Journal of Kerbala University , Vol. 8 No.1 Scientific . 2010

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

تقييم مستويات مستقبل الانترلوكين الذائب في المصل في تشخيص التهاب المفاصل الرثوي

Eham Amir Ali (PhD)
Department of Medical Biochemistry, College of Medicine, Al-Mustaniriya University, Iraq.
E-mail:reham.khalid@yahoo.co.uk

Abstract

Background: 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). This study investigate the association between serum sIL-2R levels with other parameters used for estimation 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 RF. SIL-2R values were compared with those of 25 normal controls and the correlation with the other parameters was 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. Positive serum CRP (CRP level>6mg/dl) were found in 58% of patients. The sIL-2R level was positively correlated with RF and ESR, while a slight positive correlation with uric acid. Serum sIL-2R showed a highly sensitivity and specificity for the patients with positive RF.

Conclusions: sIL-2R levels may be useful, sensitive, and specific marker for diagnostic of RA.

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

الخـــــلاصة

المقدمة: أجريت هذه الدراسة لتقييم الفائدة السريرية المحتملة في مستويات (sIL-2R) كمؤشر ذو حساسية وفعالية لتشخيص مرض التهاب المفاصل الرثوي، كذلك للتقصي عن مدى الترابط ما بين مستويات (sIL-2R) في مصل الدم وبعض المعايير المستخدمه في تقدير التهاب المفاصل الرثوي وهي سرعة ترسب كريات الدم الحمر والبروتين الفعال-C (CRP) و العامل الروماتيزمي (RF) و حامض البوليك.

الطريقة: قيست مستويات (sIL-2R) بواسطة تقنية Enzyme Linked Immunosorbent Assay (ELISA) في مصل الدم لخمسة وعشرين من مرضى التهاب المفاصل الرثوي وقورنت قيم (sIL-2R) بتلك العائدة إلى (25) شخصا سليما (مجموعة السيطرة) وتم تحليل الارتباط ما بين (SIL-2R) والمتغيرات الاخرى.

النتائج: أظهرت النتائج زيادة معنوية ($p<0.001$) في تراكيز كل من ESR و sIL-2R المقاسة في مصل الدم لمرضى التهاب المفاصل الرثوي مقارنة بمجموعة السيطرة، في حين لم تكن هناك اية اختلافات معنوية ما بين كلا المجموعتين لحامض اليوريك في مصل الدم.
وجد أن CRP في مصل دم المرضى كان ذا قيمه ايجابيه بمقدار 58% وان مستوى الارتباط للعامل (sIL-2R كان عاليا مع كل من (RF و ESR) بينما كان الارتباط بقيمه طفيفة مع حامض البوليك. اظهر (sIL-2R) خصوصية وحساسية عاليتين للمرضى الذين لديهم قيمه أكيدة للعامل الروماتيزمي.
الاستنتاج: يمكن ان يكون تقدير مستويات (sIL-2R) مؤشر دقيق لتشخيص التهاب المفاصل الرثوي.

مفاتيح الكلمات: البروتين الفعال-C (CRP) ، سرعة ترسب كريات الدم الحمر، العامل الروماتيزمي (RF)، التهاب المفاصل الرثوي.

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 a 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 present 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]. On the other hand, recent research has uncovered the 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 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, 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], 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]. However, the 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 control (ii) estimate the sensitivity and specificity of sIL-2Rin diagnosis of RA, and (iii) evaluate whether sIL-2R levels correlate with the other parameters used for estimation of RA.

Patients and Methods:

1-Patients

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

The patients with RA were enrolled from the outpatient private clinics and laboratory. 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 if 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-Methods:

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 value <8IU/ml)[13]

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). An antibody specific for Human sIL-2R has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Human sIL-2Rcontent, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated second antibody. During the first incubation, the Human sIL-2R antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site. After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Human sIL-2Rpresent in the original specimen [14].

Estimation of ESR:

Westergren's method used for estimation of ESR for patients. Briefly, 2ml of blood is mixed with 0.4 ml of 3.8% solution of sodium citrate. Blood is drawn into the Westergreen's tube (which is 2.5mm in diameter upto '0' mark). The tube is placed vertically in a stand in which a spring clip on the top firmly holds the tube against the rubber at the lower end. At the end of 1 hr, the reading corresponding to the top of the red cell layer is noted in mm. 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, 6 mg/l) [15].

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 [16].

3-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 comparing with healthy controls as shown in Table (1), while no significant difference between both groups in serum uric acid.

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	2632±1274	935±511	4.059E-05*
ESR	38.3±18.1	8.7±2.6	1.629E-08*
Uric acid	6.1±1.9	4.6±0.9	0.9*

(*): Significantly different.

Positive serum CRP (CRP level>6mg/dL) were found in 58% of patients (29/50). 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).

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.

Because of all patients are positive RF and all controls are 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.

Discussion:

Elevated sIL-2Rconcentration in RA patients in comparison with healthy control group (Table 1) is in accordance with many other researches [10, 17]. 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 [18] and 1855pg/ml [19]. The reason about 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) [20] suggest 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 [21]. Suenaga *et al* (1998) [20] have demonstrated that an increased concentration of sIL-2Rin the serum of patients with

joint pain is a predictor for the future development of RA. Spadaro *et al (*1997) [22] 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 are disagreed with the results of one research. Fro¨de *et al* (2002) [23] 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 [23]. The reason of the indifference in the mentioned paper may be due to the low number of patients (n=21) and control group (n=7 only).

An increase of sIL-2R levels during RA has been noted, both in serum/plasma and in synovial fluid [24, 25]. 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 [26].

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 [27]. Tebib *et al* (1995) [25] 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 [28]. 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.

ESR is a diagnostic test for inflammation. An increased ESR corresponds to increased non-specific inflammation in the body. In active RA, nonspecific indicators of acute inflammation, such as the ESR and CRP level, are elevated in most (but not all) patients. Some reports indicate relationships between sIL-2R and laboratory markers of inflammation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [25, 26, 29, 30, 31]. In patients with RA, IL-2R correlated weakly with ESR (r = 0.24), and CRP (r = 0.24) [17]. 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 increase in ESR level in RA patients (Table (1)) reflects inflammation that due to the immune response in RA patients. In rheumatoid arthritis (RA) the rate appearance of bony erosions in the early phase of the disease correlated with the mean serum concentration of CRP in some studies. A recent study examining the rate of spinal trabecular bone loss in the first year of rheumatoid disease found a strong correlation between bone loss and serum CRP concentrations. It appears that CRP concentrations reflect the level of "systemic osteoclast-activating factor" [32].
The normal serum uric acid in most RA patients are in accordance with general knowledge about serum uric acid in RA ((Clinical Laboratory Medicine: Clinical))and may be useful as first step for differentiation between gout and RA.

References:

1. Richardson C, Emery P. Laboratory markers of disease activity. J.Rheumatol 1996; 23: 23–30.
2. Tighe H, Carson DA. Rheumatoid factors. In: Kelley WN, Ruddy S, Harris Jr, Sledge CB, eds. Textbook of Rheumatology. Philadelphia, PA: WB Saunders, 1997: 241–249.
3. The Mayo Clinic.com Rheumatoid Factor: Definition.
4. Kuby, Kuby_Immunology; Ch.20 Autoimmunity: p.467.
5. National institute of arthritis and musculoskeletal and skin diseases (NIAMS); http://www.niams.nih.gov/Health_Info/Rheumatic_Disease/default.asp.
6. Taylor PC. Anti-cytokines and cytokines in the treatment of rheumatoid arthritis. Curr Pharm Des 2003; 9:1095–106.
7. Castillo FM, Romero TA, Est´evez J, *et al*. Concentrations of cytokines, soluble interleukin-2 receptor, and soluble CD30 in sera of patients with hepatitis B virus infection during acute and convalescent phases. Clinical and Diagnostic Laboratory Immunology. 2002;9(6):1372–5.
8. Wolf RE, Brelsford WG, Hall VC, Adams SB. Cytokines and soluble interleukin-2 receptors in rheumatoid arthritis. J Rheumatol 1992; 19:524-8.
9. Sawada S, Hashimoto H, Iijma S, *et al*. Increased soluble IL-2 receptor in serum of patients with systemic lupus erythematosus. Clin Rheumatol 1993;12:204-9
10. Vincenzo Pasceri and Edward T. H. Yeh. A Tale of Two Diseases: Atherosclerosis and Rheumatoid Arthritis. Circulation 1999; 100; 2124-6.
11. Corrigan VM, Arastu M, Khan S, *et al*. Functional IL-2 receptor beta (CD122) and gamma (CD132) chains are expressed by fibroblast-like synoviocytes: activation by IL-2 stimulates monocyte chemoattractant protein-1 production. J Immunol 2001; 166: 4141–7.
12. American College of Rheumatology Subcommittee. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Arthritis Rheum 2000; 43: 1905–1915.
13. Robert W Dorner et al. Clinica Chimica Acta 1987; 167: 1 – 21.
14. Rubini, A., et al. (1990) The soluble interleukin-2: biology, function and clinical application. Ann. Inter. Med. 113:8.
15. Chetana Vaishnavi. Immunology and Infectious Diseases 1996; 6: 139 – 144)
16. Schultz A. Uric acid. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis.Toronto. Princeton 1984; 1261-1266 and 418
17. Steiner G, Studnicka-Benke A, Witzmann G, *et al*. Soluble receptors for tumor necrosis factor and interleukin-2 in serum and synovial fluid of patients with rheumatoid arthritis, reactive arthritis and osteoarthritis. J Rheumatol. 1995; 22(3):406-12.
18. Lee GL, Chen MY, Chuang CY, Chen CY. Serum interleukin-2 receptor in systemic lupus erythematosus and rheumatoid arthritis. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi. 1988;21(1):16–22. (English abstract).
19. Pountain G, Hazleman B, Cawston TE. Circulating levels of IL-1 β, IL-6 and soluble IL-2 receptor in polymyalgia rheumatica and giant cell arteritis and rheumatoid arthritis. Br J Rheumatol. 1998;37(7):797–8.
20. Suenaga Y, Yasuda M, Yamamoto M, *et al*. Serum interleukin-2 receptor for the early diagnosis of rheumatoid arthritis. Clin Rheumatol. 1998;17(4):311–7.
21. Kuuliala A, Söderlin M, Kautiainen H, *et al*. Circulating soluble interleukin-2 receptor level predicts remission in very early reactive arthritis. Scand J Rheumatol. 2005 Sep-Oct;34(5):372-5.
22. Spadaro A, Taccari E, Riccieri V, *et al*. Relationship of soluble interleukin-2-receptor and interleukin-6 with class-specific rheumatoid factors during low-dose methotrexate treatment in rheumatoid arthritis. Rev Rheum Engl. 1997; 64: 89–94.

23. Fro¨ de TS, Tenconi P., Debiasi MR. *et al*. TNF-α and sIL-2R levels in rheumatoid arthritis. Mediators of Inflammation. (2002)11; 345–349.
24. Nassonov EL, Samsonov MY, Chichasova NV, *et al*. Soluble adhesion molecules in rheumatoid arthritis. Rheumatology (Oxford). 2000; 39(7):808–10.
25. Tebib JG, Letroublon MC, Noel E, *et al*. sIL-2Rlevels in rheumatoid arthritis: poor correlation with clinical activity is due in part to disease duration. Br J Rheumatol. 1995; 34(11):1037–40.
26. Rubin LA, Snow KM, Kurman CC, *et al*. Serial levels of soluble interleukin 2 receptor in the peripheral blood of patients with rheumatoid arthritis: correlations with disease activity. J Rheumatol. 1990;17(5):597–602.
27. Wood NC, Symons JA, Duff GW. Serum interleukin-2-receptor in rheumatoid arthritis: a prognostic indicator of disease activity? J Autoimmun.1988;1(4):353–61.
28. Lab Tests Online; American Association for Clinical Chemistry http://www.labtestsonline.org/understanding/analytes/crp/glance.html.(2009)
29. Symons JA, Wood NC, Di Giovine FS, DuffGW. Soluble IL-2 receptor in rheumatoid arthritis. Correlation with disease activity, IL-1 and IL-2 inhibition. JImmunol. 1988;141(8):2612–8.
30. Itoh M, Goto Y, Ohta Y, *et al*. Relations between surface expression of the interleukin-2 receptor and release of the soluble form of the receptor in cultured mononuclear cells from patients with rheumatoid arthritis or systemic lupus erythematosus. Clin Rheumatol. 1998;17(1):26–30.
31. Klimiuk PA, Sierakowski S, Latosiewicz R, *et al*. Interleukin-6, soluble interleukin-2 receptor and soluble interleukin-6 receptor in the sera of patients with different histological patterns of rheumatoid synovitis. Clin Exp Rheumatol. 2003;21(1):63–9.
32. Galeazzi M, Morozzi G, Veronesi M, *et al*. [Usefulness of the determination of C reactive protein and other acute phase proteins in rheumatoid arthritis] : Recenti Prog Med. 1995;86(11):456-62.
33. Richard Ravel, Clinical Laboratory Medicine: Clinical Applications of Laboratory Data. Ch.23 Bone, Joint, and Collagen-Vascular Disorders 6th edition (1995) by Mosby Co USA.