MONOCYTE CHEMOATTRACTANT PROTEIN-1, INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR-α IN CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

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ABSTRACT
Objective: to measure the levels of MCP-1, IL-6, and TNF-α in the serum and synovial fluid of a group of patients with JRA, and to correlate these levels with the clinical and laboratory parameters of disease activity.

Results: MCP-1 was significantly elevated in the serum (1021.7±181.9 pg/ml) and synovial fluid (1311.2±185.5 pg/ml) of patients with JRA compared with controls (473.7±56.4, p<0.05 and 217.7±19.8, p< 0.001 respectively). IL-6 was significantly elevated in the serum and synovial fluid of patients compared with controls (p<0.01 and p< 0.001 respectively). Also, TNF-α was significantly elevated in the serum and synovial fluid of patients compared with controls (p<0.01 and p< 0.001 respectively). Levels were significantly higher in patients with systemic-onset JRA (s-JRA) compared with polyarticular and pauciarticular JRA, but not significantly different between pau-
ciarticular and polyarticular JRA. Serum and SF IL-6 correlated significantly with ESR and RAI in patients with s-JRA and polyarticular JRA.

Conclusion: Serum and SF levels of MCP-1, IL-1β and TNF-α were significantly elevated in different JRA-onset types compared with controls, suggesting that they may play an important pathogenic role in all subtypes of JRA. IL-6 and possibly MCP-1 concentrations may be used as markers of disease activity.

Key words: Monocyte Chemoattractant Protein - Interleukin-6 - Tumor Necrosis Factor-α - Juvenile Rheumatoid Arthritis - Cytokines.

INTRODUCTION

Juvenile rheumatoid arthritis (JRA) is one of the most common rheumatic diseases of children and a major cause of chronic disability. It is characterized by an idiopathic synovitis of the peripheral joints, associated with soft tissue swelling and effusion. While systemic-onset JRA (s-JRA) is characterized by the presence of chronic arthritis associated with systemic symptoms, polyarticular- and pauciarticularonset JRA are mainly characterized by joint manifestations.

They are subdivided based on the number of joints involved during the first 6 months of disease (4 or less in the pauciarticular form, 5 or more in the polyarticular form)\(^2,3\).

Although a variety of immunological abnormalities have been described in the various clinical forms of JRA\(^4\), the etiopathogenesis of these diseases is still unknown. Much data, obtained both in humans and animals, suggest that cytokines play an important role in inducing and maintaining chronic inflammation\(^5\).

Monocyte chemoattractant protein-1 (MCP-1) is a chemokine, which acts primarily on monocytes\(^6\). Its levels have been found to be elevated in serum and in synovial fluid (SF) of patients with rheumatoid arthritis (RA), and synoviocytes and synovial macrophages from rheumatoid synovium express MCP-1\(^7,8\). MCP-1 production is induced in a variety of cell types by interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)\(^8\), and in endothelial cells by interleukin-6 (IL-6)\(^9\). MCP-1 appears to mediate at least part of the effects of IL-1, IL-6, and TNF-α: indeed, IL-6 deficient mice (IL-6/- mice) show decreased MCP-1 production and marked impairment.
of leukocyte infiltration into inflammation sites 9.

The aim of the present study was to measure the levels of MCP-1, IL-6, and TNF-α in the serum and synovial fluid of a group of patients with JRA, and to correlate these levels with the clinical and laboratory parameters of disease activity.

MATERIAL AND METHODS

A total of 30 patients (12 males and 18 females, mean age 10.9 years, range 6-16 years) diagnosed according to American Rheumatism Association3 and EULAR2 criteria were included in the study. They were selected from the pediatric and adult rheumatology clinics at El-Minia University Hospital. All patients were subjected to careful history taking and full clinical examination including disease onset type, subsequent articular course, and any complication present. The following investigations were done: complete blood picture, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF) and serum and synovial fluid (SF) levels of MCP-1, IL-6, and TNF-α. All patients had radiographs of the involved joints, and the presence or absence of bony erosions or significant joint space narrowing was determined from these radiographs.

The extent of articular involvement was assessed using Ritchie articular index (RAI), the number of painful joints, the number of swollen joints, functional class, all are assessed in our examined patients and control groups.

Out of 30 patients, 16 children (53.3%) had polyarticular-onset disease and they are characterized by involvement of more than 4 small joints. Eight children (26.7%) had pauciarticular-onset disease and they are characterized by arthritis that remains limited to four or fewer large joints. Six cases (20%) had systemic-onset disease (s-JRA), and systemic features (fever, skin rash) were encountered in the 6 cases.

At time of sampling, all patients were being treated with nonsteroidal anti-inflammatory drugs (NSAIDs) in addition to the following: methotrexate (18 patients), steroids (8 patients), antimalarials (22 patients), and gold therapy in 8 patients.

Serum and synovial fluid levels of MCP-1 were measured with an im-
munoassay, employing a rabbit anti-serum to human MCP-1 for capture and the monoclonal antibodies (Mab) 5D3-F7 to human MCP-1, as described. Enzyme-linked immunosorbent assays (ELISA) for IL-6 and TNF-α were performed using commercially available ELISA kits (R&D system, Minneapolis, MN, USA). These assays utilized a standard "sandwich" technique, with "capture" antibody.

Fifteen apparently healthy, age and sex matched children were included as a control group. Control samples of synovial fluid were obtained from 10 patients undergoing arthroscopy and arthrocentesis for orthopedic disorders. Non of these control cases had local or systemic diseases or infections.

Statistical methods:
Data were expressed as mean±standard error of the mean (Mean ±SEM) for parametric variables, and as numbers and percentages for nonparametric variables. Comparisons between groups were done by independent samples t-test or ANOVA (between 2 or more groups), or Mann-Whitney U or chi-square tests when appropriate. For measurement of linear relations, Pearson's correlation coefficient was considered. But if the relation is not linear, Spearman's rank correlation was used.

RESULTS
Clinical characteristics of the patients were summarized in tables (1 and 2). Rheumatoid factor was positive in 6 cases (20%), 20 patients (66.7) had functional class I, while 10 (33.3) cases had class II. Six cases (20%) had x-ray class I, 22 patients (73.3) had class II, and only 2 cases (6.7%) had x-ray class III.

Table (3) shows the blood elements, ESR, serum creatinine, and SGPT in 30 patients with JRA and in controls. Hemoglobin level was significantly lower (9.9±0.3 gm/dl) in JRA patients compared with controls (11.9±0.22, p <0.01). Platelet count, total leukocytic count and ESR were significantly higher in patients compared with controls (p<0.01 for each).

Table (4) Shows levels of MCP-1, IL-6 and TNF-α in serum (S) and synovial fluid (SF) of patients with JRA and controls. MCP-1 was significantly elevated in the serum (1021.7±181.9
pg/ml) and synovial fluid (1311.2±185.5 pg/ml) of patients with JRA compared with controls (473.7±56.4, p<0.05 and 217.7±19.8, p<0.001 respectively).

IL-6 was significantly elevated in the serum (351.7±40.5 pg/ml) and synovial fluid (5299.5±396.5 pg/ml) of patients with JRA compared with controls (7.4±0.7, p<0.01 and 8.7±1.9, p<0.001 respectively).

Also, TNF-α was significantly elevated in the serum (57.1±5.7 pg/ml) and synovial fluid (62.8±4.4 pg/ml) of patients with JRA compared with controls (15.2±1.8, p<0.01 and 0.9±0.2, p< 0.001 respectively). SF levels of the 3 cytokines were higher than the corresponding serum levels, indicating local production of the chemokines in inflamed joints.

Tables (5 and 6) show serum and synovial fluid cytokines levels in different JRA onset-types and controls. Serum levels of MCP-1 were significantly higher in patients with s-JRA (2308.3±686.7) compared with controls (473.7±56.4, p<0.001), with pauciarticular (500.2±86.5, p<0.05) and with polyarticular JRA (630.1±110.5, p<0.01). SF MCP-1 levels were significantly higher in different disease subtypes compared with controls (p<0.01 for each), and in s-JRA compared with pauciarticular (p<0.05)

Serum and synovial fluid IL-6 and TNF-α levels were significantly higher in the 3 onset subtypes compared with controls, and higher in s-JRA compared with pauciarticular and polyarticular JRA. No significant difference was detected between polyarticular and pauciarticular JRA as regard any of the studied variables (tables 5 and 6).

Results of correlation of serum and synovial fluid cytokines levels with variables of disease activity in patients with JRA onset subtypes are shown in table (7). Serum levels of MCP-1 correlated significantly with ESR in patients with pauciarticular JRA, and with RAI in patients with s-JRA (p<0.05 respectively), and synovial fluid levels correlated significantly with ESR and RAI only in patients with s-JRA (p<0.05 for each)

Serum IL-6 correlated significantly with ESR and RAI in s-JRA and polyarticular JRA (p<0.01 for all), and synovial fluid levels correlated significantly with ESR and RAI in patients.
with s-JRA (p<0.01 for each) and in patients with polyarticular JRA (p<0.05 for each). No significant correlation was found between serum or synovial fluid levels of TNF-α and ESR or RAI in the 3 onset types of JRA.

Table (1): Clinical characteristics of 30 patients with JRA:

<table>
<thead>
<tr>
<th></th>
<th>No. (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
</tr>
<tr>
<td>Male:</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Female:</td>
<td>18 (60)</td>
</tr>
<tr>
<td><strong>Onset:</strong></td>
<td></td>
</tr>
<tr>
<td>Poly:</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Pauci:</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Systemic:</td>
<td>6 (20)</td>
</tr>
<tr>
<td><strong>Systemic Features:</strong></td>
<td></td>
</tr>
<tr>
<td>Present:</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Absent:</td>
<td>24 (80)</td>
</tr>
<tr>
<td><strong>Rh. Factor:</strong></td>
<td></td>
</tr>
<tr>
<td>Positive:</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Negative:</td>
<td>24 (80)</td>
</tr>
<tr>
<td><strong>Functional class:</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td>II</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td><strong>X-Ray Class:</strong></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6 (20)</td>
</tr>
<tr>
<td>II</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>III</td>
<td>2 (6.7)</td>
</tr>
</tbody>
</table>
Table (2): Clinical evaluation of patients with JRA:

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6.0 – 13.0</td>
<td>10.9 ± 0.35</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>6.0 – 60.0</td>
<td>30.8 ± 2.81</td>
</tr>
<tr>
<td>Number of painful Joints</td>
<td>3 – 12</td>
<td>7.4 ± 0.49</td>
</tr>
<tr>
<td>Number of swollen Joints</td>
<td>3 – 10</td>
<td>6.0 ± 0.47</td>
</tr>
<tr>
<td>RAI</td>
<td>6 – 20</td>
<td>12.5 ± 0.77</td>
</tr>
</tbody>
</table>

Table (3): Blood element, ESR, S. creatinine, and SGPT in patients with JRA and controls (Mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>JRA patients n = 30</th>
<th>Controls n =15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>9.9 ± 0.30</td>
<td>11.9 ± 0.22</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Platelets (1000/m³)</td>
<td>324.5 ± 9.1</td>
<td>268.8 ± 17.6</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>WBCs (1000/m³)</td>
<td>6.97 ± 0.28</td>
<td>5.70 ± 0.23</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>64.27 ± 4.44</td>
<td>14.00±1.44</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>S. creatinine (mg/dl)</td>
<td>0.97 ±0.12</td>
<td>0.81 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>38.7 ± 2.3</td>
<td>30.6 ± 3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table (4): Levels of MCP-1, IL-6 and TNF-α (pg/ml) in serum and synovial fluid of patients with JRA and controls (Mean ± SEM).

<table>
<thead>
<tr>
<th></th>
<th>JRA patients n =30</th>
<th>Controls n =15</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. MCP-1</td>
<td>1021.7 ± 181.9</td>
<td>473.7 ± 56.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S. IL-6</td>
<td>351.7 ± 40.5</td>
<td>7.4 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>S. TNF-α</td>
<td>57.1 ± 5.7</td>
<td>15.2 ± 1.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SF. MCP-1</td>
<td>1311.2 ± 185.5</td>
<td>217.7 ± 19.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SF. IL-6</td>
<td>5299.5 ± 396.5</td>
<td>8.7 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SF. TNF-α</td>
<td>62.8 ± 4.4</td>
<td>0.9 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table (5): Serum and synovial fluid cytokine levels (pg/ml) in different JRA onset types and controls (M± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Polyarticular n = 16</th>
<th>Pauciarticular n = 8</th>
<th>s-JRA n = 6</th>
<th>Controls n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. MCP-1</td>
<td>630.1 ± 110.5</td>
<td>500.0 ± 86.5</td>
<td>2308.3 ± 686.7</td>
<td>473.7 ± 56.4</td>
</tr>
<tr>
<td>S. IL-6</td>
<td>354.9 ± 53.1</td>
<td>287.5 ± 67.2</td>
<td>478.3 ± 128.1</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>S. TNF-α</td>
<td>51.56 ± 7.3</td>
<td>45.4 ± 9.03</td>
<td>75.3 ± 17.0</td>
<td>15.2 ± 1.8</td>
</tr>
<tr>
<td>SF. MCP-1</td>
<td>1882.8 ± 346.7</td>
<td>834.0 ± 136.7</td>
<td>2383.3 ± 172.1</td>
<td>217.7 ± 19.8</td>
</tr>
<tr>
<td>SF. IL-6</td>
<td>4899.8 ± 552.2</td>
<td>4173.8 ± 792.7</td>
<td>6866.7 ± 878.5</td>
<td>8.7 ± 1.9</td>
</tr>
<tr>
<td>SF. TNF-α</td>
<td>60.9 ± 5.1</td>
<td>50.9 ± 8.5</td>
<td>85.0 ± 11.1</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

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Table (6): Significance levels (p) of comparison between different JRA onset types and controls.

<table>
<thead>
<tr>
<th></th>
<th>Polyarticular JRA vs Control</th>
<th>Pauci vs s-JRA</th>
<th>Pauci vs s-JRA vs Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. MCP-1</td>
<td>NS</td>
<td>NS &lt;0.01</td>
<td>NS &lt;0.05</td>
</tr>
<tr>
<td>S. IL-6</td>
<td>&lt;0.01</td>
<td>NS &lt;0.05</td>
<td>&lt;0.01 &lt;0.01</td>
</tr>
<tr>
<td>S. TNF-α</td>
<td>&lt;0.01</td>
<td>NS &lt;0.01</td>
<td>&lt;0.01 &lt;0.05</td>
</tr>
<tr>
<td>SF. MCP-1</td>
<td>&lt;0.01</td>
<td>NS NS &lt;0.05</td>
<td>&lt;0.01 &lt;0.05</td>
</tr>
<tr>
<td>SF. IL-6</td>
<td>&lt;0.01</td>
<td>NS &lt;0.05</td>
<td>&lt;0.01 &lt;0.05</td>
</tr>
<tr>
<td>SF. TNF-α</td>
<td>&lt;0.01</td>
<td>NS NS &lt;0.05</td>
<td>&lt;0.01 &lt;0.05</td>
</tr>
</tbody>
</table>

*Pauci: pauciarticular JRA, Poly: polyarticular JRA, s-JRA: systemic-onset JRA

Table (7): Correlation coefficient (r) and significance level (p) of the association of studied cytokines levels with ESR and RAI in patients with different JRA onset types.

<table>
<thead>
<tr>
<th></th>
<th>ESR s-JRA</th>
<th>ESR Poly</th>
<th>ESR Pauci</th>
<th>RAI s-JRA</th>
<th>RAI Poly</th>
<th>RAI Pauci</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. MCP-1: r</td>
<td>0.375</td>
<td>0.333</td>
<td>0.830*</td>
<td>0.685*</td>
<td>0.090</td>
<td>0.319</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS &lt;0.05</td>
<td>NS &lt;0.01</td>
<td>NS &lt;0.01</td>
<td>NS &lt;0.01</td>
<td>NS &lt;0.01</td>
</tr>
<tr>
<td>S. IL-6: r</td>
<td>0.774*</td>
<td>0.823*</td>
<td>0.545</td>
<td>0.884*</td>
<td>0.702*</td>
<td>657</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
<tr>
<td>S. TNF-α: r</td>
<td>0.434</td>
<td>0.216</td>
<td>0.362</td>
<td>0.430</td>
<td>0.317</td>
<td>0.462</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SF. MCP-1: r</td>
<td>0.822*</td>
<td>0.139</td>
<td>0.543</td>
<td>0.830*</td>
<td>0.200</td>
<td>0.513</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SF. IL-6: r</td>
<td>0.897*</td>
<td>0.689*</td>
<td>0.407</td>
<td>0.782*</td>
<td>0.685*</td>
<td>0.516</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>SF. TNF-α: r</td>
<td>0.073</td>
<td>0.301</td>
<td>0.085</td>
<td>0.071</td>
<td>0.090</td>
<td>0.164</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Pauci: pauciarticular JRA, Poly: polyarticular JRA, s-JRA: systemic-onset JRA
DISCUSSION

It has long been recognized that the activity of JRA is reflected in a systemic acute phase response characterized by increased concentrations of a variety of different plasma proteins\(^1\). Hematologic abnormalities often reflect the degree of systemic or articular inflammation, with elevated white blood cell and platelet counts and decreased hemoglobin concentration and mean corpuscular volume\(^1\). In our study, hemoglobin level was significantly lower, while platelet count, total leukocytic count, and ESR were significantly elevated compared with controls (table 3). It was found that the ESR and CRP are often elevated in JRA, and the degree of elevation correlates roughly with disease activity\(^11,12\).

Our results showed that serum and synovial fluid (SF) levels of MCP-1 were significantly higher in patients with JRA compared with controls (table 4). MCP-1 is a chemokine, which acts primarily on monocytes\(^6\). Its levels have been found to be elevated in serum and in synovial fluid (SF) of patients with rheumatoid arthritis (RA), and synoviocytes and synovial macrophages from rheumatoid synovium express MCP-1\(^7,8\). We observed that serum levels were significantly higher in patients with s-JRA compared with controls and with other subtypes (tables 5 and 6). To the best of our knowledge, there is only one paper on MPC-1 in JRA published by De Benedetti et al\(^6\). Our results are in agreement with those of those investigators who found that serum levels of MCP-1 were significantly increased in patients with s-JRA than in controls, and higher than in patients with active polyarticular or pauciarticular JRA\(^6\). We found that in patients with polyarticular or pauciarticular JRA, serum levels of MCP-1 were not significantly different from those of controls (tables 5 and 6). De Benedetti et al\(^6\), reported that in patients with s-JRA, elevated serum levels of MCP-1 was found to be associated with the presence of systemic features at time of sampling. In patients without systemic features, they found that circulating levels of MCP-1 were comparable to those of patients with polyarticular or pauciarticular JRA\(^6\). This observation suggests that systemic production of MCP-1 may occur during systemic phases of the disease.

SF MCP-1 levels were significantly higher in patients with s-JRA compared with controls but not with other
onset-types (tables 5 and 6), and this is in agreement with De Benedetti et al.\(^6\). We found that SF levels of MCP-1 were higher than the corresponding serum levels, indicating local production of the chemokine in inflamed joint.

Our results showed that SF MCP-1 levels correlated significantly with ESR and platelet count, but not with RAI, number of painful or swollen joints (data not shown). In patients with s-JRA, serum and SF MCP-1 correlated significantly with RAI and SF levels correlated significantly with ESR, and in pauciarticular JRA, serum MCP-1 correlated with ESR (table 7). De Benedetti et al.\(^6\) found that in the 3 JRA onset types, no significant correlations were found between MCP-1 and the joint swelling score, ESR, CRP, or platelet count, different patient selection may explain these apparent discrepancies.

Our results showed that serum and SF levels of IL-6 and TNF-\(\alpha\) were significantly higher in JRA patients compared with controls (table 4). Levels were significantly higher in different JRA onset types compared with controls, and in s-JRA compared with polyarticular and pauciarticular JRA (tables 5 and 6). Also, our results showed that in patients with JRA, SF levels of IL-6 and TNF-\(\alpha\) correlated significantly with ESR, and platelet count but not with number of swollen or painful joints (data not shown). Serum and SF levels of IL-6 correlated significantly with ESR and RAI in patients with polyarticular and s-JRA, no significant correlation was detected between TNF-\(\alpha\) levels (serum or SF) and ESR or RAI in all 3 onset types (table 7).

Several studies have shown the important role played by IL-6 in JRA\(^{13-15}\), with elevated serum levels of IL-6 in all three forms of the disease, and a significant correlation between serum IL-6 levels and disease activity, the extent and severity of joint involvement, and laboratory parameters\(^{15,16}\). De Benedetti et al. reported that this proinflammatory cytokine seems to play a pivotal role in the pathogenesis of JCA, and represents a reliable marker of disease activity\(^{15}\).

Our results are in agreement with the results of Hafez et al who found that different types of JRA were associated with significant elevations in the serum levels of many cytokines.
including IL-6 and TNFα. They reported that TNF-α was higher in polyarticular JRA, and IL-6 was higher in s-JRA, and stepwise regression revealed that TNF-α was the first among the studied cytokines, according to their level of significance, that may be important in the pathogenesis of JRA. They concluded that TNF-α might be essential in the induction and maintenance of JRA.

Also, our results are in accordance with the results of Kutukcüler et al who found that SF levels of IL-6 were extremely high in all the patients examined, and that there was a statistically significant difference between patients and control group. They also found that SF TNF-α levels showed significant elevation. As in our results, they reported a significant correlation between ESR value and serum IL-6 levels. Lepore et al reported that in pauciarticular JRA, serum levels of IL-6 and TNF-α were elevated compared to controls, and that SF IL-6 levels were extremely high in all the patients examined. They found no correlation between serum levels of these cytokines with the number of swollen joints, platelet counts, RAI, hemoglobin, ESR, CRP and serum immunoglobulins. In accordance with our results, they found a significant correlation between SF IL-6 and RAI, and no correlation between TNF-α with the laboratory parameters and RAI. It was reported previously that not only CRP and ESR values, but also plasma IL-6 and TNF-α concentrations may be used as markers of disease activity. However, it was clear that conventional inflammation indicators such as ESR appeared to correlate better, and are also cheaper.

De Benedetti et al found that in patients with s-JRA, circulating levels of IL-6, measured as HGF activity, are markedly elevated and significantly higher than those in patients with polyarticular or pauciarticular JRA, and these findings have been confirmed by studies employing immunoassays. Another study reported that in s-JRA, SF levels of IL-6, measured as HGF activity, are significantly higher than in pauciarticular JRA or in adult rheumatoid arthritis (RA). Studies on circulating TNF-α levels provided contradictory results, possibly due to the inaccuracy of available immunoassays for TNF-α measurements. This fact may explain the discrepancy between our results and those of Hafez et al.
who found that serum levels of TNF-α were higher in polyarticular JRA, while in our results, it was higher in s-JRA (Tables 5 and 6).

Eberhand et al studied the number of SF cells producing TNF-α, TNF-β and IL-6 in patients with JRA: they reported that 3/3 samples from patients with systemic JRA contained cells producing IL-6, while this was the case in only 9/29 and 4/13 samples from patients with pauciarticular or polyarticular JRA, respectively22. These findings indicate that, in comparison with other chronic arthritides, systemic JRA is characterized by prominent production of IL-6; moreover, increased production of IL-6 appears to explain several clinical and laboratory features of this systemic disease15,23,24. An increase in circulating levels of IL-6 (2 to 8-fold), but not of IL-1 or TNF-α, precedes the fever peak12,15. Serum IL-6 levels are also significantly correlated with platelet counts15, and IL-6 has been shown to promote megakaryocyte growth and thrombopoiesis in vivo25. Prominent IL-6 production appears also to explain characteristic features of systemic JRA, such as microcytic anemia and impairment in linear growth25. The same investigators reported that the large amount of circulating IL-6 found in patients with s-JRA is the main factor responsible for the characteristic extraarticular features of this disease26.

Regarding joint involvement, previous studies have observed in patients with s-JRA, a direct correlation of serum levels of IL-6, but not of IL-1β or TNF-α, with the extent and severity of joint involvement as measured by joint scores15,20,26,27. In patients with s-JRA, SF levels of IL-6 are markedly elevated and significantly higher than those found in patients with pauciarticular or with rheumatoid factor positive RA, while no significant differences in IL-1β, or TNF-α levels were found20. IL-6 induces endothelial cell production of IL-8 and MCP-1, 2 chemokines responsible for recruitment and activation of granulocytes and monocytes, respectively, and IL-6−/− mice show markedly impaired leukocyte accumulation at the inflammatory site9. Moreover, IL-6 is involved in the recruitment of mesenchymal vascular cells, and neoangiogenesis in vivo, has been shown to induce proliferation of synovial fibroblasts and to play a key role in the generation and activation of osteoclasts in vitro, and in bone resorption in vivo26.
In addition, there are finding indicating that IL-6 in the synovial compartment may also play a role in joint inflammation and in the damage to articular and periarticular tissues; indeed, IL-6 has been suggested to play a role in decreased proteoglycan production by chondrocytes, and has been shown to induce and activate osteoclasts, and to promote synovial fibroblast proliferation.

In agreement with our results, De Benedetti et al found that TNF-α was measurable in the majority of SF fluid samples they studied, and no significant differences in its levels were found among the 3 forms of chronic arthritides they studied (polyarticular JRA, pauciarticular JRA, and RA). The results of a controlled study on the efficacy of the treatment of RA with a monoclonal antibody (Mab) to TNF-α show not only the potential efficacy of treatment specifically aimed at inhibiting inflammatory cytokines, but also the important role of TNF-α in the pathogenesis of RA. The same investigators reported that treatment of one patient with severe s-JRA with the same Mab to TNF-α was not associated with any evident change in arthritis activity, although an important effect on fever was observed.

These findings suggest that, although similar levels of a cytokine may be present in different diseases, their relevance in the clinical manifestations may be different.

In conclusion, serum and SF levels of MCP-1, IL-6 and TNF-α were significantly elevated in different JRA-onset types compared with controls, suggesting that they may play an important pathogenic role in all subtypes of JRA. Levels were significantly elevated in s-JRA compared with polyarticular and pauciarticular JRA. SF levels of the 3 cytokines were higher than the corresponding serum levels, indicating local production of the chemokines in inflamed joints. In addition, not only ESR and platelet count, but also IL-6, and possibly MCP-1 concentrations may be used as markers of disease activity.

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