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THE ROLE OF INTERLEUKIN-8 AND MONOCYTE CHEMOTACTIC PROTEIN-1 IN RHEUMATOID ARTHRITIS

***Vjeroslava Slavić*[1], *Aleksandra Stanković*[2], *Borislav Kamenov*[3]**

[1]*"Dr Simo Milošević" Institute, Igalo*
[2]*Institute for Prevention and Treatment of Rheumatic and Cardiovascular Diseases, Niška Banja*
[3]*Pediatric Clinic, Niš*
Serbia and Montenegro

Summary. *The paper discusses the significant roles of IL-8 and MCP-1 as possible serum and synovial markers of joint inflammation in rheumatoid arthritis (RA). The study involved 30 patients, divided on the basis of parameters of disease activity into (a) those with a highly active disease and (b) those with a moderately active disease. In the highly active RA group, significantly higher concentrations were obtained in both serum (IL-8 $p<0.001$; MCP-1 $p<0.01$) and synovial fluid (IL-8 $p<0.001$; MCP-1 $p<0.001$), compared to the values in the moderately active RA group. The obtained significant differences between concentrations in synovial fluid and serum were indicative of IL-8 and MCP-1 local production. In patients with a highly active RA, the obtained differences were highly statistically significant for both IL-8 and MCP-1 ($p<0.001$), whereas the differences in the moderately active RA form showed to be less sensitive ($p<0.05$) for both of the chemokines. We have demonstrated that IL-8 and MCP-1 local production in synovial fluid is associated with a significant positive correlation ($p<0.05$) obtained in patients with a highly active disease. On the basis of the obtained results we conclude that concentrations of IL-8 and MCP-1 in serum and synovial fluid can serve as good indicators of local inflammation in RA.*

Key words: *Interleukin-8, monocyte chemotactic protein-1, rheumatoid arthritis*

Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by symmetric polyarticular inflammation that is accompanied by destruction of joints, their deformation and functional disorders. The inflammed RA joints show hyperplasia of the intimal lining layer and increased cellularity of the synovial sublining. Predominant cell types involved in synovial inflammation include activated T-cells (1), monocytes/macrophage (2) and neutrophils (3). Increased cellularity is accompanied by increased expression of adhesion molecules involved in cell trafficking and of pro-inflammatory mediators such as cytokines and chemokines. Recently it has become clear that chemokines and their receptors are closely involved in the regulation of organ-specific leukocyte trafficking and inflammation in RA (5, 6, 7).

Interleukin-8 (IL-8) belongs to the CXC subfamily of chemokines, whereas monocyte chemotactic protein-1 (MCP-1) is part of CC subfamily. They are responsible for development and sustenance of chronic inflammatory joint disorders in RA. Recent studies have confirmed that IL-8 and MCP-1 are elevated in synovial fluid and serum of RA patients. IL-8 as a neutrophile chemoattractant is responsible for the increased number of neutrophils in RA joints and, therefore, for the clinical manifestation of joint swell and pain. *In vivo,* it has been shown that innoculation of only one intra-articular injection of IL-8 induces synovial hyperplasia similar to the human RA (8). The role of MCP-1 in RA is chemoattraction of marophages in the inflamed RA joint, which is confirmed *in vivo* where innoculation of only one intra-articular injection of MCP-1 was responsible for a significant increase in the number of neutrophils in the synovial tissue (9). Recent studies have confirmed that MCP-1 concentration in the synovial fluid and serum correlates with clinical manifestations of joint swell and pain, so it can act as a marker of RA joint inflammation (10, 11).

Chemokines have a very important role in angiogenesis, as they induce the formation of new blood vessels in RA synovium. It is this increased vascularity that enables increased extravasation of different leucocytes and sustained synovitis. Therefore RA is considered as "angiogenic" diseases (7, 12, 13).

The Aim of the Study

Our aim was to investigate the possibility of using concentration values of IL-8 and MCP-1 in serum and synovial fluid in RA as a parameter for the assessment of diseased activity, as well as a marker of synovial inflammation.

Patients and Methods

Our research included 30 patients with definitive diagnosis of RA (ACR criteria 1987). Women accounted for 80% of the total number of patients. Mean age was 53.03±13.57 years (min 18, max 73) and mean duration of disease 8.27±8.16 years (min 0, max 30). All patients recruited in this study were RF-positive and with clinical signs of active arthritis. They were subdivided into a group with moderate disease activity (mRA) n=9 and a group with high disease activity (hRA) n=21. The criteria for disease activity included: erytrocyte sedimentation rate (ESR) at least 30 mm/h, Ritchie index at least 10, number of swollen joints at least 5 and duration of morning stiffnes at least 1 h. Moderate disease activity included the presence of one or two of the above criteria and high disease activity included presence of more than two or all of the above mentioned criteria.

IL-8 and MCP-1 concentrations in serum and synovial fluids of RA patients were measured by enzyme-linked immunosorbent assay (ELISA) using the commercial quantakine kits (R&D Systems Inc., MN, USA). The sensitivity immunoassay for IL-8 was within the range 1.5–7.5 pgr/ml (mean 3.5 pgr/ml) and less than 5 pgr/ml in immunoassay for MCP-1. Concentrations under 31.2 pgr/ml in both immunoassay were considered a negative result. Colour intensites were detected on Microplate Manager (Bio-Rad Laboratories, Inc).

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, Version 10.0 (SPSS Inc., IL, USA). The difference in serum and synovial fluid IL-8 and MCP-1 concentrations were assessed by Student's t test, χ^2–test and Pearson's correlation test. Probability (p) less than 0.05 and 0.01 was considered significantly different and less than 0.001 highly significantly different.

Results

Serum IL-8 concentration was within the range 16.89–49.03 pgr/ml (mean 30.04 pgr/ml) in hRA patients, and 18.06–41.05 pgr/ml (mean 24.63 pgr/ml) in mRA patients. In the mRA group, the patients had a significantly frequent regular serum IL-8 concentration (χ^2=2.778, p<0.05).

Serum IL-8 concentration was significantly higher in hRA patients, compared to the mRA patients (t=4.4115, p<0.001) (Fig. 1).

Synovial IL-8 concentration in the hRA group ranged between 678.16-3582.26 pgr/ml (mean 2441.95 pgr/ml) and between 51.61-3430.62 pgr/ml (mean 785.17 pgr/ml). Patients with hRA had a significantly frequent synovial concentration at above 2000 pgr/m (χ^2=8.857, p<0.01).

The concentration of IL-8 in synovial fluid was significantly higher in hRA patients, compared to the mRA patients (t=7.6912, p<0.001) (Fig. 2).

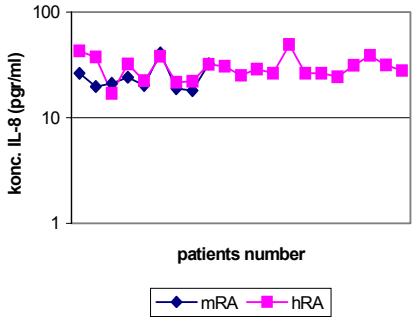

Fig. 1. Serum IL-8 concentration in hRA and mRA

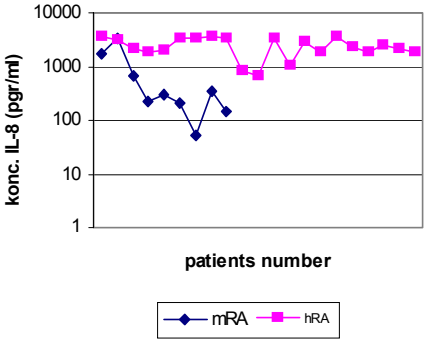

Fig. 2. Synovial concentration in hRA and mRA

The concentration of IL-8 in synovial fluid was significantly higher compared to its serum concentration in hRA patients (t=11.841, p<0.001) and mRA patients (t=2.04, p<0.05). These results confirm IL-8 local production in RA joint and the possibility of its being used as a marker of synovial inflammation.

Serum MCP-1 concentration in hRA patients was within the range 82.40–320.06 pgr/ml (mean 178.22 pgr/ml) and 85.99–276.51 pgr/ml (mean 140.38 pgr/ml). Patients with hRA frequently had a statistically significant serum concentration under 125 pgr/ml (χ^2=10.714, p<0.001). Serum MCP-1 concentration was significantly higher in hRA patients, compared to the mRA patients (t=2.5464, p<0.01) (Fig. 3).

The concentration of MCP-1 in synovial fluid in hRA patients was between 332.72-2798.09 pgr/ml (mean 1467.11 pgr/ml) and between 89.97-694.28 pgr/ml (mean 254.19 pgr/ml). Patients with hRA had a significantly higher synovial concentration, compared to the mRA patients (t=4.8127, p<0.001) (Fig. 4).

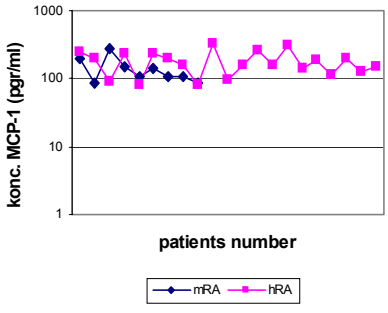

Fig. 3. Serum MCP-1 concentration in hRA and mRA

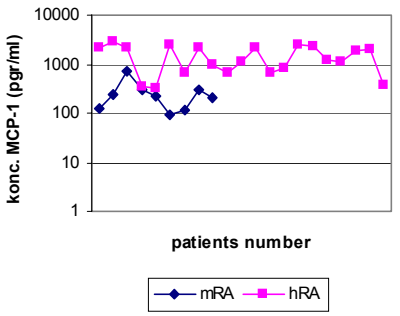

Fig. 4. Synovial MCP-1 concentration in hRA and mRA

Synovial MCP-1 concentration was significantly higher compared to its serum concentration in hRA patients ($t=7.20$, $p<0.001$) and mRA patients ($t=2.30$, $p<0.05$). This results confirm its local production in inflammed RA joint.

Concentrations of IL-8 and MCP-1 in serum and synovial fluid did not correlate in either of the patients group. Meanwhile IL-8 and MCP-1 synovial concentration was in a significant positive correlation in hRA patients ($r=0.492$, $p<0.05$). This correlation is confirmed to be possibly relevant for IL-8 and MCP-1 production in inflammed RA joint.

Discussion

It was previously demonstrated that chemokines have a significant role in the pathogenesis of RA, especially in the patterns of myelo-monocytes migration into an inflamed RA joint. In our investigation we focused on IL-8 and MCP-1. They show clear structural differences and a certain functional homology. First of all, IL-8 is a potent chemoattractant for neutrophils and less potent for monocytes, when compared to MCP-1. On the other hand, MCP-1 is a potent chemoatracttant for monocytes and less potent for neutrophils, compared to IL-8 (14). Next, both of these chemokines exert an angiogenic activity, which has been demonstrated by numerous *in vitro* and *in vivo* studies (15, 16). They are also produced in the same cells. Major cell sources include synovial fibroblasts, synovial stromal cells (10), subchondral bone marrow stromal cells (17), chondrocytes (18), and endothelial small blood vessels (19). Some of them constitutively express IL-8 and MCP-1. The endothelia of micro-vessels exert lower but significant basal secretion of IL-8 and MCP-1 (19). Similar secretion of both of these chemokines has been confirmed in subchondral bone marrow stromal cells (17). Synovial fibroblasts and macrophages constitutively express MCP-1 (20). Pro-inflammatory cytokines, such as IL-1 and TNF-α, can induce the synthesis of the two chemokines. IL-1 is a more potent inductor for IL-8 synthesis, whereas TNF-α is more potent for MCP-1 synthesis (18, 20, 21, 22).

RA synovium is involved in the synthesis and secretion of inflammatory mediators. After being produced in synovium, inflammatory mediators reach the circulation. Mean concentration of serum IL-8 was 18±14 pgr/ml or regular, unlike serum MCP-1 concentration which was 797±142 pgr/ml or increased as suggested by Patel *et al* (23). Our results have demonstrated a significantly higher serum IL-8 concentration in hRA than mRA patients. We also demonstrated that IL-8 has a significantly regular serum concentration in mRA patients. All patients in our analysis had an increased serum MCP-1 concentration, but it was significantly higher in hRA than in mRA patients.

The presence of inflammatory cells in synovial fluid can be explained by complex interactions between cell surface molecules, the extracellular matrix, and chemokines. This suggests that inflammatory RA synovium also has tissue-specific immunological properties that may be influenced by the local microenvironment. IL-8 and MCP-1 were confirmed in RA synovial fluid. However, their synovial concentrations were significantly higher compared to other forms of inflammatory arthritis and OA, as suggested by various authors (20, 24, 25, 26). In synovial fluid, the concentrations of IL-8 and MCP-1 in RA patients were significantly higher in hRA than in mRA. This result confirms the presence of a local inflammation of higher RA intensity that is, in addition, accompanied by severe and more progressive RA. Koch *et al.* demonstrated that concentrations of IL-8 and MCP-1 in synovial fluid were in a significant positive correlation in RA patients, which has been confirmed in our study as well (20).

To summarize, concentration of IL-8 and MCP-1 in serum and synovial fluid are significant markers of disease activity and progression of synovial inflammation in RA joints.

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ULOGA INTERLEUKINA-8 I MONOCITNOG HEMOTAKTIČNOG PROTEINA-1 U REUMATOIDNOM ARTRITISU

Vjeroslava Slavić[1], Aleksandra Stanković[2], Borislav Kamenov[3]

[1]*Institut "Dr Simo Milošević", Igalo*
[2]*Institut za prevenciju i lečenje reumatičnih i kardiovaskularnih bolesti, Niška Banja*
[3]*Dečja interna klinika, Niš*

Kratak sadržaj: *U našem ispitivanju smo ukazali na značajne uloge IL-8 i MCP-1 kao mogućih serumskih i sinovijalnih markera zglobne inflamacije u rematoidnom artritisu (RA). Ispitivanje je obuhvatilo 30 bolesnika sa RA, koji su na osnovu parametara aktivnosti bolesti podeljeni na one koji su imali visoko aktivno i na one koji su imali umereno aktivno oboljenje. U visoko aktivnom RA su dobijene značajno veće koncentracije u serumu (za IL-8 p<0,001 i za MCP-1 p<0,01) ali i u sinovijalnoj tečnosti (za IL-8 p<0,001 i za MCP-1 p<0,001) u odnosu na bolesnike sa umereno aktivnom bolešću. Na njihovo lokalno stvaranje u inflamiranim zglobovima su ukazale dobijene značajne razlike između koncentracija u sinovijalnoj tečnosti i u serumu. Kod bolesnika sa visoko aktivnim RA dobijene razlike su bile visoko statistički značajne i za IL-8 i za MCP-1 (p<0,001), a u umereno aktivnoj formi su dobijene razlike bile manje senzitivne (p<0,05) za oba hemokina. Pokazali smo da je njihova lokalna produkcija povezana u sinovijalnoj tečnosti kod bolesnika sa visoko aktivnim oboljenjem dobijanjem značajne pozitivne korelacije (p<0,05). Na osnovu dobijenih rezultata smo zaključili da koncentracije IL-8 i MCP-1 u serumu i u sinovijalnoj tečnosti mogu da budu dobri pokazatelji lokalne inflamacije u reumatoidnom artritisu.*

Ključne reči: *Interleukin-8, monocitni hemotaktični protein -1, reumatoidni artritis*