Levels of Antioxidant Proteins and Soluble Intercellular Adhesion Molecule-1 in Serum of Patients with Rheumatoid Arthritis

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Abstract. Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1), ceruloplasmin (Cp), and transferrin (Tf) were measured in patients with rheumatoid arthritis (RA) and the correlations of these parameters with disease activity were investigated. Serum sICAM-1 levels were determined by a sandwich enzyme-linked immunosorbant assay (ELISA) in serums from 42 patients with RA and 30 healthy controls. Erythrocyte sedimentation rate (ESR) was determined by the Westergren method and C-reactive protein (CRP), Cp, and Tf by nephelometric methods. Disease activity was assessed by standard criteria. Serum Tf levels were significantly diminished and serum levels of sICAM-1 and Cp were significantly increased in patients with RA, compared to healthy controls. Serum sICAM-1 levels showed negative correlation with serum Tf levels \(r = -0.47, p < 0.01\), and positive correlation with serum Cp levels \(r = 0.49, p < 0.001\). There was weak positive correlation between sICAM-1 levels and the Ritche articular index (RAI) scores \(r = 0.32, p < 0.05\) and serum CRP levels \(r = 0.44, p < 0.01\), but no significant correlations of sICAM-1 levels with ESR, patient's age, or duration of disease. There were no significant correlations between values of serum CRP, RAI score, or ESR with serum CP or Tf levels. This study indicates that serum sICAM-1, together with other parameters, is a useful and novel marker for evaluating the disease status and activity of patients with RA. (received 10 January 2002, accepted 28 February 2002)

Keywords: soluble intercellular adhesion molecule-1, ceruloplasmin, transferrin, rheumatoid arthritis

Introduction

Inflammation is characterised by the accumulation of leukocytes and other mesenchymal cells at sites of injury or infection. Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology. The inflammed synovium in RA is characterized by marked hyperplasia of the synovial membrane, neovascularization, and massive infiltration of leukocytes [1,2].

Adhesion molecules are defined as substances that provide the contact and interaction of cell-cell or cell-extracellular matrix. These molecules play a role in many functions of the immune system. On the basis of certain features, adhesion molecules are generally categorized in three groups: the immunoglobulin supergene family, the integrins, and the selectins. Intercellular adhesion molecule-1 (ICAM-1) is a cytokine-inducible single-chain glycoprotein belonging to the immunoglobulin supergene family [3]. ICAM-1 is a protein of 55,000 kDa that is expressed in different tissues because of differential glycosylation [2]. It is induced on epithelial cells, fibroblasts, and endothelial cells by cytokines that are secreted by monocytes and T lymphocytes, including tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), TNF-\(\beta\), interleukin-1\(\alpha\), IL-\(\beta\), and interferon-\(\gamma\) (IFN-\(\gamma\)) [4-9].

Adhesion molecules expressed on vascular endothelium and on circulating leukocytes play an important role in the inflammatory response by regulating adhesion of leukocytes to vascular...
endothelial cells, transmigration through endothelium, and cell-cell interactions in the immune response [10,11]. Leukocyte-endothelial cell interaction molecules mediate the adhesion of inflammatory cells to the vessel walls, as well as their extravasation. Expression of selectins and members of the immunoglobulin superfamily (ICAM-1, VCAM-1) by microvascular endothelial cells is a common hallmark of inflammatory reactions [12]. Like E-selectin, ICAM-1 is expressed in abundance on vascular endothelium after several hours of stimulation by IL-1, TNF, and other cytokines [13].

Soluble cell adhesion molecules are released from cells and are found in the circulation as well as in body fluids [14,15]. Interestingly, ICAM-1 has been shown to serve as the ligand for rhinoviruses, some strains of Coxsackie virus, and leukocytes [16]. Interest has been generated in the measurement of soluble adhesion markers as possible indicators of rheumatic disease activity, and several show promise. Among the rheumatic diseases, the adhesion molecules have been most extensively studied in RA, but with conflicting results [17,18].

Antioxidants are compounds that inhibit lipid peroxidation by interfering with the chain reaction of peroxidation and/or by scavenging reactive oxygen radicals. Human blood serum behaves as a powerful antioxidant in certain in vitro systems, and most of this activity has been attributed to the presence of transferrin (Tf) and ceruloplasmin (Cp) [19-21].

Ceruloplasmin (Cp) is a plasma glycoprotein that is primarily synthesized in the liver and secreted into the blood [22]. A primary physiologic role of Cp involves plasma redox reactions. Cp permits the incorporation of iron into transferrin (Tf) without the formation of toxic Fe products [23,24]. Under physiologic conditions, Cp is also important in the control of membrane lipid oxidation, probably by direct oxidation of cations, thus preventing their catalysis of lipid peroxidation [24,25].

To our knowledge, there has been no previous study that measured serum levels of both antioxidant proteins and sICAM-1 in patients with RA. Therefore, we aimed to investigate serum antioxidant proteins and sICAM-1 levels in RA to ascertain whether these parameters were correlated with the clinical features of this disease.

Materials and Methods

We studied 42 patients with RA (8 men, 34 women; mean age, 45.8 ± 9.2 yr; range 28-65 yr), and 30 healthy subjects (10 men, 20 women; mean age, 42.7 ± 8.4 yr; range 25-65 yr). The mean duration of the disease was 59.8 ± 44.8 mo (range 5-240 mo). Patients with RA were examined and the diagnosis confirmed by at least two experienced rheumatologists, according to the 1987 revised criteria of the American College of Rheumatology. All patients had active disease, defined as the presence of at least 3 of the following features: >6 swollen joints, CRP >9.6 mg/L, morning stiffness >45 min duration, and Ritchie Articular Index (RAI) score >10. None of the patients had received glucocorticoids within the prior 3 mo. Fifteen patients were taking a combination of methotrexate and hydroxychloroquine and 32 patients were receiving nonsteroidal anti-inflammatory drugs at the time of sampling. Ten patients were not receiving any anti-rheumatic therapy at the beginning of this study. None of the subjects in this study consumed alcohol and none had an absorption defect. The control subjects were all healthy hospital personnel. The rheumatoid arthritis patients and healthy controls were recruited into the study after obtaining their informed consent.

Erythrocyte sedimentation rate (ESR) was determined by the Westergren method using anticoagulated whole blood. Venous blood was collected in Vacutainers without additive, allowed to clot for 30 min at room temperature, and centrifuged at 2000 x G for 5 min. Serum aliquots were stored at -80°C prior to analysis. Hemolysed samples were excluded.

Levels of sICAM-1 were measured by a sandwich ELISA kit using two monoclonal antibodies, directed against different epitopes of sICAM-1 (Catalog #1-573-659, Roche Diagnostics). During the first incubation step, sICAM-1 in standards and samples is simultaneously bound by a biotin-labeled antibody and a peroxidase-conjugated detection antibody. The sandwich complex binds via the biotin-labeled antibody to the streptavidin-coated surface of a microtiter plate. Subsequent to a washing step, the peroxidase enzyme in the complex reacts with tetramethylbenzidine as a substrate to form a chromogen that is assayed photometrically. The
developed color is proportional to the concentration of sICAM-1. A standard curve is prepared by plotting absorbance versus concentration and the sICAM-1 level of the test sample is calculated from the calibration curve.

Serum C-reactive protein (CRP), Cp, and Tf were determined by nephelometric methods (Array-360 Protein System, Beckman Instruments).

Statistical analyses were performed by t-test and the results were expressed as mean ± SD. Correlations between the variables were tested by Spearman’s rank correlation coefficient (r), with p <0.05 considered to be significant.

Results

The data for sICAM-1, CRP, ESR, Cp, and Tf in specimens from the patients with RA and healthy controls are summarized in Table 1. When compared with the levels in controls, the values of sICAM-1, Cp, CRP, and ESR in the RA patients were significantly elevated and the level of Tf was significantly decreased. Negative correlation between sICAM-1 and Tf, and positive correlation between sICAM-1 and Cp were observed (r = -0.47, p <0.01 and r = 0.49, p <0.001 respectively).

In the RA patients, serum sICAM-1 levels were compared with various clinical variables and measures of disease activity, including the RAI score, ESR, and serum CRP level. There were relatively weak but significant positive correlations between the sICAM-1 level and RAI score (r = 0.32, p <0.05) and between the serum sICAM and CRP levels (r = 0.44, p <0.01). However, the sICAM-1 level was not significantly correlated with ESR, age of the patient, or disease duration. There were no significant correlations between values of serum CRP, RAI score, or ESR with serum CP or Tf levels.

Discussion

Rheumatic diseases are characterized by inflammation of connective tissue involving different organs in a multisystemic way. The diagnosis of these disorders and the evaluation of disease activity are based on clinical findings and some laboratory tests showing the presence of autoantibodies, changes in serum immunoglobulins, and acute phase reactant proteins [9,26].

Cp is a prominent serum antioxidant that can scavenge a variety of oxygen-derived free radicals [27-29]. It is important for normal release of cellular iron because of its ferroxidase activity, a catalytic oxidation of Fe²⁺ to Fe³⁺. Thus, Cp permits the incorporation of Fe into Tf without the formation of toxic Fe products [23,24,29]. By keeping iron in Fe³⁺ state, Cp prevents it from undergoing the redox cycles that initiate toxic effects [24,29]. We found increased serum Cp levels and decreased serum Tf levels in patients with RA. In agreement with our findings, Kose et al [30] observed increased plasma Cp and decreased Tf levels in RA patients. On the other hand, Ashour et al [31] reported decreased plasma Cp levels in children with juvenile RA.

During the past few years, cell-cell interactions have been discovered that are critically involved in the development of inflammatory lesions. Regardless of the primary immunopathogenic mechanisms, the development of vascular inflammatory infiltrates requires dynamic interactions between leukocyte surface receptors and their ligands on the endothelial...

Table 1. Clinical features and biochemical parameters in healthy controls versus patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 30)</th>
<th>RA Patients (n = 42)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>42.7 ± 8.4</td>
<td>45.8 ± 9.2</td>
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<tr>
<td>Disease duration (mo)</td>
<td>-</td>
<td>59.8 ± 44.8</td>
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<tr>
<td>RAI (score)</td>
<td>-</td>
<td>38.2 ± 11.8</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>16.2 ± 8.9</td>
<td>56.9 ± 17.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CRP (mg/L)</td>
<td>1.4 ± 0.6</td>
<td>26.4 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>sICAM-1 (ng/ml)</td>
<td>231.9 ± 24.8</td>
<td>496.9 ± 64.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cp (mg/dl)</td>
<td>35.5 ± 8.4</td>
<td>51.4 ± 33.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tf (mg/dl)</td>
<td>286.3 ± 38.6</td>
<td>210.0 ± 33.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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Data are means ± SD.

<sup>a</sup>p <0.0001 versus controls; <sup>b</sup>p <0.001 versus controls.
Fig. 1. (above) Correlation between serum sICAM-1 and serum Cp in RA patients ($r = 0.49$, $p < 0.001$)
Fig. 2. (below) Correlation between serum sICAM-1 and serum Tf in RA patients ($r = -0.47$, $p < 0.01$)

Antioxidant proteins and sICAM-1 in rheumatoid arthritis
cell surface. In order to infiltrate tissues, circulating leukocytes roll over the endothelial cell membrane through interactions between carbohydrates and selectins. Additional stimuli (chemokines and other co-stimulatory signals) activate leukocyte integrins, which bind tightly to their endothelial counter-receptors of the immunoglobulin superfamily, intercellular adhesion molecule-1 [(ICAM-1, ICAM-2, and vascular cell adhesion molecule-1 (VCAM-1)]. These interactions are also involved in leukocyte transmigration through the endothelial layer [9,32].

A common feature of autoimmune diseases such as juvenile chronic arthritis and systemic lupus erythematosus is dysregulation of normal immune responses, which leads to chronic inflammation and tissue damage. Adhesion molecule expression and interactions are probably involved in the initiation and propagation of autoimmune diseases [33].

sICAM-1 levels have been reported to be elevated in inflammation, infections, and cancer. Changes of sICAM-1 levels have been reported in adult rheumatic diseases [34]. Levels of soluble adhesion molecules have been shown to reflect their cell surface expression in vitro, and thus may provide a useful surrogate marker of surface expression at inflammatory sites [35].

Interestingly, combinations of cytokines can differentially modulate the induction of ICAM-1, VCAM-1, and E-selectin on endothelium and the induction of ICAM-1 on other cell types. Therefore, the various patterns of cytokines or inflammatory mediators associated with a specific inflammatory disease may lead to differential induction of endothelial and leukocyte adhesion molecules, which in turn may influence the pathogenesis of that disease [36]. The present study revealed that serum sICAM-1 is elevated in patients with RA. Our results confirm the similar findings of Mason et al [36] in RA patients. Littler et al [37] also reported high sICAM-1 levels in RA patients, as well as positive correlation between the disease activity and sICAM-1 levels, and usefulness of sICAM-1 levels in following the clinical course of patients with RA. In the present study, we found significant positive correlation between the serum sICAM-1 level and CRP and RAI values, which are also indicative of disease activity.

Although an immunoregulatory role of sICAM-1 has not been elucidated, the presence of increased levels of sICAM-1 occurs in a various inflammatory disorders [37]. Some investigators have proposed that soluble adhesion molecules be used as markers for disease activity in autoimmune diseases [38].

In the present authors’ opinion, serum sICAM-1 level may be a useful and novel marker for evaluating the disease status and activity of RA, when considered together with other parameters. Further studies are needed to elucidate the specific relationships between antioxidant proteins and sICAM-1 in patients with RA.

References


