory markers as ESR and CRP, and serological marker as anti-CCP but not with RF. Similar to IL-15, IL-17 level showed a strong direct correlation with DAS28, tender joint count (TJC) and swollen joint count (SJC). Finally we found a direct correlation between serum IL-15 and IL-17 concentration ($r= 0.228$, $p<0.05$) (Table 8).

Table 8: Correlations of IL-15 and IL-17 with different laboratory parameters in RA patients.

	Serum IL-15	Serum IL-17
RF	r= 0.141 P= NS	r=0.194 P=NS
CRP	r=0.027 P=NS	r=0.243 P<0.05
ESR	r=0.212 P=NS	r=0.497 P<0.01
Anti-CCP	r=0.184 P=NS	r=0.579 P<0.01
DAS28	r= 0.265 P<0.05	r=0.793 P<0.01
TJC	r=0.242 P<0.05	r=0.644 P<0.01
SJC	r=0.227 P<0.05	r=0.524 P<001
Serum IL-17	r=0.228 P<0.05	r= 1

RF=Rheumatoid factor, CRP= C-reactive protein, ESR=Erythrocyte sedimentation rate, Anti-CCP=anti-cyclic citrullinated peptide, DAS28= disease activity score28, TJC=tender joint count, SJC=swollen joint count, r= correlation coefficient, NS= not significant.

DISCUSSION:

The mean age of patients in the present study was approximately similar to many local studies in Basra [18] and in Baghdad [19,20] but slightly higher than that reported in Kuwait by Al-Salem et al[21] and younger than that observed in Turkish patients with RA[22]. This difference could possibly be explained by demographic characteristics which are specific to the region or by real differences in the age of symptom onset.

Not surprisingly, RA patients were dominantly females in the study group, this is similar to the general agreement that the incidence of the disease is more common in females than in males, hormonal factors like estrogen and progesterone could potentially explain some of the gender effect. Estrogen might have detrimental effects through its ability to decrease apoptosis of B cells , potentially permitting the selection of auto-reactive clones. Hormones also have a complex influence on the balance of T-cell subsets with distinct cytokine profiles [23].

The age distribution of patients group showed that the majority of patients were between 30-50 years .Berglin[24] found that the peak age of incidence between 4th and 6th decades, Tehlirian et al [2] found that The onset of disease can occur at any age but peak incidence occurs within the 4th and 5th decades of life which is consistent with our observation.

Investigation of various laboratory parameters in assessment of RA patients as anti-CCP and RF reflect a general agreement that anti-CCP antibodies are important serologic diagnostic marker for RA [25], since the figure (77.5%) of anti-CCP seropositivity observed in this study is almost similar to that reported from other studies, from Turkey[22], Netherlands [26] and Iran [27]. However, the prevalence of anti-CCP reported in south Iraq to be 61.2% [18], this variation in anti-CCP seropositivity is due to that these antibodies are directed against different epitopes in citrulline-containing peptides and sera from individual

patients may contain different subsets of anti-CCP antibodies [28].

RF seropositivity varies among RA patients in different studies ranged from 57% [18], 90.3% [22] and 100% [29]. In this study, 75% of RA patients were rheumatoid factor positive. This may be attributed to the size of study population and the method of assessment used beside the time of patient's selection.

The current study demonstrated significantly higher levels of IL-15 and IL-17 among RA patients than the control group. This observation is consistent with several studies[30-33]. On the contrary, no significant difference was found in the frequency of peripheral blood Th17 cells between RA patients and controls [34].other reports suggested that IL-15 was detected in the RA synovial membrane and present but at low levels in sera of up to 40% of patients with RA [35]. The discrepancy between those and the present study may relate to patient selection, received therapy and assay conditions when measuring cytokine levels.

The time of cytokine estimation and duration of illness have a considerable impact on the obtained values and the levels of cytokines as the values of serum IL-15 was significantly lower in early RA patients as compared to patients with long standing disease. Consistent with this, D:\pubmedGonzalez-Alvaro et al [36] observed that RA patients with a disease duration less than 2 years showed significantly lower IL-15 levels than those with long-term disease (p=0.004) and concluded that IL-15 is involved in the perpetuation of RA synovitis, while no important differences in serum IL-15 levels according to disease duration were found by Lamana et al [37]

No significant difference was found in the mean serum IL-17 levels of our patients in relation to disease duration. The same result was obtained by Metawi et al [30] . In contrast to our results, Raza et al [38] reported a significant negative correlation between synovial IL-17 levels and disease duration of RA patients. These discrepancies in both IL-15 and IL-17 studies can be justified as their studies included patients with early active RA (<12 months) rather than established RA.

In this analysis, serum IL-15 level was found to be significantly higher in RA patients with active disease than those in remission state, in contrast with other report [36], which showed no association between serum IL-15 levels and disease activity in their RA patients group. On the other hand serum IL-15 level was found to be higher among RA patients with high and moderate disease activity (MDA) in comparison to low disease activity (LDA) but this increase was not significant. Petrovic-Rackov et al [32] observed that RA patients with high disease activity (HAD) had significantly higher levels of serum IL-15 compared to moderate (MDA) and low disease activity (LDA) groups. A possible explanation for these differences might be due to different demographics and treatment regime in the selected RA patients in these studies.

A statistically significant difference was found in serum IL-17 level between RA patients with active disease and those in remission state, in addition , patients with HDA showed significantly higher serum IL-17 levels than MDA and LDA groups. In agreement with our observation, Gullick et al [39] observed that patients with active disease had a higher percentage of Th17 cells, which is the main source of IL-17, in synovial tissue than patients in remission, suggesting a possible role for Th17 cells in active synovitis in RA.

Metawi et al [30] reported an increase in both serum and synovial IL-17 levels with higher DAS-28 scores. Although this increase was not statistically significant regarding synovial IL-17 level but, similar to our finding, it was significant in the serum levels of IL-17 among the three RA patients groups.

The current work revealed no significant correlations between IL-15 level and laboratory parameters including RF, CRP, ESR and Anti-CCP. However, significant positive correlations were found between IL-15 and DAS28, tender joint count (TJC), swollen joint count (SJC) and IL-17 concentration. Similarly, González-Álvaro et al [40] showed that serum IL-15 levels did not overlap extensively with RF or anti-CCP but they were significantly and independently associated with a higher DAS28 during follow-up.

In the same trend, significant positive correlation between serum level of IL-15 and DAS 28 was reported by Petrovic-Rackov et al [32] . In addition, significant positive correlation between IL-15 and IL-17 levels in serum and synovial fluid of RA patients was observed by Ziolkowska et al [8]. This finding is especially relevant as previous data have already demonstrated that IL-15 has been involved in the survival and activation of human Th17 cells and the production of IL-17 [41].

The present study revealed a non-significant correlation between IL-17 level and RF, in accordance with the findings of Raza et al [38], they also observed a non-significant correlation between RF titer and IL-17 level in RA patients. Furthermore, no significant difference in serum and

synovial IL-17 levels in relation to the presence of RF was observed by Metawi et al [30]. In contrast, significant correlation between the RF and IL-17 level was found by other studies [42]. This is possibly due to differences in the methods used for estimation of RF positivity and titer, and the time of patient's selection.

An Interesting finding was the correlation existing between circulating IL-17 level and measures of disease activity – CRP and ESR. This finding is not surprising since previous animal model studies have suggested that IL-17 acts independently of TNF-α under arthritic conditions and drives disease activity [43]. Furthermore, IL-17 is a potent inducer of CRP from human smooth muscle cells and hepatocytes [44]. Other recent study showed that the percentage of Th17 cells in SF positively correlated with CRP ($r = 0.51$, $p = 0.04$)[39].

Data of this study agree with other studies [42] by showing significant direct correlation between circulating IL-17 levels and anti-CCP positivity and titers .This finding is especially important because in addition to its use as a diagnostic marker, anti-CCP has also been identified as a predictor of poor prognosis in terms of disease severity and joint damage[45]. In addition, synovial IL-17 concentrations were significantly higher in patients with ACPA-positive RA than in those with ACPA-negative RA [46].

Serum IL-17 levels were found to correlate directly with DAS28, TJC and SJC which is in agreement with Metawi et al [30] who noted an increased serum IL-17 levels with higher TJC and SJC and significant positive correlation with DAS28. Moreover, poorer functional status was associated with higher IL-17 levels. In addition, Leipe et al [47] reported that serum IL-17 production and Th17 cell numbers and activity were strongly correlated with the extent of disease activity and systemic inflammation as assessed according to the DAS28 scores and levels of CRP both in early and established RA. On the contrary, Yamada et al [48] showed that the frequency of Th17 cells was neither increased in RA nor correlated with DAS28.

There are some limitations in this study. Firstly the recruitment of patients with established, rather than very early disease. However, identification of patients with very early disease is particularly difficult in the health care setting of a developing country. Secondly, this is only a cross-sectional study and we did not follow the response to treatment and the serum IL-17 and IL-15 levels after treatment which is difficult because of short period of the study and this 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 as well as the range of circulating markers of disease activity and severity which were evaluated.

CONCLUSION:

Serum IL-15 and IL-17 are significantly higher in RA patients than in healthy controls confirming their important role in the pathogenesis of RA and possible target for future therapy. Elevated IL-17 levels could be considered as a possible indicator of more severe clinical course, this is underscored by relatively strong correlations between almost validated disease activity and severity markers including CRP, ESR, anti-CCP, DAS28, TJC and SJC.

REFERENCES:

1. Saxena A, Raychaudhuri SK, Raychaudhuri SP. Rheumatoid Arthritis: Disease Pathophysiology, In:Bagchi D, Moriyama H, Raychaudhuri SP(eds.) *Arthritis pathophysiology, prevention, and therapeutics*. Boca Raton, Taylor& Francis Group: CRC Press; 2011:55-74.
2. Tehlirian CV , Bathon JM . Rheumatoid Arthritis: Clinical and Laboratory Manifestations ,In: Stone J, Crofford LJ , White PH (eds.). *Primer on the Rheumatic Diseases* . 13th ed. USA, springer science;2008:114-21.
3. McInnes IB, Schett G. The Pathogenesis of Rheumatoid Arthritis. N Engl J Med. 2011;365:2205-19.
4. Brennan FM , McInnes IB . Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest. 2008;118:3537–45.
5. Rückert R, Brandt K, Ernst M, Marienfeld K, Csernok E, Metzler C , et al . Interleukin-15 stimulates macrophages to activate CD4+ T cells: a role in the pathogenesis of rheumatoid arthritis? Immunology. 2009;126:63–73.
6. McInnes IB, Liew FY. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis . Immunol Today. 1998;19:75-79.

7. Gonzalez-Alvaro I, Dominguez-Jimenez C, Ortiz AM, Núñez-González V, Pedro Roda-Navarro, Fernández-Ruiz **568** t al . Interleukin-15 and interferon-gamma participate in the cross-talk between natural killer and monocytic cells required for tumor necrosis factor production. Arthritis Res Ther. 2006;8:R88.
8. Ziolkowska M, Koc A, Luszczykiewicz G, Ksiezopolska-Pietrzak K, Klimczak E, Chwalinska-Sadowska H, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. J Immunol. 2000;164:2832-38.
9. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, et al.IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J Immunol. 1998;160:3513-21.
10. Hwang SY, Kim JY, Kim KW, Park MK, Moon Y, Kim WU, et al . IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways, Arthritis Res Ther. 2004;6:R120–R8.
11. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest. 1999;103:1345–52.
12. Chabaud M, Lubberts E, Joosten L, van den Berg W, Miossec P. IL-17 derived from juxta-articular bone and synovium contributes to joint degradation in rheumatoid arthritis. Arthritis Res.2001;3:168–77.
13. Van Bezooijen RL, Van Der Wee-Pals L, Papapoulos SE, Löwik CW, Interleukin 17 synergises with tumor necrosis factor alpha to induce cartilage destruction in vitro, Ann Rheum Dis. 2002; 61:870–76.
14. Davis JM , Matteson EL. Cytokine biomarkers and the promise of personalized therapy in rheumatoid arthritis . Reumatol Clin. 2009;5:143–46
15. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315-24.
16. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum.1995;38:44-48.
17. Osei-Bimpong A, Burthem J. Supplementary techniques including blood parasite diagnosis, In: Lewis SM, Laffan MA, Bain BJ, Bates I. *Dacei & Lewis practical hematology*.11th ed. Germany, Churchill Livingstone Elsevier; 2012:101-18.
18. Barakat AM , Hasony HJ, Al-Khafaji AT . Diagnostic value of antibodies to citrullin-containing peptides in patients with rheumatoid arthritis in southern Iraq. Basrah Journal of Surgery 2009; 15:43-50.
19. Ameer KA, Alosami MH , Salih ES . Comparative study of predicting the risk of cardiovascular diseases in active rheumatoid arthritis iraqi patients by traditional and nontraditional method. G.J.B.B. 2013 ;2:522-26 .
20. Gorial FI .Validity and reliability of CDAI in comparison to DAS28 in Iraqi patients with active rheumatoid arthritis. Fac Med Baghdad. 2012;54:231-33.
21. Al-Salem IH, Al-Awadhi AM .The expression of rheumatoid arthritis in Kuwaiti patients in an outpatient hospital-based practice. Med PrincPract.2004;13:47–50.
22. Kobak S. Demographic, clinical, and serological features of Turkish patients with rheumatoid arthritis: evaluation of 165 patients. Clin Rheumatol. 2011;30:843–47.
23. Firestein GS. Etiology and pathogenesis of rheumatoid arthritis. In: Firestein GS , Budd RC , Harris ED , McInnes IB , Ruddy S, Sergent JS(eds.) . *Kelly's Textbook of Rheumatology*. 8th edition Philadelphia, PA: Saunders Elsevier; 2009:1035-86.
24. Berglin E. Predictors of Disease Onset and Progression in Early Rheumatoid Arthritis A Clinical, Laboratory and Radiological Study. Thesis submitted to Department of Public Health and Clinical Medicine, Rheumatology, Umeå University, Umeå, Sweden.2006.
25. Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. Ann Rheum Dis. 2006;65:845-51.

26. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, et al . The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum.2000 ;43:1831-35.

27. Sharif SK, Eghbal S, Gharibdoost F, Kbarian MA, Shahram F, Nadji A et al. Comparative study of anti-CCP and RF for the diagnosis of rheumatoid arthritis. APLAR Journal of Rheumatology 2007; 10:121– 24

28. Pruijn GJ, Wiik A , Venrooij W J . The use of citrullinated peptides andproteins for the diagnosis of rheumatoid arthritis. Arthritis Res Ther. 2010;12:203.

29. Manhal FS. Cytokine Profile in Patients with Rheumatoid Arthritis . Fac Med Baghdad. 2009;51: 433-36.

30. Metawi SA , Abbas D , Kamal MM, Ibrahim MK. Serum and synovial fluid levels of interleukin-17 in correlation with disease activity in patients with RA . Clin Rheumatol. 2011;30:1201–7.

31. Pavkova Goldbergova M, Pavek N, Lipkova J, Jarkovsky J, Stouracova M, Gatterova J et al .Circulating cytokine pattern and factors describing rheumatoid arthritis: IL-15 as one of the biomarkers for RA? Biomarkers.2012;17:655-62.

32. Petrovic-Rackov L .Pejnovic N. Clinical significance of IL-18, IL-15, IL-12 and TNF-α measurement in rheumatoid arthritis. Clin Rheumatol. 2006;25:448–52.

33. Moran EM, Mullan R, McCormick J, Connolly M, Sullivan O, Fitzgerald O, et al. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumor necrosis factor-alpha, Oncostatin M and response to biologic therapies, Arthritis Res. Ther. 2009;11: R113.

34. Jandus C, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. Arthritis Rheum. 2008;58:2307–17.

35. McInnes IB, Gracie JA, Harnett M, Harnett W, Liew FY.New strategies to control inflammatory synovitis: interleukin 15 and beyond. Ann Rheum Dis.2003;62:ii51-54.

36. Gonzalez-Alvaro I, Ortiz AM, Garcia-Vicuña R, Balsa A, Pascual-Salcedo D, Laffon A. Increased serum levels of interleukin-15 in rheumatoid arthritis with long- term disease. Clin Exp Rheumatol.2003;21:639-42.

37. Lamana A, Ortiz AM, Alvaro-Gracia JM, Díaz-Sánchez B, Novalbos J, García-Vicuña R, et al **.** Characterization of serum interleukin-15 in healthy volunteers and patients with early arthritis to assess its potential use as a biomarker. European Cytokine Network . 2010;21:186-94.

38. Raza K, Falciani F, Curnow SJ, Ross EJ, Lee C-Y, Akbar AN, et al . Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. Arthritis Res Ther. 2005;7: R784-R95.

39. Gullick NJ, Evans HG, Church LD, Jayaraj DM, Filer A, Kirkham BW et al . Linking power Doppler ultrasound to the presence of Th17 cells in the rheumatoid arthritis joint. PLoS One. 2010;5:e12516.

40. González-Álvaro I, Ortiz AM, Alvaro-Gracia JM, Castañeda S, Díaz-Sánchez B, Carvajal I, et al . Interleukin 15 levels in serum may predict a severe disease course in patients with early arthritis. PLoS One. 2011;6:e29492.

41. Annunziato F, Cosmi L, Santarlasci V, Maggi L , Liotta F , Mazzinghi B, et al. Phenotypic and functional features of human Th17 cells. J Exp Med. 2007;204:1849.

42. Roşu A , Mărgăritescu C , Stepan A, Muşetescu A , Ene M. IL-17 patterns in synovium, serum and synovial fluid from treatment-naïve, early rheumatoid arthritis patients. Rom J Morphol Embryol. 2012;53:73–80.

43. Koenders MI, Lubberts E, Loo FA, Oppers-Walgreen B, Bersselaar L , Helsen MM, et al. Interleukin-17 Acts Independently of TNF-a under Arthritic Conditions. J Immunol. 2006;176:6262-69.

44. Patel DN, King CA, Bailey SR, Holt JW, Venkatachalam K, Agrawal A, et al . Interleukin-17 stimulates C-reactive protein expression in hepatocytes and smooth muscle cells via p38 MAPK and ERK1/2-dependent NF-kappa B and C/EBP beta activation. J Biol Chem. 2007;282:27229-38.

45. Meyer O, Labarre C, Dougados M, Goupille P, Cantagrel A, A Dubois, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis. 2003;62:120-26.

46. Suurmond J, Dorjée AL, Boon MR, Knol EF, Huizinga TWJ, Toes REM, et al. Mast cells are the main interleukin 17-positive cells in anticitrullinated protein antibody-positive and -negative rheumatoid arthritis and osteoarthritis synovium. Arthritis Research & Therapy 2011;13:R150.

47. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, Skapenko A, Role of Th17 cells in human autoimmune arthritis, Arthritis Rheum. 2010;62:2876– 85.

48. Yamada H, Nakashima Y, Okazaki K, Mawatari T, Fukushi JI, Kaibara N , et al. Th1 but not Th17 cells predominate in the joints of patients with rheumatoid arthritis. Ann Rheum Dis. 2008;67:1299–304.