Evaluation of Serum Soluble Interleukin -2 Receptor level in Diagnosis of Rheumatoid Arthritis.

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Abstract

Background: soluble interleukin-2 receptor (sIL-2R) is secreted by lymphocytes upon activation and has been used as a marker of immune activation in several diseases.

Objective: This study aimed to assess the potential clinical utility of serum level of serum soluble interleukin-2 receptor (sIL-2R) as a diagnostic tool in rheumatoid arthritis disease (RA). The study investigated also the association between serum sIL-2R levels with other parameters used for assessment of RA such as rheumatoid factor (RF), erythrocytes sedimentation rate (ESR), C-reactive protein (CRP), and uric acid.

Methods: Serum sIL-2R levels, measured by ELISA, were evaluated in 25 RA patients who have positive rheumatoid factor (RF) and compared with those of 25 normal controls. The correlations with the other parameters were analyzed.

Results: Compared with the healthy control group, RA patients tended to have significantly higher serum sIL-2R and ESR concentrations ($P<0.001$). While no significant difference between both groups in serum uric acid was seen. Positive serum CRP (CRP level>6mg/dl) was found in 56% of patients. The sIL-2R level was positively correlated with RF and ESR, while a slight positive correlation with uric acid was noticed. Serum sIL-2R showed a high sensitivity and specificity for the patients with positive RF.

Conclusions: A sIL-2R level is a sensitive and specific marker and can be useful for diagnosis of RA.

Key words: C-reactive protein, Erythrocyte sedimentation rate, Rheumatoid arthritis, Soluble interleukine-2 receptors.

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that primarily attacks the synovial membrane of the minor joints leading to joint stiffening, swelling, and loss of function in the joints. Its aetiology is unknown, and definitive diagnosis depends predominantly on characteristic clinical features, typical radiographic findings, the presence of auto-antibodies called rheumatoid factors (RF), elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [1, 2]. Failure to meet these criteria does not therefore exclude the diagnosis, especially during the early stages of the disease. There is no single test for the disease and only few symptoms may be present in the early stages. The common test is rheumatoid factor (RF) is present in 80% of adults who have RA [3], an antibody that is presented eventually in the blood of most people with RA. Rheumatoid factors can bind to normal circulating IgG, forming complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a hypersensitive reaction, which leads to chronic inflammation of the joints [4].

Other common laboratory tests include complete blood picture, ESR, which measures inflammation in the body. C-reactive protein is another common test that measures disease activity [5] .Recent research has uncovered an important role of cytokines which promote inflammation, such as tumor necrosis factor-α (TNF-α) and interleukin-1ß (IL-1ß). Also, TNF-α and IL-1 are

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considered to be the key cytokines in the development of RA [6]. Soluble interleukin-2 receptor (sIL-2R; previously known as Tac) is a surrogate marker of T-lymphocyte activation and proliferation [7]. A soluble fraction of the IL-2 receptor is released from the cell membrane. It is the released extracellular domain of the IL-2Rα by activated cells during a variety of autoimmune disorders including rheumatoid arthritis (RA) [8], and systemic lupus erythematosus [9]. SIL-2R and CRP increases in RA [10]. In RA, IL-2 protein and the IL-2 soluble receptor (sIL-2R) are preferentially expressed at disease onset, in comparison with later stages of the disease [11]. Studies that have addressed this theme have shown discordant results since they have reported evidence pro and against an association between the current proposed markers of this disease (CRP and ESR) and sIL-2R [11].

The aim of the present study is (i) to determine whether there is a difference in sIL-2R levels between RA patients (who have positive RF) and healthy controls (ii) to estimate the sensitivity and specificity of sIL-2Rin diagnosis of RA, and (iii) to evaluate whether sIL-2R levels correlate with the other parameters used for assessment of RA.

Materials and Methods

1-Subjects

The patients included in the study appeared free of the conditions that may cause raised serum RF including cancerous diseases, cirrhosis, and inflammatory lung diseases. Other connective tissue diseases that raise RF were excluded by serologic tests such as systemic lupus erythematosus.

The patients with RA were recruited from the private clinics and laboratory of outpatient department. Only patients with positive results for RF who fulfilled the American College of Rheumatology diagnostic criteria for RA [12] were selected for inclusion in the study.

Criteria for exclusion. Pregnant women, patients with cancer, diabetes mellitus, or autoimmune illnesses, patients with hepatitis, or patients under dialysis were excluded from the study.

The study includes 25 women with RA in addition to 25 healthy control women. The patients were assessed by a rheumatologist on presentation. Apparently healthy persons were asked to participate as controls and they had negative RF values and an absence of acute and chronic diseases.

Sample collection: Ten milliliters of peripheral blood was withdrawn from each individual. Two milliliters of fresh blood poured in a tube containing 0.4ml of sodium citrate as anticoagulant in order to estimate ESR. Serum for serological tests was obtained by centrifugation at 3000rpm for 10 minutes and coded serum aliquots were stored at -20°C until it was analyzed.

2-Measurements:

Estimation of RF:

The RF-latex kit supplied by Spinreact ® Company-Spain was used for diagnosis of RF in serum of the individuals The RF-latex is a slide agglutination test for the qualitative and semiquantitative detection of RF in human serum. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF. RF (cut-off, <8IU/ml)

Estimation of serum sIL-2R:

The concentrations of sIL-2Rs in serum samples were measured by the Invitrogen® Human sIL-2Rkit according to the solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA).

Estimation of ESR:

Westergren's method used for estimation of ESR for patients. The

ESR cutoff values, measured by the Westergreen method, are: female< 20mm/h; male< 15mm/h

Estimation of CRP:

The CRP-latex kit supplied by Spinreact ® Company-Spain was used for diagnosis of CRP in serum of the individuals. This kit is a slide agglutination test for the qualitative and semiquantitative detection of CRP in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP. CRP (cut-off value is 6 mg/l),

Estimation of Uric acid:

Uric acid in serum is oxidized by uricase to allantoine and hydrogen peroxide, which under the influence of peroxidase enzyme, 4–aminophenazone, and 2-4 dichlorophenol sulfonate forms a red quinoneimine compound. The intensity of the red color formed is proportional to the uric acid concentration in the sample.

Statistical Analysis

All statistics were carried out using Excell® program-Microsoft Corporation-USA. The results expressed as mean±standard deviation. Correlation coefficient values were estimated by regression analysis. Predictive value, sensitivity and specificity for measured parameters were estimated by the following formulas:

Predictive value of positive result=TP/ (TP+FP)*100%

Specificity=TN/ (TN+FP)*100%

Sensitivity=TP/ (TP+FN)*100%

Where TP=true positive, TN=true negative, FP=false positive, FN=false negative.

Cutoff value for sIL-2Rwas expressed as (mean +2×standard deviation) that equal 1957pg/ml. The difference between groups was estimated using Pooled Student t-test. The difference is said to be difference if p-value is less than 0.05.

Results

The mean sIL-2R concentrations and ESR level were significantly higher ($p<0.001$) in patients with positive RF patients in comparison with healthy controls as shown in Table (1), while no significant difference between both groups in serum uric acid.

Positive serum CRP (CRP level>6mg/dL) were found in 56% of patients (14 / 25). Correlation coefficients (r) values for the patients group showed a positive correlation between the sIL-2R vs. RF (r=0.64) and sIL-2R vs. ESR (r=0.57). A slight positive correlation between sIL-2R vs. uric acid (r=0.34). There is no correlation between each pair of the compared parameters in the control group.

The sensitivity and specificity of the measured parameters are shown in Table (2). Because all patients were positive RF and all controls were negative RF, the sensitivity and specificity for RF=100%. Serum sIL-2R had 84% sensitivity and 96% specificity for the patients with positive RF which is higher sensitivity than other measured parameters. Every sample with values more than the cutoff value of healthy controls (mean + 2×standard deviation) was defined as positive for increased sIL-2Rconcentration. Predictive values showed that the cut off value for sIL-2R=1957pg/ml is an excellent medical decision limit for the prediction of RA.

Table 1: Serum concentration of sIL-2R, ESR, and uric acid of patients and control groups expressed as mean±standard deviation.

Parameter	Patient	Control	p-value
sIL-2R (pg / ml)	2632±1274	935±511	P<0.001*
ESR (mm / hr)	38.3±18.1	8.7±2.6	P<0.001*
Uric acid (mg / dl)	6.1±1.9	4.6±0.9	p>0.05

(*): Significantly different.

Table 2: Sensitivity, Specificity, and Predictive values of the measured parameters.

Parameter	Sensitivity	Specificity	Predictive value
RF	100%	100%	100%*
sIL-2R	84%	96%	94
ESR	78%	96%	95
CRP	64%	92%	86
Uric acid	42%	73%	78

(*): Because all patients have positive RF value and healthy control have negative RF test intentially.

Discussion

Elevated sIL-2Rconcentration in RA patients in comparison with healthy control group (Table 1) is in accordance with many other researches [10, 13]. Iraqi RA patients showed high mean sIL-2Rlevel (2632 ± 1274 pg/ml), while the increase in sIL-2R concentration in other studies were 1532pg/ml [14] and 1855pg/ml [15]. The reason for these differences may be due to the severity of disease or effects of medication on the sIL-2R level in serum as noted in various studies.

Suenaga et al (1998) [16] suggested that sIL-2R measurements to be helpful for the early diagnosis of RA in patients with joint pain, but without symptoms of bone or joint destruction. A high serum sIL-2R level at baseline is a predictor of remission in patients with acute RA [17]. Suenaga et al (1998) [16] have demonstrated that an increased concentration of sIL-2R in the serum of patients with joint pain is a predictor for the future development of RA. Spadaro et al (1997) [18] observed that treatment of RA patients with methotrexate for 6 months was able to decrease the levels of sIL-2R. However, the results of sIL-2R in the present work disagreed with the results of one research. Fro¨de et al (2002) [19] showed that the median levels of sIL-2R did not significantly differ in comparison with those of controls, whereas ESR levels but not CRP were significantly increased. Altogether, these inflammatory indices seem to independently reflect a final pathway of multifactorial events [19]. The reason of the indifference in that study may be due to their low number of patients (n=21) and controls (n=7 only).

An increase of sIL-2R levels during RA has been noted, both in serum/plasma and in synovial fluid [20, 21]. Detailed clinical trials showed that serum sIL-2R levels are related to disease duration and a decline in sIL-2Rconcentration may result from joint improvement [22].

Findings from clinical trials raise a question on whether sIL-2R concentration in serum provides a reliable immunological marker to assess disease activity in RA. Earlier studies reported the possible advantages of sIL-2R measurements for these purposes [23]. Tebib et al (1995) [21] do, however, question the utility of sIL-2R as such a marker, since it is neither specific nor sensitive

to measure disease activity in an outpatient RA population.

The most commonly measured laboratory markers of disease activity in RA are the ESR and CRP. In recent studies, it was reported that CRP is more sensitive than ESR as a marker of disease activity because ESR is additionally affected by several factors, such as age, sex, anaemia, elevated fibrinogen and immunoglobulin levels, renal failure, pregnancy, and abnormal red blood cell morphology [(1)]. Both CRP and ESR give similar information about non-specific inflammation. A high or increasing amount of serum CRP suggests an acute infection or inflammation. CRP appears and disappears more quickly than changes in ESR. Therefore, CRP level may drop to normal following successful treatment, whereas ESR may remain elevated for a longer period [(24)]. As a blood test, CRP is not specific. A high result serves as a general indication of acute inflammation. In cases of inflammatory rheumatic diseases, such as rheumatoid arthritis and lupus, doctors can utilize the CRP test to assess the effectiveness of a specific arthritis treatment and monitor periods of disease flare-up. Its value is as a general indicator, not specific

Some reports indicate relationships between sIL-2R and laboratory markers of inflammation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [(21, 22, 25, 26, 27)]. In patients with RA, IL-2R correlated weakly with ESR (r = 0.24), and CRP (r = 0.24) [(13)]. The finding of our research showed higher r-value for IL-2R with ESR (r=0.57) indicating the reliability of ESR as a diagnostic tool for RA.

The normal serum uric acid in most RA patients are in accordance with general knowledge about serum uric acid in RA[(28)] and may be useful as first step for differentiation between gout and RA.

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