# Measuring of Interleukin -22 and IL -17a Levels in Seropositive and Seronegative Rheumatoid Arthritis Patients

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## Abstract

***Background***: "Rheumatoid arthritis (RA) is an inflammatory disease leading to joint disruption". The instant study designed to detect serum markers in RA that could discriminate between seropositive (SP) and seronegative (SN) by evaluating levels of IL- 22and IL -17a in patients with rheumatoid arthritis. ***Methods***: In this cross-sectional study, a total of sixty Rheumatoid arthritis (RA ) patients' age and sex-matched with healthy controls were involved in the present study. The serum IL- -22 and IL -17a levels were measured using an ELISA kit. Results: The mean ±SD age in seropositive and seronegative was (37.22± 11.29 and 34.28±20.3 years, respectively), while in control group was (27.14±9.33 years). Furthermore, serum IL- 22 and IL -17a level was significantly higher in seropositive and seronegative RA patients compared to healthy controls ($P<0.001$). There was no significant variation in serum IL -22 and IL -17a level according to the seropositive and seronegative in RA patients ($p>0.05$), but there was a positive correlation between them ROC test representing a highly sensitive and specific . **Conclusion:** The present study exhibited higher serum IL- 22 and IL -17a levels in seropositive and seronegative in RA patients compared to healthy controls. Therefore, IL- 22 and IL -17a can be considered as a biomarker for RA disease, with high sensitivity and specificity but these cytokines couldn't be used as discriminated between SP and SN in RA patients.

***Keywords:*** *- Rheumatoid arthritis, Seropositive, Seronegative IL22, and IL17a*

## Introduction

"Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial membrane [(1)] Multiple studies have been demonstrated that levels of disease-related biomarkers may be elevated prior to the onset of symptomatic rheumatoid arthritis [(2),(3)] . These biomarkers include rheumatoid «factor (RF), antibodies to «citrullinated protein antigens (CCP), as well as multiple cytokines/chemokines», C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [(4), (5).] Releasing "pro-inflammatory cytokines, as well as other pro-inflammatory molecules, reaches to the mediated of cellular differentiation, inflammation, immune pathology, and regulation of the immune response [(6)]. Selectively Th17 cells generate the signature cytokines such as interleukin 17 (IL-17), IL-21 and IL-22, and have been reported to play a vital role in the chronic inflammatory response and affected consequently joints and led to tissue damage in RA patients [(7)]. The interleukin (IL-22( was a member of the IL-10 family that significantly controls tissue responses to inflammation 2". A few studies propose the pro-inflammatory/ pathogenic role of IL-22 in the onset and progress of RA [(8)]. "The object of this research was to assess the role of certain disease markers such as autoantibodies (ACCP, RF), "cytokines (IL-17 and IL-22) and (hs-CRP, ESR) in the pathogenesis of RA as panel useful in assessing disease with seronegative and seropositive besides efforts to search whether of these cytokines could distinguished between SP and SN included in the of RA .

## Subjects, Materials and Methods

"The considered investigation included 60 patients

with RA (50 females and 10 males), including new diagnoses without treatment divided to 36 seropositive, 24 seronegative providing the "American College of Rheumatology (ACR) criteria for RA". These RA patients were diagnosed by the clinic's rheumatologists and participation of the "Rheumatology Department / Baghdad Teaching Hospital" within the period of Feb 2019 to June 2019. Each Rheumatoid arthritis patients were chosen after excluding patients with any of the following states such as "malignancy, other autoimmune diseases, pregnancy, medications including steroids, antibiotics, CNS" depressants, hormonal therapy, and others. Unrelated, fifty healthy individuals were correspondent to the patient's group in their ages and genders were randomly selected as controls. During the morning five milliliter of Blood was collected from all members of the study groups. Each sample was separated into 2 parts; the first part is the serum for the serological tests, while the second is whole blood for ESR. The concentrations of the serum IL-22 and IL-17 levels in patients and healthy controls were determined by using ELISA Kit, according to the manufacturer's guidance (MyBiosource .USA). On the other hand, RF and ACCP measured by ELISA (Chorus, Italy) the highly sensitive C –reactive protein (hs-CRP) were detected by ELISA (Demedltec diagnostics) and ESR was tested by Westergren method

## Statistical Analysis

Statistical analyses were conducted using the SPSS statistical package for Social Sciences (version 20.0 for Windows, SPSS, Chicago, IL, USA). Data are displayed as mean ± SD for quantitative variables.While number and percentage for qualitative variables. Quantitative data were tested using ANOVA and Kruskal-Wallis test for differences between groups, Pearson's correlation for the relation between groups; while qualitative relations were evaluated using the Chi-square test. P-value of <0.05 was considered statistically significant. Cut-off values were estimated according to ROC [9].

## Result

Demographic characteristics of the studied groups:

Table 1 shows the baseline characteristics of the studied samples according to age and gender with a comparison of significance in studied groups shows that a total of 100 individuals were divided into two groups 60 patients with RA [35 seropositive (SP) and 25 seronegative (SN)], while 40 individual chosen as apparently healthy controls. Results in this table demonstrated that there was no statistically significant difference between males and females in RA patients and control at P>0.05. Moreover, no significant difference was reported in the current study according to age groups between RA groups and control at P>0.05.

**Table 1: The baseline characteristics of the studied groups.**

| Variables | | RA Patients | | Control | P-value |
|---|---|---|---|---|---|
| | | seropositive | Seronegative | | |
| Age (years) | Range | (25-64) | (20-61) | (20-50) | P=0.677 NS |
| | Mean ± SD | 37.22± 11.29 | 34.28±20.3 | 27.14±9.33 | |
| Gender | Male No. (%) | 7 ( 20 %) | 6 (24 %) | 20(50%) | P=0.955 NS |
| | Female No. (%) | 28 ( 80 %) | 19 ( 76 %) | 20(50%) | |
| Total No. | | 35 | 25 | 40 | |

**(*) NS: Non -Significant. at P>0.05.**

The concentration levels of ESR, RF, hs-CRP, and ACPA among studied groups

Table 2 representing the Mean± SD of ESR, RF, hs-CRP, and ACPA, according to studied groups. Analysis study shows that there were no significant differences in the Mean± SD between seropositive (76±23.5;

16.43±9.98, respectively) and seronegative (68±86.2; 17.76±10.29, respectively) in RA patients according to ESR, hs-CRP at P>0.05. Hence, that there was a significant difference in the Mean± SD of RF, and ACPA in RA patients between seropositive (372.5± 34.7; 22.6± 11.6.9, respectively) and seronegetive (12.8± -8.7; 6.6± 1.9, respectively) at P> 0.001. While, the mean concentrations of «ESR, RF, hs-CRP and ACPA» in seropositive RA patients were significantly higher than healthy control (P < 0.001).

**Table 2: distribution of serological and blood marker in the studied groups.**

| Parameters | Patient seropositive | Patients seronegatie | Control | P-value |
|---|---|---|---|---|
| ESR mm/h M ± SD | 7 6± 23.5 | 68 ± 36.2 | 1 6± 2.5 | 0.000 |
| hs-CRP mg/L M ± SD | 16.43 ± 9.98 | 17.7 6± 10.29 | 3.47 ± 10.65 | 0.000 |
| Anti-CCP AU/ml M ± SD | 22.6± 11.6.9 | 6.6± 1.9 | 5.7± 7.2 | 0.000 |
| RF AU/ml M ± SD | 372.5± 34.7 | 12.8± -8.7 | 11.8± 6.7 | 0.000 |

Highly Sig. P=0.000 among patients groups and healthy control

**Mean levels of IL-22 and IL-17a among the studied groups**

The mean concentration of serum IL-22 level was significantly higher in RA patients (SP and SN) than healthy control. The same was true for serum IL-17a level exhibited a significantly higher mean concentration in RA patients versus control P<0.000) .Notably that no differences in mean concentration of both IL-22 and IL-17 between RA patient (SP and SN) as showing in table 3.

**Table 3: Mean concentration of serum cytokines IL-22 and IL-17a among studied groups**

| Parameters | RA Patient seropositive | RA Patients seronegatie | Control | P-value |
|---|---|---|---|---|
| IL-22 pg/ml M ± SD | 26.6 ± 38.7 | 21.2 ± 27.5 | 7.4 ± 8.0 | 0.000 |
| IL-17a pg/ml M ± SD | 18.4 ± 15 | 19.8 ± 11.2 | 9.6 ± 6.7 | 0.000 |

Highly Sig. at P=0.001 among patients groups and healthy control

**Pearson's Correlation Coefficients between IL-22 & IL-17a among RA seropositive**

In the current study, there were significant correlations between IL-22 with IL-17a (r=0.353 with P=0.032), in seropositive RA patients and (r=0.297 with P=0.024 ) in seronegative as show in table 4.

**Table 4 : Pearson's Correlation Coefficients between IL-22 & IL-17a among RA groups**

| Groups | Studied parameter | Pc &p-value | IL-22 | IL-17a |
|---|---|---|---|---|
| Seropositive RA Patients | IL-22 | r | | 0.353* |
| | | p-value | | 0.042 (S) |
| Seronegative RA Patients | IL-17a | r | 0. 297* | |
| | | p-value | 0.024 (S) | |

Pearson Correlation. S.: significant *: Significant at Ps values (0.05)

Estimation of cut-off values, ROC curve, sensitivity and specificity of the IL-22 &IL-17a among RA groups:

In RA patients , the IL-22 cuts-off values was 27.6 pg/ml with sensitivity 86.8 % , specificity 93.9%, and the AUCROCs of s0.952 (P = 0.000) ; while in IL-17a cuts-off values was 16.24 pg/ml with sensitivity 93.4% ,specificity 99 %, with AUROCs of 0.934 (P = 0.000). Table 5

**Table 5 : Estimation of cutoff points, sensitivity, specificity and AUROC of the parameters in RA patients .**

| Parameters | Cut-off points | Sensitivity (%) | Specificity (%) | AURO | P-values |
|---|---|---|---|---|---|
| IL-22 pg/ml | 27.6 | 86.8 | 93.9 | 0.952 | 0.000 |
| IL-17 a pg/ml | 16.24 | 93.4 | 99 | 0.934 | 0.000 |

AUCROC: Area Under receivers operating characteristic, , Sig. at P value < 0.05.

## Discussion

Rheumatoid arthritis (RA) refers to systemic chronic inflammatory disease leading to joint destruction. [10]. information on the differentiation in the systemic inflammatory serum markers between SP and SN RA is insufficient .Consequently, in the current study preferred of serum markers were evaluated in self-governing groups of SP and SN RA patients. It has been suggested that different inflammatory pathways are involved in the development of seronegative (SN RA) and seropositive RA (SP RA). The existing study exposed that female patients were nearly three times more predisposed to RA than men this result was agreeable to some extent among that of local prior studies in Iraq [11], [12]. The preponderance of particular disease activity markers representing the appearance of autoantibodies in early RA these including ACCP and RF that have been conferred a higher risk of further aggressive and prognostic markers of an erosive disease compared to the population had negative for both autoantibodies [13]. The immune complexes containing –ACPA established to induced production of pro-inflammatory cytokines and triggering of macrophages via Fcγ R-dependent augmented by the presence of IgM RF in this process [14]. Regarding ESR and reactivity of h-CRP, it was noted that ESR, significantly raised in seropositive (SP) and seronegative (SN) RA patients, which is nearly harmonious with results of previous works of literature [15],[16] .Here rise in the seropositivity of active disease markers has been additionally connected with continued active inflammatory registered by the long-term height of erythrocyte sedimentation rate (ESR) and disease activity score [17]. The highly-sensitivit CRP (hs-CRP) assay can be applied to distinguish mild disease activity that was associated with inflammation but that was not detectable by routine CRP testing [18]. The results of hs-CRP in this study correspond with other studies [19], [20]. Those studies were proved that hs-CRP consider as a cardiovascular (CV) disease brand in RA patients plus dropped a spot of light on the high incidence of patients with or at the hazard of progressing to CV diseases in RA. Aforementioned study result of increased IL-17a level in RA patients would coincide with many other studies [21],[22], and established its important role in RA pathogenesis . Previous studies found the concentrations level of IL-17a in both serum and synovial fluid were greater in patients with RA than in healthy and recommend that high IL-17a levels may be correlated with a more severe clinical development as mentioned by [23],[24]. Meanwhile, another study observed strong associations of serum and synovial fluid IL-17a levels found with ESR, CRP, RF, and anti-CCP [25], those findings imply that raised serum and synovial IL-17a levels in RA patients correspond to the development of the disease activity and severity. Besides, Metawi *et al* observed that levels of IL-17a in serum and synovial fluid revealed significant positive associations with Disease Activity Score in 28 joints (DAS28) in a cohort of 30 patients with active RA with knee diffusions, while the worse functional state was associated with greater IL-17a levels [26]. In contradiction with the study on RA Egyptian patients, reported that serum level of IL-17a didn't correlate with the DAS28 score but it was significantly greater among critically active patients as corresponded to patients with modest activity [27].

**conclusion**: There was an elevation in both IL-17 and IL-22 in seropositive and seronegative RA patients with high sensitivity and specificity that means IL-22 and IL-17 would act as pro-inflammatory cytokine during the disease course of RA but they couldn't use for discriminated between SP and SN RA.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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