Increased Serum Sialic Acid Levels in Primary Osteoarthritis and Inactive Rheumatoid Arthritis

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ALTURFAN, A.A., USLU, E., ALTURFAN, E.E., HATEMI, G., FRESKO, I. and KOKOGLU, E. Increased Serum Sialic Acid Levels in Primary Osteoarthritis and Inactive Rheumatoid Arthritis. Tohoku J. Exp. Med., 2007, 213 (3), 241-248 — Accumulation of oxidized proteins and impaired antioxidant system have been shown to be associated with arthritis. Serum sialic acid (SA) is known as a parameter of inflammation. In the present study, to explore the potential role of SA in arthritis, we measured serum SA levels, plasma protein oxidation, and antioxidant status in patients with primary osteoarthritis (POA) and inactive rheumatoid arthritis (RA). Inactive RA (iRA) was defined upon the American College of Rheumatology criteria for clinical remission of RA. A total of 40 patients (20 POA patients, including 4 male subjects, and 20 iRA female patients) and 20 healthy female subjects were included in this study. SA, antioxidants, and protein oxidation levels were determined spectrophotometrically in serum or plasma samples. Serum SA levels were significantly increased in POA (3.34 ± 0.37 mM, $p < 0.0001$) and iRA (3.11 ± 0.47 mM, $p < 0.05$), compared with healthy controls (2.41 ± 0.16 mM). Plasma total antioxidant activity, plasma superoxide dismutase activity and serum reduced glutathione levels were significantly decreased in patients with POA and those with iRA, whereas plasma carbonyl content and serum total protein were increased in those patients. Moreover, plasma total thiol levels were significantly increased in iRA and decreased in POA. Thus, increased SA and protein oxidation levels are associated with the decreased antioxidant levels in POA and iRA patients. These results suggest that SA may be considered as a potent defense molecule against oxidative damage in arthritis. Antioxidant therapy may halt or ameliorate the progression of arthritis. ———— osteoarthritis; rheumatoid arthritis; oxidative stress; antioxidants; sialic acid

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Osteoarthritis (OA) results from the pathological imbalance of degradative and reparative processes (Abramson and Krasnokutsky 2006).

In OA, the entire joint structure is affected. The cartilage, synovium, and bone can all be major sites for production of cytokines, growth factors,

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chemokines, and mediators, all classically associated with inflammation, which eventually promote progressive joint destruction (Pelletier et al. 2001; Loeser 2006). Cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6, IL-8, and nitric oxide (NO), act on chondrocytes to cause this catabolic state, creating a positive amplification loop leading to protease production. Eventually, many of these cells, via NO and other oxygen species production, undergo apoptosis and die (Ayral et al. 2005). On the other hand, primary osteoarthritis (POA) is also a degenerative joint disease, which occurs in the absence of any underlying abnormality (Glass 2006).

Rheumatoid arthritis (RA) is a complex systemic disease and the most common inflammatory arthritis, affecting from 0.5 to 1% of the general population worldwide. Despite intensive work, only modest progress has been achieved in determining the cause of RA. The mediators of inflammation, cytokines, growth factors, chemokines, adhesion molecules and matrix metalloproteinases have been carefully defined. These products attract and activate cells from the peripheral blood and evoke proliferation and activation of synoviocytes. Proteases can subsequently lead to behaviour resembling a localized tumor, which invades and destroys articular cartilage, subchondral bone, tendons and ligaments (Muller-Ladner et al. 2005; Sommer et al. 2005).

OA is commonly described as a non-inflammatory disease in order to distinguish it from RA (Benito et al. 2005). However, in OA, the synovial compartment is also regarded as important since synovial proliferation and inflammatory changes are reported in some patients undergoing acute episodes of synovitis (Ayral et al. 2005). Despite this, it is still unclear whether inflammation is a feature of all patients with OA at some stage of their disease (Bonnet and Walsh 2005).

The term oxidative stress refers to the situation of a serious imbalance between production of reactive oxygen species/reactive nitrogen species (ROS/RNS) and antioxidant defense. Attack of ROS upon proteins produces carbonyls as a marker of oxidative protein damage. It is based on the fact that several ROS attack amino acid residues in proteins to produce products with carbonyl groups. Protein carbonyl content (CC) is actually the most general indicator of peroxidation intensity and it has been observed in several diseases including Alzheimer’s disease, diabetes, inflammatory bowel disease and arthritis (Renke et al. 2000; Dalle-Donne et al. 2003).

Another way of expressing oxidative damage to proteins is reflected by increased level of thiol groups (-SH) in proteins. They are often essential for protein stability and function, but at the same time exceptionally vulnerable to oxidation (Renke et al. 2000; Meyer and Hell 2005).

On the other hand, the body’s defense mechanisms would play an important role in the form of antioxidants and try to minimize the oxidative damage. Antioxidant is a substance that, when present at low concentrations compared with those of an oxidizable substrate, may either prevent the generation of toxic oxidants or intercept the propagation of chain reaction produced by the oxidants (Renke et al. 2000; Surapaneni and Venkataramana 2007). These might be referred to as enzymatic antioxidants such as superoxide dismutase (SOD), metabolic antioxidants like glutathione (GSH) and antioxidant molecules like thiols (Mahajan and Tandon 2004). In blood plasma, chain breaking antioxidants can trap free radicals directly as well, thereby interrupting chain propagating reactions. Chain breaking antioxidants are SOD, uric acid, protein sulphydryls, ascorbate, alpha tocopherol, GSH and bilirubin (Koracevic et al. 2001). The determination of total antioxidant activity (TAA) has been used for scientific purposes to examine the medical importance of free oxygen radicals and antioxidative defense (Rumley and Paterson 1998; Woodford and Whitehead 1998).

Sialic acid (SA) is an acetylated derivative of neuraminic acid and it is attached to non reducing residues of carbohydrate chains of glycoproteins and glycolipids. Serum SA has been reported as a marker of the acute phase response; increased SA concentrations have been observed in several diseases such as myocardial infarction, diabetes, tumors and alcoholism. Serum SA is also increased during inflammatory processes as a
consequence of elevated concentrations of richly sialylated acute phase glycoproteins (Ponnio et al. 1999). Serum SA was reported to be useful as a parameter of inflammation, and synovial fluid SA was also considered to be useful for differentiation between RA and OA (Kosakai 1991). On the other hand, Iijima et al. (2004) reported that monomeric SA is the potent defense molecule against oxidative damage and the cell death caused by $H_2O_2$ was suppressed by SA in a dose-dependent manner. However its significance has not been discussed in various pathological conditions.

Inflammation is among the reasons which make the prognosis difficult in the arthritis cases. The exact reason and mechanism of inflammation have not yet been understood. Furthermore, differing from RA, it is not clear if OA is an inflammatory disease. Therefore, in our present study, we hypothesized that primary OA (POA) might be an inflammatory disease. To test this hypothesis, SA levels were measured as an inflammatory marker.

Since oxidative damage caused by free radicals is a pivotal mechanism implicated in the progression of arthritis, the relation between SA levels and oxidant-antioxidant status was evaluated. For this purpose, plasma protein oxidation and the antioxidant status in patients with POA and inactive RA (iRA) were determined and compared with healthy controls. In addition, SA levels were measured as an inflammatory marker, since we hypothesized that POA might be an inflammatory disease.

**Patients and Methods**

**Patients**

The first group of this study consisted of 20 patients (16 female and 4 males, age range 25-61 years) with POA of the knee who fulfilled the American College of Rheumatology (ACR) criteria for knee OA (Altman et al. 1986) (Table 1). OA patients were in preoperative period of knee arthroplasty. To measure the degree of functional impairment and to identify the severity of disease in patients with knee OA, we included joint specific Knee-Society Score (Insall et al. 1989) and Oxford knee score (Dawson et al. 1998).

The second group consisted of 20 female iRA patients (age range 26-58 years), who were at the inactive state of RA (Table 1). Inactive RA was defined upon the ACR criteria for clinical remission of rheumatoid arthritis (Pinal et al. 1981). Inactive RA was present for at least 6 months prior to inclusion in the study. The duration of the RA disease was 3 years to 21 years.

The control group consisted of 20 healthy female subjects (age range 25-34) (Table 1). They were recruited for the study after giving their informed consent as approved by the ethics committee of Marmara University Medical School. Everyone filled out a questionnaire giving information about their gender, age, past health problems, smoking and alcohol habits and taking vitamins or antioxidants. Patients in POA group were taking non-steroidal anti-inflammatory drugs and patients in iRA group were taking disease-modifying antirheumatic drugs such as methotrexate and sulfasalazine. The persons with diabetes, cancer, heart or liver disorders were not included in the study. The study was performed in accordance with the ethic standards laid down in the Declaration of Helsinki.

**Sample collection**

Venous blood samples were collected in the morning after an overnight fast then centrifuged at 2,000 $\times$ g for 10 min at +4°C. Plasma samples were stored at −70°C until the analysis was carried out.

**Chemicals**

All chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO, USA) and were of analyti-
cal grade or the highest grade available.

Plasma CuZn SOD activity. CuZn SOD activity was determined by the method of Sun et al. (1998) based on the inhibition of nitroblue tetrazolium (NBT) reduction. The absorbance of reduction product was read at 560 nm in a spectrophotometer. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction 50%.

Plasma protein carbonyl content. CC levels were measured according to the method based on spectrophotometric detection of the reaction of 2,4-dinitrophenylhydrazine (DNPH) with protein carbonyl to form protein hydrazones (Reznick and Packer 1994).

Serum glutathione levels. GSH levels were determined by the method of Beutler et al. (1963).

Plasma antioxidant activity. Antioxidant activities were determined by the method of Koracevic et al. (2001). A standardized solution of Fe-EDTA complex reacts with H2O2 by a phenton type reaction, leading to the formation of hydroxyl radicals (OH•). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the plasma samples cause suppression of the production of TBARS. Finally, absorbance values were measured by a spectrophotometer at 532 nm.

Serum SA concentrations. SA levels were assayed using Warren’s thiobarbituric acid assay (1959). Samples were incubated with 0.9 mL 0.1 N H2SO4 at 80°C, for 1 hr, and total SA were determined in hydrolysate.

Plasma protein sulphydryls. Membrane protein sulphydryl concentration was measured spectrophotometrically using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as described by Ellman (1959).

Serum protein content. Total protein concentration was estimated by Biuret method (Peters et al. 1982) using bovine albumin as a standard.

Statistical methods. The results were calculated as mean ± s.e.m. Kruskal-Wallis nonparametric one-way analysis of variance was used to compare the group differences. Correlations between SOD and GSH levels were performed using Spearman’s rank correlation test. The statistical software package SPSS 10.0 (SPSS Inc., Chicago, IL, USA) was used and p ≤ 0.05 was considered significant.

RESULTS

TAA, SOD, and GSH levels were decreased significantly both in POA patients ([p < 0.0001], [p = 0.0004], [p < 0.0001] respectively) (Table 2) and in iRA patients ([p = 0.002], [p = 0.0002], [p = 0.005] respectively) (Table 3), when compared with healthy controls.

TT levels were increased in iRA patients and decreased in POA patients significantly when compared with the control group (p < 0.05, p < 0.0001). SA, TP and CC levels were increased significantly both in POA patients ([p < 0.0001], [p < 0.0001], [p < 0.0001] respectively) and iRA patients ([p = 0.02], [p = 0.0082] and [p = 0.007]) when compared with healthy controls (Tables 2 and 3).

Moreover, there was a positive correlation between GSH and SOD levels in the iRA group.

| Table 2. Oxidant and antioxidant parameters in POA. Shown are the mean ± s.d. values. |
|---------------------------------|-----------------|-----------------|
|                                | Control group   | POA             |
|                                | (n = 20)        | (n = 20)        |
| TAA (mmol/l)                   | 1.04 ± 0.14     | 0.67 ± 0.10*    |
| SA (mM)                        | 2.41 ± 0.16     | 3.34 ± 0.37*    |
| TT ( μM)                       | 581.25 ± 51.5   | 315.77 ± 7.16*  |
| SOD (U/ml)                     | 8.08 ± 0.72     | 5.22 ± 0.95*    |
| GSH (% mg)                     | 8.1 ± 0.52      | 5.53 ± 0.24*    |
| TP (g/dl)                      | 4.67 ± 1.79     | 7.10 ± 1.68*    |
| CC (nmol/mg protein)           | 0.61 ± 0.10     | 0.91 ± 0.07*    |

*p < 0.0001 significantly different from the control group.

*p < 0.001 significantly different from the control group.

TAA, total antioxidant activity; SA, sialic acid; TT, total thiols; SOD, superoxide dismutase; GSH, reduced glutathione; TP, total protein; CC, carbonyl content.
Antioxidant Activity and Sialic Acid Levels in Arthritis

DISCUSSION

A major conclusion of this study is that depletion of antioxidant activity and oxidation of proteins in plasma are common in POA and iRA. Furthermore, SA might be a potent defense molecule against oxidative stress.

Although there are several studies in the literature about oxidative damage and antioxidant parameters in RA (Bauerova and Bezek 1999; Parades et al. 2002; Hadjigogos 2003; Hitchon and El-Gabalawy 2004), only a few studies have examined these parameters in POA (Ostalowska et al. 2005; Regan et al. 2005). Indeed, to our knowledge, our study is the only one in recent relevant literature, which evaluates these parameters in POA. In the present study, we preferred to measure the CC and TT levels rather than more common oxidative damage markers such as nitrite, nitrate and lipid peroxidation. The reason was that nitrite, nitrate and lipid peroxidation have already been reported to be increased in RA and OA (Kaur and Halliwell 1994; Hilliquin et al. 1997; Novaes et al. 1997; Ersoy et al. 2002; Paredes et al. 2002; Karan et al. 2003; Ostalowska et al. 2005, 2007; Sahin et al. 2006).

We had something to self-criticise. The number of patients were limited in our study since we aimed to gather the groups with subjects having similar clinical properties.

In the present study, we observed decreased TAA in patients with POA and iRA when compared to healthy controls. Yet, Mantle et al. (1999) reported insignificant differences in total antioxidant status in plasma between RA, OA and control cases. We also found decreased SOD activity in iRA and POA patients. Additionally, Imadaya et al. (1998) and Regan et al. (2005) found decreased SOD activity in RA and OA patients as well. However, Sarban et al. (2005) found no significant differences in erythrocyte SOD activities between the groups. Furthermore, decreased GSH levels in patients with RA was formerly reported by other researchers (Hassan et al. 2001; Jaswal et al. 2003). In accordance with the previous studies, we observed decreased GSH levels in patients with iRA. There was a positive correlation between GSH and SOD levels in the iRA group. ROS are produced in many normal and abnormal processes in humans, including atheroma, asthma, joint diseases, aging, and cancer. The superoxide anion ($O_2^-$) is the main ROS. Increased ROS production leads to tissue damage associated with inflammation. SODs convert superoxide to $H_2O_2$, which is then removed by glutathione. SODs prevent the formation of highly aggressive ROS, thus its level decreases

Table 3: Oxidant and antioxidant parameters in iRA. Shown are the mean ± s.d. values.

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<th>Control group</th>
<th>iRA</th>
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<tr>
<td></td>
<td>($n = 20$)</td>
<td>($n = 20$)</td>
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<tr>
<td>TAA (mmol/l)</td>
<td>1.04 ± 0.14</td>
<td>0.82 ± 0.13*</td>
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<tr>
<td>SA (mM)</td>
<td>2.41 ± 0.16</td>
<td>3.11 ± 0.47*</td>
</tr>
<tr>
<td>TT (μM)</td>
<td>581.25 ± 51.5</td>
<td>828.1 ± 5.24*</td>
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<tr>
<td>SOD (U/ml)</td>
<td>8.08 ± 0.72</td>
<td>5.03 ± 1.28b</td>
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<tr>
<td>GSH (% mg)</td>
<td>8.1 ± 0.52</td>
<td>5.75 ± 0.1a</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>4.67 ± 1.79</td>
<td>5.90 ± 1.56a</td>
</tr>
<tr>
<td>CC (nmol/mg protein)</td>
<td>0.61 ± 0.10</td>
<td>0.84 ± 0.09*</td>
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* $p < 0.05$ significantly different from the control group.

* $p < 0.001$ significantly different from the control group.

TAA, total antioxidant activity; SA, sialic acid; TT, total thiols; SOD, superoxide dismutase; GSH, reduced glutathione; TP, total protein; CC, carbonyl content.
On the other hand, reduced GSH also prevents the oxidation of essential biomolecules.

Oxidative stress, an imbalance towards the prooxidant side of the prooxidant/antioxidant homeostasis is well characterized with the usage of protein carbonyl groups (Dalle-Donne et al. 2003). To our knowledge, there was no report on the serum level of protein carbonyls in patients with POA. However, parallel to our findings, experimental studies reported increased protein carbonyls in patients with RA (Kosakai 1991; Dalle-Donne et al. 2003). Moreover, consistent with our findings, increased serum TP levels have been reported in patients with RA and OA (Segami et al. 2002; Lemarechal et al. 2006).

SA is a structural component of the terminal oligosaccharide chains of glycoproteins and glycolipids. Most studies on SA have focused primarily on cell protection, fertilization, cell differentiation, cell adhesion, immunity, inflammation and tumors (Kosakai 1991; Ponnio et al. 1999). Kulkarni et al. (1986) and Kosakai et al. (1991) investigated SA as an inflammatory marker and reported increased SA levels in patients with arthritis. In the present study, SA levels were found to be increased in RA and POA when compared with the controls. Thus POA was suggested as an inflammatory disease as well as RA. However, additional inflammatory markers are necessary to support this idea.

Antioxidant property of SA as a $\text{H}_2\text{O}_2$ scavenger has been reported (Tanaka et al. 1997; Iijima et al. 2004). Iijima et al. (2004) examined the detoxification of $\text{H}_2\text{O}_2$ in cultured cells with SA and declared that the cell death caused by $\text{H}_2\text{O}_2$ was suppressed by SA in a dose dependent manner. However, SA levels were investigated together with antioxidant and oxidant parameters for the first time in the literature. In our present work, serum SA levels increased parallel to oxidative stress. Consequently we suggest that increased levels of SA might be considered as a defence molecule against oxidative stress.

TT levels were decreased in patients with POA and increased in patients with iRA. Baskol et al. (2006) found increased TT levels in patients with RA. Data concerning the level of TT in patients with OA are scarce (Hilliquin et al. 1997; Segami et al. 2002). In our study, decreased TT levels in POA may be due to inflammation since antioxidants are reported to be decreased in inflammatory joint diseases (Hassan et al. 2001; Afonso et al. 2007).

Measurement of oxidant and antioxidant parameters may be a useful way for demonstrating oxidative stress-mediated pathology and testing the effectiveness of therapeutic agents in preventing such damage in arthritis. Moreover, the present study suggests that POA may be an inflammatory disease, since SA levels were increased and TT levels were decreased significantly in POA patients. Furthermore, serum SA levels were increased in parallel to oxidative stress, supporting the role of SA as an antioxidant. Using advanced antioxidant therapies in combination with antioxidant supplements, bench to bedside strategies will be developed, thereby enabling the creation of novel treatments of RA and OA. On the other hand, additional studies investigating inflammatory properties of POA are required.

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**References**


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