Dynamic thiol-disulphide homeostasis in grade 3-4 gonarthrosis

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ABSTRACT

Objectives: We aimed to determine thiol-disulphide homeostasis, which plays a vital role and to investigate the relationship among homeostatic parameters and disease.

Methods: In this prospective study, we enrolled 38 patients with osteoarthritis (31 females and 7 males) and 38 healthy controls (30 females, 8 males volunteers). Diagnosis of osteoarthritis was made according to the American College of Rheumatology Criteria. The severity of osteoarthritis was assessed and classified according to the Kellgren-Lawrence grading scale.

Results: The mean age was 63.8 (range; 53-74) years in the osteoarthritis group and 65.6 (range; 55-75) years in the control group. There were no significant differences between the patients and controls in respect to age, gender and body mass index (p > 0.05). Serum albumin (p = 0.605) and total protein levels (p = 0.605) between patients and controls were similar. In the osteoarthritis group disulphide/ native thiol percent ratios and disulphide/ total thiol percent ratios were found to be statistically higher (p = 0.002 and p = 0.002; respectively) and native/ total thiol percent ratios were significantly lower than that of the control group (p = 0.002).

Conclusions: Thiol-disulphide homeostasis is weakened in osteoarthritis, and the balance shifts to the disulphide bond formation side. Substitution of thiol deficiency and correction of thioldisulphide imbalance may be beneficial in the managing treatment of the disease. Further studies may be needed for evaluating articular fluid thiol-disulphide homeostasis.

Keywords: Oxidative stress, osteoarthritis, thiol-disulphide homeostasis

Osteoarthritis is a chronic, progressive disorder of the synovial joints, characterized by focal loss of cartilage and changes in subchondral and marginal bone, synovium, and peri-articular structures [1]. Osteoarthritis of the knee is a relatively common condition that affects approximately 10% of the general population above the age of 55 years [2]. Radiographic appearance and clinical features are still often used for diagnosis of the disease. However, the etiology of osteoarthritis is not fully understood, although mechanical, biochemical, and genetic factors are accepted to play roles [3, 4].

One possible cause of osteoarthritis is oxidative stress. There is some evidence of the relationship be-
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The levels of pro-inflammatory mediators, such as reactive oxygen species (ROS), are elevated in osteoarthritis [7, 8]. Thus, the increased levels of these reactive species with oxidative activity mediate the effects of many pro-inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α [7-9]. IL-1 and TNF-α may play a crucial role in cartilage matrix degradation by stimulating matrix metalloproteinase (MMP) expression in patients with osteoarthritis [8, 9].

It is known that free radicals cause oxidation of –SH groups in sulfur-containing amino acids of proteins and this is the earliest observable signs of protein oxidation [10]. To protect cells against oxidative stress, certain low molecular weight antioxidant molecules, either water-soluble (e.g., ascorbic acid) or lipid-soluble (e.g., vitamin E), are present in extracellular fluids [11]. Thiols are in interaction with almost all physiological oxidants. And they are mentioned as essential antioxidant buffers. Thiols, also known as mercaptans, which consist of a sulfur atom and a hydrogen atom bound to a carbon atom, are functional sulphydryl groups [12]. A very large part of the blood plasma thiol pools consist mainly of albumin and other proteins such as glutathione, thioredoxin, cysteine and homocysteine [13]. Thiol groups of proteins are oxidized by oxygen molecules present in the medium and are reversibly converted to disulphide bonds. Formed disulphide bonds can be reduced to thiol groups again. Thus the thiol-disulphide balance is maintained [14]. Dynamic thiol-disulphide homeostasis plays a critical role in antioxidant defense, detoxification, apoptosis, regulation of enzyme activity, transcription and cellular signal transduction mechanisms [15, 16]. Only a single side of this double-sided balance has been measured since 1979 [17]. Both variable levels are measured one by one and cumulatively with a novel and automated method [18].

We aimed to determine thiol-disulphide homeostasis, which plays a vital role and to investigate the relationship among homeostatic parameters and disease.

METHODS

In this prospective study, we enrolled 38 patients with osteoarthritis (31 females and 7 males) and 38 healthy controls (30 females, 8 males volunteers). Diagnosis of osteoarthritis was made according to the American College of Rheumatology Criteria [19]. The severity of osteoarthritis was assessed and classified according to the Kellgren-Lawrence grading scale [20]. Grade 0 was accepted as normal, grade 1 as possible osteophytes only, grade 2 as absolute osteophytes and possible joint space narrowing, grade 3 as moderate osteophytes and/or absolute joint space narrowing, and grade 4 as large osteophytes, severe joint space narrowing, and/or bony sclerosis. All patients had grade III-IV knee osteoarthritis according to the radiological classification and clinical findings. Exclusion criteria included use of supplemental vitamins, smoking, diabetes mellitus, coronary artery disease, acute/chronic liver diseases, inflammatory rheumatic disease, clinically unstable medical illness, or the use of any medication within 4 weeks prior to initiation of the study.

All subjects were informed. Written consents were obtained and the study was approved by the local ethics committee. Patient and healthy groups were matched in terms of osteoarthritis grade and age.

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of patients and controls</th>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>Mean (range)</td>
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<tr>
<td>Gender, n</td>
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<tr>
<td>(male/female)</td>
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<tr>
<td><strong>BMI (kg/m²)</strong></td>
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<tr>
<td>Mean (range)</td>
</tr>
</tbody>
</table>

BMI = body mass index
Venous blood samples were collected from the subjects and centrifuged at 2300 x g for 10 min. Serum samples were separated and stored at -80 °C until analysis. Serum thiol - disulphide homeostasis was determined with a recently developed novel and automatic measurement method by using an automated clinical chemistry analyser (Roche, cobas 501, Mannheim, Germany) [18]. Native thiol (-SH) and total thiol (-SH + -S-S-) were measured directly, and -S-S/-SH, -S-S/-SH + -S-S-, -SH/-SH + -S-S- results were obtained with calculation.

Statistical Analysis
All analyses were conducted using the SPSS software (version 22; IBM SPSS Inc., Chicago, IL, USA). The normality of distributions was evaluated using the one-sample Kolmogorov – Smirnov test, revealing a uniform distribution. Mann Whitney U test was used to analyze the numerical variables. p-values of less than 0.05 were regarded as significant.

RESULTS
The mean age was 63.8 (range; 53-74) years in the osteoarthritis group and 65.6 (range; 55-75) years in the control group. Demographic characteristics of patients with knee osteoarthritis and controls are shown in Table 1. There were no significant differences between the patients and controls in respect to age, gender and body mass index (BMI). Serum albumin and total protein levels of the between patients and controls were similar (p = 0.605 and p = 0.652; respectively).

There is no difference in native thiol levels and total thiol levels between the groups (p = 0.06 and p = 0.07; respectively). In addition there is no difference in disulphide values between the groups (p = 0.07). In the osteoarthritis group disulphide/ native thiol percent ratios and disulphide/ total thiol percent ratios were found to be statistically higher (p = 0.002 and p = 0.002; respectively) and native/ total thiol percent ratios were significantly lower than that of the control group (p = 0.002) (Table 2).

DISCUSSION
Thiol groups have a significant role in the cell by minimizing the toxic effects of oxygen activation processes. Fundamentally sulphydryl groups are associated with proteins. So, when thiol levels decreases in serum its antioxidant power will decrease too. Because reactive species organized near the sides of their formation, increases in the expression of protein levels of thioldisulphide will protect the tissular oxidative damage and cannot prevent the oxidation of thioldisulphide groups in serum [10-14].

Dynamic thiol-disulphide homeostasis has a critical role in the organism. Changes in the thiol-disulphide balance serve as components for antioxidant protection, detoxification, regulation of enzymatic activity and cellular signaling mechanisms [16, 21]. Changes in thioldisulphide homeostasis have been associated with various diseases such as diabetes mellitus, cancer, chronic kidney disease, liver disorders and chronic obstructive pulmonary disease [22-24].

Osteoarthritis is a process of progressive deterioration of articular cartilage and formation of osteophyte at the joint surface. Osteoarthritis is often associated with significant disability and an impaired

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Osteoarthritis group</th>
<th>Control group</th>
<th>p value</th>
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<tbody>
<tr>
<td>-SH (µmol/L)</td>
<td>375.07 ± 34.512</td>
<td>407.66 ± 48.99</td>
<td>0.603</td>
</tr>
<tr>
<td>-S-S- (µmol/L)</td>
<td>15.50 ± 4.507</td>
<td>18.76 ± 4.59</td>
<td>0.003</td>
</tr>
<tr>
<td>-SH + -S-S (µmol/L)</td>
<td>405.992 ± 37.493</td>
<td>407.66 ± 48.99</td>
<td>0.0712</td>
</tr>
<tr>
<td>-S-S-/SH + -S-S- (%)</td>
<td>3.806 ± 1.030</td>
<td>4.61 ± 1.090</td>
<td>0.002</td>
</tr>
<tr>
<td>-SH/-SH + -S-S- (%)</td>
<td>92.407 ± 2.006</td>
<td>90.80 ± 2.21</td>
<td>0.002</td>
</tr>
<tr>
<td>-S-S- / -SH (%)</td>
<td>4.145 ± 1.217</td>
<td>5.11 ± 1.093</td>
<td>0.002</td>
</tr>
</tbody>
</table>

-SH = native thiol, -S-S- = disulphide, (-SH + -S-S-) = total thiol
quality of life. Pathologically, the disease is characterized by fissuring and focal erosive cartilage lesions, as well as cartilage loss and destruction [5]. Oxidative stress leads to increased risk for osteoarthritis but the precise mechanism remains unclear. Studies suggested that oxidative stress causes chondrocyte senescence and cartilage ageing [24, 25].

Soran et al. [5] and Altindag et al. [6] found that oxidative stress extremely increased in osteoarthritis, which may be responsible for the etiopathogenesis of the disease. In our study, we observed decreased antioxidant parameters in subjects with knee osteoarthritis compared to the controls. These results confirmed the presence of oxidative stress.

CONCLUSION

In conclusion, thiol-disulphide homeostasis is weakened in osteoarthritis, and the balance shifts to the disulphide bond formation side. Substitution of thiol deficiency and correction of thio-disulphide imbalance may be beneficial in the managing treatment of the disease. Further studies may be needed for evaluating articular fluid thiol-disulphide homeostasis.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES


