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Oxidative profile of patients with osteoarthrosis undergoing total knee arthroplasty

Perfil oxidativo de pacientes com osteoartrose submetidos à artroplastia total de joelho

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Abstract

Introduction: Total knee arthroplasty may be the most appropriate method of treatment in several cases of osteoarthritis. This disease causes tissue damage, which is closely related to the production of free radicals, leading to oxidative stress and to lipid damage. Because of that, the body has several antioxidative defense systems involved in detoxification (antioxidants). **Objective**: Based on the previous information, the goal of this study was to establish the systemic and local oxidative profile of individuals with osteoarthritis submitted to total knee arthroplasty. **Materials and methods**: The sample consisted of four female patients (65.5 ± 0.7 years) with osteoarthritis of the knee. Blood and synovial fluid (SF) samples were collected from the patients 15 minutes before surgery. The concentrations of flavonoids, catalase, and TBARS were then quantified. **Results**: The results indicate a higher catalase activity in the SF than in the serum (S), (SF = 1 S = 14.3 ± 3.1 ± 0.8). The concentration of TBARS proved to be higher in the SF (SF = 0.29 ± 0.02 S = 0.09 ± 0.05),

whereas the concentration of phenols was higher in the serum (SF = 3.2 S = 5.2 ± 0.2 ± 0.6). **Conclusion**: Osteoarthritis is a disease that increases the oxidative stress markers in the serum and in the SF.

Keywords: Osteoarthritis. Knee. Oxidative stress. Antioxidants. Free radicals.

Resumo

Introdução*: A artroplastia total de joelho pode ser o método de tratamento mais indicado em casos severos de osteoartrose. No organismo a consequência dessa patologia implica em danos teciduais e estes tem uma ligação direta com a produção de radicais livres, que podem gerar o estresse oxidativo e dano lipídico. Devido a isso, o organismo possui vários sistemas de defesa antioxidante que atuam na detoxificação (antioxidantes).* ***Objetivo****: Partindo destas informações o objetivo do trabalho foi estabelecer o perfil oxidativo sistêmico e local de indivíduos com osteoartrose submetidos à artroplastia total de joelho.* ***Materiais e métodos****: A amostra foi constituída de 4 indivíduos do gênero feminino (65,5 ± 0,7 anos), com osteoartrose do joelho. Amostras de sangue e liquido sinovial dos pacientes foram coletadas 15 minutos antes da cirurgia. Procedeu-se a quantificação bioquímica de flavonóides, catalase e a medida de peroxidação lipídica (TBARS).* ***Resultados****: A análise dos resultados aponta uma maior atividade catalase no líquido sinovial (LS) quando comparado com o soro (S), (LS = 14,3 ± 1 S = 3,1 ± 0,8). Ao analisar a concentração de TBARS, essa se mostrou maior no líquido sinovial (LS = 0,29 ± 0,02 S = 0,09 ± 0,05), já a concentração de polifenóis foi maior no soro (LS = 3,2 ± 0,2 S = 5,2 ± 0,6).* ***Conclusão****: Os dados obtidos apontam que a osteoartrose é uma doença que aumenta os marcadores de estresse oxidativo (EO) no sangue e no líquido sinovial.*

Palavras-chave*: Osteoartrite. Joelho. Estresse oxidativo. Antioxidantes. Radicais livres.*

Introduction

Osteoarthrosis (OA), also referred to as arthrosis or osteoarthritis, used to be known as a degenerative rheumatic disease. However, in 1992, the term was phased out and the disease was then denoted as a disorder characterized by degradation mechanisms driven by cells and by reparative cartilaginous processes (1).

The choice of proper treatment must be based upon the several manifestations of the disease, such as: level of impact of the pain, emotional aspects, socioeconomic background, quality of life, extent of joint damage, and instabilities and deformities, which requires the expertise of a multidisciplinary team (2). Total knee arthroplasty can be the most widely recommended treatment method in severe cases of OA and it is selected when more conservative approaches have been ruled out (3).

Osteoarthrosis leads to tissue damage and such damage is closely related to the production of free radicals, being likely to cause oxidative stress. Free radicals are atoms or molecules with one or more unpaired electrons in their outermost shell, being highly unstable, which try to stabilize themselves at any cost. Under adverse circumstances, the increase in free radicals prevents the body from inhibiting their production, resulting in structural cell changes and in pathologies such as rheumatic diseases, degenerative diseases, cancer, aging, among others (4).

The production of free radicals is a physiological process, but under certain conditions, there may be increased production of reactive oxygen species (ROS) or depletion of antioxidative defenses, thus triggering oxidative stress. Because of that, the body has numerous antioxidative defense systems involved in the detoxification of ROS in different ways. As an example, we can cite the enzyme system, with the participation of antioxidant enzymes such as glutathione reductase and catalase (5).

The assessment of oxidative stress relies on the ability to detect the presence of reactive species (6, 7). These species can be measured directly by their concentration in body fluids and tissues or indirectly by determination of the damage they cause (8).

Production of free radicals takes place as part of the physiological process. Hence, the cell metabolism produces free radicals under physiological conditions, and these active radicals may have several useful effects, for instance, by acting as a defense mechanism

against the attack of microorganisms through the control of molecular stimuli and signs (9).

Oxidation is paramount to aerobic life and to our metabolism, and as free radicals are naturally produced, they play a role in many diseases, such as inflammatory processes (9).

As soon as inflammatory cells (macrophages, neutrophils, lymphocytes and endothelial cells) are isolated and stimulated in the case of an inflamed joint, they are able to produce oxygen radicals. These radicals, in the presence of lipid, DNA, protein, carbohydrate or proteoglycan molecules, cause oxidative damage (9).

Oxidative damage is also triggered by another mechanism: acute phase responses that lead to oxidative stress. The gradual increase in the production of ROS induced by exercising can trigger adaptations to a larger production of these free radicals (10).

Likewise, the relationship between oxygen radicals and joint cartilage damage, which can be local or become systemic (9), is well established.

Thanks to advances in research, synovial fluid (SF) analyses indicate that inflammatory processes are correlated with biochemical changes in this fluid, and it is then possible to gain a better understanding of the disease (11).

The dearth of reports about biochemical markers in SF can be explained by the fact that its collection is invasive and traumatic and, therefore, blood sampling is preferred, as it is simpler and allows for coherent and representative results.

Based on that, our goal in this study is to determine the systemic and local oxidative profile of osteoarthrosis patients undergoing total knee arthroplasty.

Materials and methods

Study design

This is a cross-sectional study aimed at assessing the intensity and difference of oxidative damage between SF and blood collected from patients undergoing total knee arthroplasty.

Patient population

The sample consisted of four female patients (65.5 ± 0.7 years) with osteoarthrosis of the knee associated or not with meniscal lesion (average lesion time 9 ± 1.4 years). Those patients who had not taken any anti-inflammatory drug 48 hours before the collection and who also had not used antioxidants were included in the study.

Ethical aspects

All of the subjects volunteered to participate in the study after being informed about its objectives and possible risks. After that, pursuant to the Nuremberg Code (1947), to the Universal Declaration of Human Rights (1948) and to the Declaration of Helsinki, they signed the informed consent form. The study protocol was approved by the Research Ethics Committee following the Brazilian National Health Council's regulation 196/1996, and registered as CAAE – 0164.0.398.000-11.

Experimental model

Initially, the medical history of each patient was taken and inclusion criteria, such as refrainment from the use of anti-inflammatory and antioxidative drugs 48 hours before the collection, were checked. Those patients who met the inclusion criteria signed the informed consent form, thereby authorizing the collection of biological samples during the surgical procedure.

The surgical procedures for sample collection were carried out at Hospital da Cidade de Passo Fundo, state of Rio Grande do Sul, southern Brazil, by orthopedists from the Orthopedic Hospital of Passo Fundo.

Morning blood samples were collected under aseptic conditions from the antecubital fossa of fasted patients before they underwent surgery; 8 mL of blood was drawn and later stored in an anticoagulant-free tube and centrifuged at 1,500 rpm for 15 minutes. The plasma was extracted and stored in Eppendorff tubes for biochemical analysis.

The SF was aspirated by needle insertion (arthrocentesis) into the outer parapatellar and suprapatellar pouches during knee arthroplasty and 4 mL of the fluid was stored in Eppendorf tubes. The samples were collected in the morning from fasted patients. All aliquots were stored at -18 ^{o}C until analysis.

After collection, the collected material was analyzed at the Biochemical Laboratory of Universidade de Passo Fundo.

Biochemical analyses

The levels of lipid peroxidation were measured as described by Esterbauer and Cheeseman (12). Catalase (endogenous antioxidant) activity occurred in a medium containing hydrogen peroxide as previously described by Aebi (13). The serum amount of phenolic compounds (exogenous antioxidants) was measured by the Folin-Ciocalteau's method as described by Waterman and Amole (14) and analyzed on a Biosystem BTS 350 spectrophotometer.

Statistical analysis

Each variable was submitted to the Kolmogorov-Smirnov test and to Levene's test, assuming a 95% confidence interval and setting a $p < 0.05$ as statistically significant.

The results were transcribed onto a worksheet and then submitted to descriptive and inferential statistical analysis. The means were compared by the Wilcoxon-Mann-Whitney test (nonparametric data), using SPSS 16.0, and setting $p < 0.05$ as statistically significant. The results are expressed as mean ± standard error.

Results

The Figures 1, 2 and 3 show a higher statistically significant concentration of polyphenols in the serum (S) compared to the SF (SF = 3.2 ± 0.2 S = 5.2 ± 0.6), indicating flavonoid (exogenous antioxidant) consumption in the SF via free radicals obtained from local oxidative stress, compared to serum.

The analysis of lipid damage by determination of thiobarbituric acid reactive substances (TBARS) in the serum and SF of patients submitted to total knee arthroplasty reveals a statistically higher increase of TBARS in the SF than in the serum (SF = 0.29 ± 0.02 S = 0.09 ± 0.05). This indicates that the inflammation caused by this disorder increases the oxidative damage to lipids via free radicals in the fluid, which can spread to areas that underlie the tissues lubricated by the SF and aggravate the complications arising from osteoarthrosis.

The statistical analysis of catalase activity in the serum and SF of patients submitted to total knee arthroplasty demonstrates larger catalase activity in the SF than in the serum (SF = 14.3 ± 1 S = 3.1 ± 0.8), indicating possible metabolic adaptation, since it is an endogenous antioxidant and in view of the increase in the demand for free radicals produced by inflammatory cells.

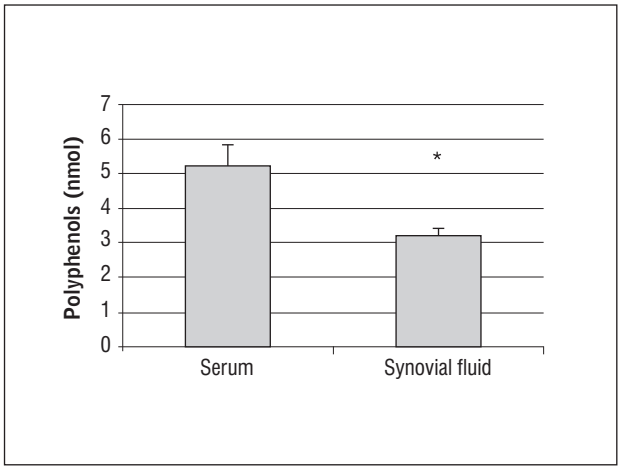

Figure 1 - Comparative analysis of polyphenol concentration in serum and in SF

Note: Results expressed as mean ± standard error. * = $p < 0.05$ using the Wilcoxon-Mann-Whitney test for nonparametric data.

Source: Research data.

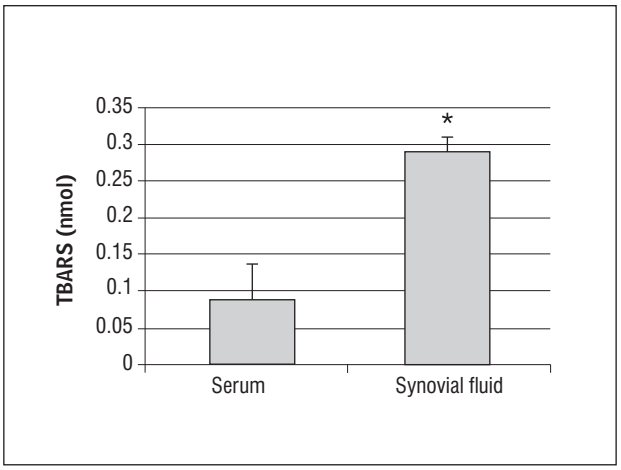

Figure 2 - Comparative analysis of TBARS concentration in serum and in SF

Note: Results expressed as mean ± standard error. * = $p < 0.05$ using the Wilcoxon-Mann-Whitney test for nonparametric data.

Source: Research data.

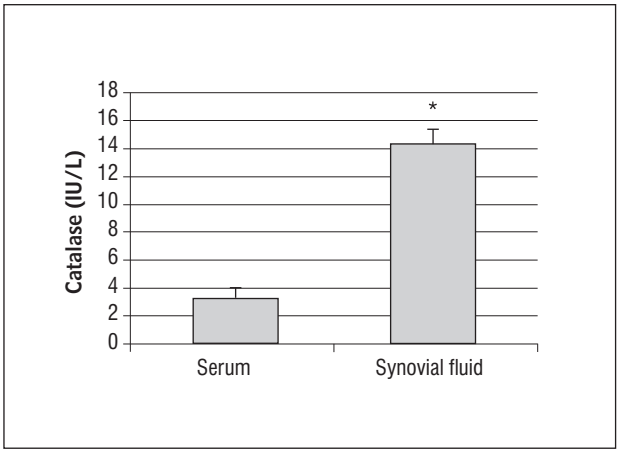

Figure 3 - Comparative analysis of catalase activity in serum and in SF

Note: Results expressed as mean ± standard error. * = $p<0.05$ using the Wilcoxon-Mann-Whitney test for nonparametric data.

Source: Research data.

Discussion

According to Ramos (9), OA produces oxygen free radicals and may contain defense cells in the inflamed joint. Once isolated and stimulated, these cells can produce ROS. Johnston (15) claims that the inflammation of the synovial membrane (knee) causes inflammatory cells to leak into the SF, producing oxygen free radicals.

Halliwell and Gutteridge (16) affirm that the half life of a free radical can be expressed in microseconds, which demonstrates its high electron instability. Thus, the direct determination of these free radicals in biological samples necessitates highly sophisticated equipment and techniques. On the other hand, it is possible to measure the extent of oxidative damage of free radicals in organic compounds such as lipids, proteins, and nucleic acids. This way, the analyses mentioned here were chosen to assess lipid damage through the analysis of lipid peroxidation measured by TBARS and of the endogenous antioxidative catalase activity and exogenous flavonoid activity.

This study demonstrates that patients with osteoarthrosis have oxidative stress, with possible increase of catalase and TBARS, combined with a decrease of flavonoids in the SF compared to their serum.

The results show a larger catalase activity in the SF than in the serum, (SF =1 4.3 ± 1 S = 3.1 ± 0.8). This enzyme is responsible for the detoxification of oxidative peroxides, as it is a component of the primary antioxidative defense mechanism (17). Catalase activity is important for monitoring because it is an enzyme with elevated activity when the body is under oxidative stress (17). The elevation of catalase activity in the SF may be an adaptive process of the body as a way to increase the activity of this enzyme in order to neutralize the elevation of peroxides induced by the local inflammatory process (18, 19, 20).

By assessing oxidative damage to lipids, TBARS was higher in the SF than in the serum (SF = 0.29 ± 0.02 S = 0.09 ± 0.05). According to Silva et al. (20), the reaction of ROS with components found in cell membranes and in lipoproteins triggers a chain process known as lipid peroxidation. The increase in TBARS concentration in the SF indicates that the inflammatory reaction produced by the disease (OA) induced oxidative stress and, consequently, led to local lipid peroxidation. The impact of this lipid peroxidation can spread to all underlying tissues lubricated by the SF, compounding local damage.

On the other hand, the concentration of polyphenols was statistically higher in the serum than in the SF (SF = 3.2 ± 0.2 S = 5.2 ± 0.6). Polyphenols, also known as flavonoids, are exogenous antioxidative substances that significantly inhibit or delay oxidative processes (21). Consumed in large amounts in a human diet, flavonoids have pharmacological properties previously observed by Szent-Gyorgi in 1936. They act upon biological systems through their antioxidative property, which has been the focus of studies so far (21). Most of flavonoids at hand are exogenous, and are substantially influenced by the diet. The higher concentration of flavonoids in serum is due mainly to their larger bioavailability in the serum than in the SF. Since patients were in the preoperative stage, some balance between the serum and fluid levels of flavonoids is expected. Nevertheless, that did not occur probably because of excessive consumption via free radicals in the inflamed joint (22, 23, 24).

While there are no literature references to this sort of biochemical comparison, this result shows us that several oxidants and antioxidants play a role in the pathogenesis of an inflammatory process in individuals with OA (25, 26, 27), revealing an oxidant/antioxidant imbalance that might cause considerable complications for these patients, given that free radicals are involved in a myriad of cell structural changes and in pathologies such as rheumatic diseases, degenerative diseases, cancer, aging, among others (28, 29, 30).

A limitation of this study concerns its small sample size, as it is considered to be a pilot study for future research with more rigorous sample calculation.

The knowledge about and confirmation of oxidative stress in knee lesions allow future studies to minimize (or not) oxidative damage through the use of antioxidants or even contribute towards local anti-inflammatory activity in patients with joint damage.

Conclusion

The results of this study show a large increase in catalase activity in the SF, combined with a low concentration of polyphenols, which demonstrates the imbalance between antioxidants, thus denoting oxidative stress.

Moreover, TBARS concentration was high in the SF, indicating that oxidative damage was limited to the site of inflammation.

The data collected herein demonstrate that OA is a disease that provenly increases the number of oxidative stress markers (ROS), decreases antioxidative defenses, and induces oxidative damage in the SF, compared to the serum. This provides some subsidies so that studies on the use of antioxidants may or may not minimize oxidative damage in patients with joint lesions.

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