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T. Şentürk · G. Kınıklı · M. Turgay · H. Tutkak
M. Duman · G. Tokgöz

Evaluation of interleukin-6 in rheumatoid arthritis as an activity criterion

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Abstract This study evaluated interleukin-6 levels as an activity criterion in rheumatoid arthritis (RA) and compared if with other activity criteria. We evaluated 35 patients with active RA, 31 with inactive RA, and 25 patients with osteoarthritis, in addition to 28 healthy individuals. Serum interleukin-6 levels were higher in active RA patients than in those with inactive RA, or osteoarthritis and healthy individuals ($P<0.001$). Serum interleukin-6 levels of patients with active RA were positively correlated with the erythrocyte sedimentation rate, C-reactive protein, and α_2-globulin levels ($P<0.001$), but there was a negative correlation with serum albumin levels ($P<0.05$). We conclude that interleukin-6 can be responsible for both the most systemic manifestations of RA and for its local manifestations.

Key words Rheumatoid arthritis · Osteoarthritis · Synovial fluid · Interleukin-6

Introduction

Interleukin-6 (IL-6) is a well-investigated cytokine with a multitude of biological activities [1, 2]. In particular, IL-6 mediates acute-phase protein synthesis and terminal B cell differentiation [3]. Since IL-6 has been shown to be a multifunctional cytokine involved in various immune and inflammatory responses, it is very likely that IL-6 plays an important role in the pathogenesis of rheumatoid arthritis (RA) [4, 5]. Several authors [1, 6, 7] have shown high levels of IL-6 to be present in serum and synovial fluids from patients with RA. RA patients' most systemic manifestations and their local manifestations may be explained by the excess production of IL-6 [5, 7–11].

IL-6 is produced from fibroblasts [12], endothelial cells [13], monocyte-macrophage cells [14], synovial cells [12, 15], T and B cells [16], and other cells. Using cytokine probes, Firestein et al. [3] have shown that synoviocyte IL-6 is derived from non-T lymphocytes, type B synovial lining cells, and fibroblasts. IL-6 may act as a protective cytokine by increasing levels of acute-phase proteins such as metalloproteinase inhibitors in patients with RA. On the other hand, it stimulates the activation of polyclonal B cells and may be rather deleterious. Serum IL-6 levels were recently measured in patients with active RA and compared to clinical and laboratory indices of disease activity, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), morning stiffness, rheumatoid factor (RF) titers [17].

The aim of this study was to evaluate whether IL-6 levels can serve as an activity criterion in RA. We measured IL-6 levels in synovial fluid and serum of patients with active RA and compared serum IL-6 levels of patients with inactive RA, patients with osteoarthritis (OA), which is known to be a noninflammatory arthritis, and healthy controls.

Patients and methods

We studied serum samples collected from 66 patients with RA who fulfilled the criteria of the American Rheumatism Association [18], 25 patients with OA (2 males and 20 females, aged 37–75, mean 60.04 years), and 28 healthy individuals (16 males and 12 females, aged 24–40, mean 30.82 years). Among the patients with RA 35 had active disease (10 males and 25 females, aged 16–65, mean 45.06 years), and 31 had remission criteria (4 males and 27 females, aged 32–64, mean 43.84 years). We accepted as inactive RA patients those who had five or more of the following criteria for at least 2 months: Duration of morning stiffness not exceeding 15 min, no fatigue, no joints pain, no joint tenderness or pain on motion, no soft tissue swelling in joints or tendon sheats, ESR less than 30 mm/h in women and 20 mm/h in men [19]. The patients without remission criteria were accepted as active RA. All of the patients with RA were being treated with nonsteroidal anti-inflammatory drugs. Most of them were

T. Şentürk (✉) · G. Kınıklı · M. Turgay · H. Tutkak · M. Duman
G. Tokgöz
Medical School of Ankara University,
Department of Immunology,
İvedik Caddesi No. 77/34, Demetevler 06300,
Ankara, Turkey

also treated with low-dose glucocorticoids (5–7.5 mg/day) and a disease-modifying drug, including hydroxychloroquine, gold salts, or methotrexate.

Synovial fluid samples were collected during knee arthrocentesis from patients with active RA and spun down at 1200 rpm for 10 min. Serum and synovial fluid samples were rapidly frozen after sampling and stored at −70 °C until IL-6 measurement. Serum and synovial fluid IL-6 levels were determined with enzyme-amplified sensitivity immunoassay kits (from Medgenix) using oligoclonal antibody systems containing monoclonal antibodies. Results were read at 450 nm in Biotech EL 311SL reader, and the values were expressed as pictograms per milliliter. ESR was measured by the Westergreen method. Serum CRP and RF levels were measured by nephelometer. α_2-Globulin and albumin were determined by serum protein electrophoresis.

The Mann-Whitney *U* test, correlation analysis, one-way analysis of variance, and the Duncan test were performed for statistical analysis of the results.

Results

Serum IL-6 levels of active RA patients were found to be statistically significant higher than in the other groups ($P<0.001$; Table 1), while those between inactive RA, OA, and controls did not differ significantly ($P>0.05$). IL-6 levels were also measured in the synovial fluid of knee from ten patients with active RA and compared to serum IL-6 levels of the same patients. The mean synovial fluid IL-6 levels in these patients was 17 806 pg/ml (560–48 000 pg/ml), which was 400–500 times higher than serum IL-6 levels.

We examined the correlations between IL-6 levels, acute-phase protein levels, and RF titers in the serum of patients with active RA. In the patients with seropositive RA there was no correlation between IL-6 levels and RF titers ($r=0.16$, $P>0.05$).

There was a positive correlation between serum IL-6 levels and ESR ($r=0.52$, $P<0.001$), CRP ($r=0.37$, $P>0.05$), and α_2-globulin ($r=0.55$, $P<0.001$) but a negative correlation between serum IL-6 and albumin levels ($r=-0.38$, $P>0.05$; Fig. 1).

Discussion

IL-6 is a cytokine that has been found to have multiple effects on the immune system and inflammatory response both in vitro and in vivo. It is likely that IL-6 plays an importance role in the pathogenesis of RA. IL-6 activates hepatocytes and B lymphocytes to produce acute-phase proteins and immunoglobulines [20, 21]. This study examined whether IL-6 is an activation criterion in active RA. We measured IL-6 levels in patients with various rheumatic diseases and compared acute-phase reactants and RF.

Table 1 Comparison of serum IL-6 levels in patients with active RA, inactive RA, osteoarthritis, and controls (all differences vs. active RA: $P<0.001$)

	IL-6
Active RA	52.51±52.91
Inactive RA	16.68±19.76
Osteoarthritis	15.34± 9.35
Control	13.84± 5.61

Our results clearly show that the serum IL-6 level in patients with active RA is significantly higher than in patients with inactive RA or OA or in healthy normals ($P<0.001$). There were no significant differences between inactive RA patients, OA patients, and controls. These results are in accord with those of previous studies [4, 22, 23].

RF is an autoantibody directed against the Fc portion of IgG. It is possible that RF may provide additional stimulation signals in collaboration with immune complexes to monocyte/macrophage to produce cytokines, including IL-6 [3]. Seronegative RA patients have been shown to have high serum IL-6 activity, although serum IgM RF was almost undetectable in these patients, suggesting that IL-6 alone is insufficient for RF production [10, 21, 24]. We also found no correlation between serum IL-6 and RF titers. These results suggest that IL-6 is not the only factor affecting the production of RF.

ESR values of active RA patients are significantly higher than those of patients with inactive RA or OA ($P<0.001$), and there is no statistical difference between patients with inactive RA and OA ($P>0.05$). These results show that ESR is an important indicator of active disease. We found a positive correlation between serum IL-6 and ESR levels in active RA patients but no correlation between serum IL-6 and ESR levels in inactive RA and OA patients.

It has been demonstrated that IL-6 stimulates all spectra of acute-phase proteins in inflammatory states [10]. In our study serum CRP and α_2-globulin levels were higher in active RA patients than in other groups, and there were positive correlations, between serum IL-6 and both CRP and α_2-globulin levels ($P<0.05$ and 0.001, respectively). Serum albumin levels of active RA patients were lower than in other patient groups ($P<0.001$). There was a negative correlation between serum IL-6 and albumin levels inactive RA patients ($P<0.05$). These results show that IL-6 is one of the major cytokines stimulating hepatic acute-phase proteins.

Serum IL-6 levels do not appear to vary with age or sex, as there was no significant difference between patients with OA and healthy controls, the mean ages being of 60.04 and 30.82 years, respectively.

Patients with active RA show clearly high levels of IL-6 in the synovial fluid [6, 20]. IL-6 levels in synovial fluid are 1000 times higher than those in serum; for this reason it is thought that IL-6 may be produced locally in synovial tissue and added to circulation from here [17, 24–26]. In the present study synovial IL-6 levels were found 400–500 times higher than serum IL-6 levels. Our findings also suggest that IL-6 is produced locally in synovial tissue and may be added to circulation from here.

IL-6 levels of patients with active RA was significantly higher than those of other rheumatic patients and healthy individuals and were shown to be correlated with other

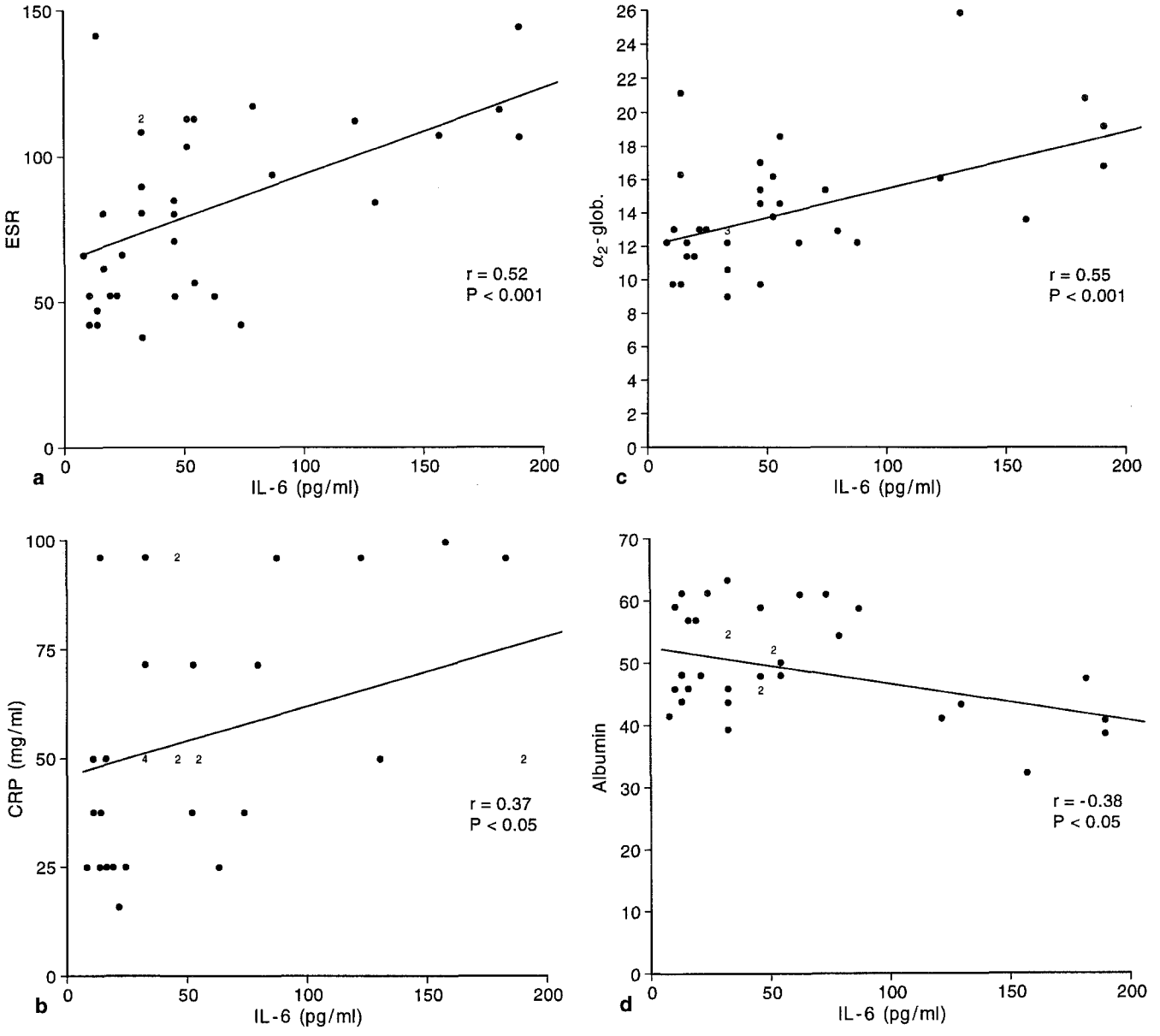

Fig. 1 Correlation in serum between IL-6 and ESR (**a**), CRP (**b**), α_2-globulin (**c**), and albumin (**d**)

acute-phase proteins used for the management of activity. There is evidence that IL-6 is responsible both for systemic symptoms of RA and for local symptoms. Measurement of serum and synovial fluid IL-6 levels may be a useful method for determining disease activity.

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