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ORIGINAL RESEARCH

Analysis of the oxidative profile of different biological samples of patients with anterior cruciate ligament injury

Análise do perfil oxidativo de diferentes amostras biológicas de pacientes com lesão de ligamento cruzado anterior

Análisis del perfil oxidativo de diferentes muestras biológicas de pacientes con lesión de ligamento cruzado anterior

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ABSTRACT | The knee is one of the most important joints for locomotion. However, due to its complexity, it becomes susceptible to several types of injuries, such as the anterior cruciate ligament (ACL) rupture. This complication triggers an inflammatory process, which can lead to the formation of free radicals and, consequently, oxidative stress (OS). The objective of this study was to compare the oxidative profiles of patients with ACL injury, analyzing two biological samples: synovial fluid and serum. Eleven male subjects with total ACL rupture, older than 18 were analyzed. Blood samples and synovial fluid were collected fifteen minutes before arthroplasty. OS catalase biomarkers, flavonoids and lipid peroxidation (TBARS) were analyzed. The results indicate a lower flavonoid concentration, combined with an increase in TBARS and serum catalase activity when compared with synovial fluid. Analysis of the results indicates that the ACL injury induces OS, characterized by antioxidant consumption and elevated lipid damage in the synovial fluid, when compared with the serum, which indicates that serumal analyses may not be adequate to measure OS in compartments such as the knee joint.

Keywords | Knee; Anterior Cruciate Ligament; Free Radicals; Biomarkers; Oxidative Stress.

RESUMO | O joelho é uma das articulações mais importantes para locomoção. No entanto, devido a sua complexidade, torna-se suscetível a diversos tipos de lesões, como a ruptura do ligamento cruzado anterior (LCA). Essa complicação desencadeia um processo inflamatório, que pode culminar em formação de radicais livres e, consequentemente, em estresse oxidativo (EO). O objetivo do estudo foi comparar o perfil oxidativo de pacientes com lesão do LCA, analisando duas amostras biológicas: líquido sinovial e soro. Foram analisados 11 indivíduos do gênero masculino, com ruptura total do LCA, com idade superior a 18 anos. Coletou-se amostras de sangue e líquido sinovial 15 minutos antes da artroplastia e se analisou biomarcadores de EO, catalase, flavonoides e peroxidação lipídica, isto é, substâncias reativas ao ácido tiobarbitúrico (TBARS). Os resultados apontam menor concentração de flavonoides, combinada a aumento de TBARS e de atividade de catalase no soro quando comparado com o líquido sinovial. A análise dos resultados indica que a lesão de LCA induz a quadro de EO, caracterizado por consumo de antioxidantes e elevação de dano lipídico no líquido sinovial quando comparado com o soro, indicando que análises séricas podem não ser adequadas para medir EO em partes como a articulação do joelho.

Descritores | Joelho; Ligamento Cruzado Anterior; Radicais Livres; Biomarcadores; Estresse Oxidativo.

RESUMEN | La rodilla es una de las articulaciones más importantes para locomoción. Sin embargo, debido a su

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complejidad, se torna susceptible a diversos tipos de lesiones, como la ruptura del ligamento cruzado anterior (LCA). Esa complicación desencadena un proceso inflamatório, que puede culminar en formación de radicales libres y, en consecuencia, en estrés oxidativo (EO). El objectivo del estudio fue comparar el perfil oxidativo de pacientes con lesión del LCA, analizando dos muestras biológicas: fluido sinovial y suero. Fueron analizados 11 individuos del género masculino, con ruptura total del LCA, con edad superior a 18 años. Se recogió muestras de sangre y fluido sinovial 15 minutos antes de la artroplastia y se analizó biomarcadores de EO, catalasa, flavonoides y peroxidación de las grasas, o sea, substancias reactivas al ácido tiobarbitúrico (TBARS). Los resultados apuntan menor concentración de flavonoides, combinada a aumento de TBARS e de actividad de catalasa en el suero cuando comparado con el fluido sinovial. El análisis de los resultados indica que la lesión de LCA induce a cuadro de EO, caracterizado por consumo de antioxidantes y elevación de daño de las grasas en el fluido sinovial cuando comparado con el suero, indicando que análisis séricas pueden no ser adecuadas para medir EO en partes como la articulación de la rodilla.

Palabras clave | Rodilla; Ligamento Cruzado Anterior; Radicales Libres; Biomarcadores; Estrés Oxidativo.

INTRODUCTION

Knees are intermediate joints of lower limbs, assuming one of the most important roles during locomotion, which makes them susceptible to several injuries mainly on sportspeople, since it is the most vulnerable target of an athlete. The most frequent injury in sportspeople is the anterior cruciate ligament (ACL) rupture[1].

ACL knee injury is responsible for approximately 50% of all ligamentary injuries[2]. The growing number of practitioners of physical activities contributes to increase occurrences of this pathology, since in the USA, around 70,000 ligament reconstructions are performed every year through surgical procedure[3].

One of the consequences of ACL rupture is the increased production of free radicals (FR), triggered by inflammation[4]. The increase of FR is associated with the increased consumption of O_2 by active tissues[5], as well as by the inflamatory process itself[4-7].

The generation of FRs occurs as part of the body physiological process as cellular respiration. These active radicals may have various physiological effects such as defense mechanism against aggression of microorganisms, stimuli control and molecular signals[8].

Inflammation mediatory cells (macrophages, neutrophiles, lymphocytes and endothelial cells) are recruited to the injury region where, in addition to produce oxygen FRs, will cause formation of proteolytic enzymes to repair the damaged tissue[9]. In addition, the ACL rupture causes increased production of inflammatory mediators and proteins of acute phase that can act as FRs[5]. Therefore, imbalance between production of free radicals and antioxidant defenses, with predominance of FRs can trigger oxidative stress (OS)[6-8,10].

During oxidative stress and in the presence of lipid molecules, DNA, proteins, carbohydrates or proteoglycans, FRs trigger amplification of oxidative injury to subjacent tissues[8], and may result in injury to normal cells adjacent to the injured location, amplifying the inflammatory and oxidative process[9,11,12].

The OS determination depends on the ability to gauge presence of reactive species[12]. These can be measured directly through their concentration in biological fluids and tissues, or indirectly, through evaluation of damage they cause[13].

For being a simple collection procedure, oxidative damage in humans is routinely determined in blood samples, once biomarkers diffuse from the inflammation location to the serum where is determined. Studies on biological fluids such as the synovial fluid are restricted, since it is an invasive and relatively traumatic procedure[14,15].

There are no reports in the literature regarding comparison between levels of biochemical biomarkers of inflammations or OS in the serum and in compartments as the synovial fluid, which keeps doubts whether OS and inflammation in joints studies can use blood samples as biological matrix of inflammation and OS diagnosis.

Thus, the objective of this study was to analyze the systemic oxidative profile (blood) and local (synovial fluid) of individuals with ACL rupture of the knee, in order to evaluate whether inflammation biochemical parameters diffuse from the tissue into the blood and are equivalent in a systemic way in individuals.

METHODOLOGY

Delineation

This study is a cross-sectional study, to evaluate intensity and difference of oxidative damage between the synovial fluid and blood of patients with ACL rupture.

Casuistry

The sample was formed by 11 men, with total ACL rupture associated or not with meniscus and/or chondral injury. This study included patients over 18 years old who did not use anti-inflammatory medications in the last 48 hours before collection, and who did not use antioxidant supplements.

Ethical aspects

All individuals were informed about the objectives and possible risks of the study and voluntarily agreed to participate. After that, they signed the informed consent form according to the Nuremberg Code (1947), the Universal Declaration of Human Rights (1948) and the Declaration of Helsinki. The study protocol was previously submitted and approved by the Research Ethics Committee according to regulation 196/1996 of the Brazilian National Health Council under the register – CAAE (Certificate of Presentation for Ethical Consideration) – 0164.0.398.000-11.

Experimental model

Initially, all participants were submitted to an anamnesis, for characterization of demographic data and determining criteria for inclusion in the study.

To carry out the arthroplasties, patients were previously sedated and fifteen minutes before the surgical procedure, 8 mL blood was collected from the antecubital fossa and placed in a test tube without anticoagulant. Subsequently, 4 ml synovial fluid was collected through puncture of the knee (arthrocentesis) with needle inserted into the parapatellar and suprapatellar external region (sub-quadricipital bag bottom) and placed in Eppendorf tubes. Collection and surgical procedures were performed in the morning.

After collection, blood samples were centrifuged at 1500 rpm for fifteen minutes. Serum was extracted and packaged in Eppendorff tubes for carrying out biochemical dosages. Serum and synovial fluid were stored at -18°C up to the moment of biochemical analyses.

Biochemical analyses

The ability to induce lipid peroxidation was measured through formation of measurement of substances reactive to thiobarbiturate acid during a heated acid reaction, as described by Esterbauer and Cheeseman[16].

Phenolic compounds content in the serum of individuals was determined by the Folin-Ciocalteau method[17].

Catalase activity happened with hydrogen peroxide described earlier by Aebi[15].

After the experimental procedure, concentrations of analytes were measured through the semi-automated spectrophotometer Biosystems BTS 350®.

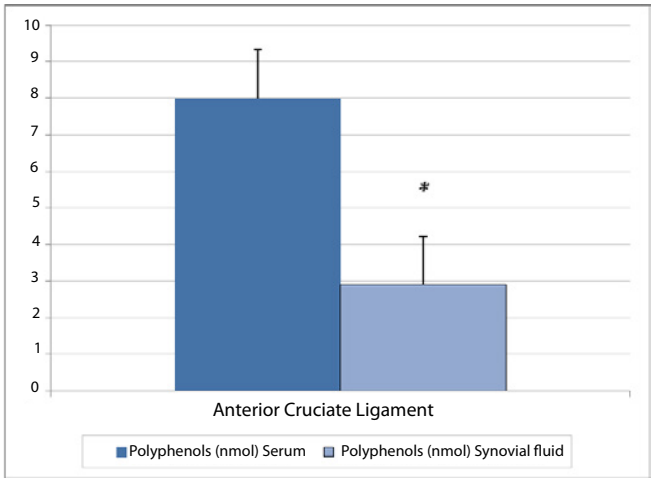
Statistical analysis

Each variable was submitted to statistical analysis using the Kolmogorov-Smirnov normality test and the Levene's test of variance. The 95% confidence interval was assumed and differences were considered statistically significant when p<0.05>

Results were transcribed in a worksheet and statistically analyzed by comparing means using the Wilcoxon-Mann-Whitney test (non-parametric data), in the statistical software SPSS 16.0, considering p<0.05 as significance minimum level. The results were expressed as mean ± standard error.

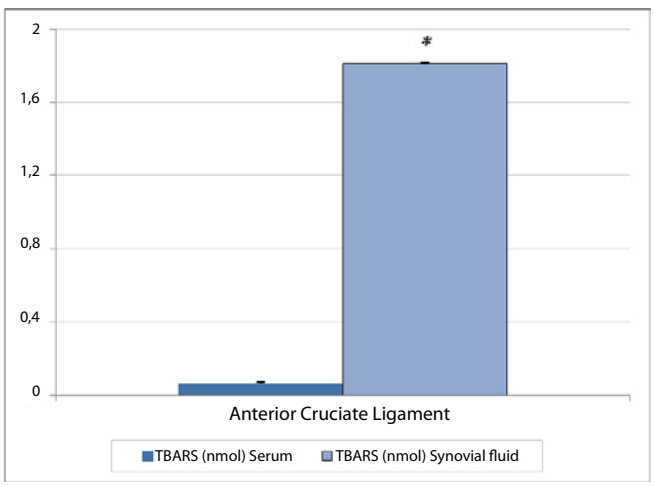
RESULTS

Statistical analysis of the results showed a statistically significant reduction in the concentration of polyphenols (flavonoids) in the synovial fluid, when comparing to the serum (SF=2.9±0.9 S=8.0±4.4). Determination of polyphenols is a type of antioxidant defense indicator, thus, this reduction indicates consumption of this substrate via FRs resulting from ACL inflammation.



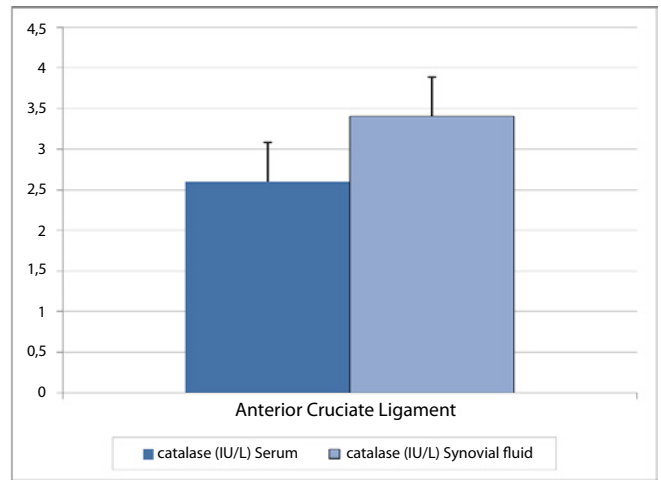
Graph 1. Comparative analysis of concentration of polyphenols in the blood and synovial fluid of patients with ACL rupture. Results expressed as mean±standard error. *p<0.01 non-parametric data analysis through the Wilcoxon-Mann-Whitney test

Analysis of TBARS concentration (substances reactive to thiobarbiturate acid) as an indicator of lipid damage via FRs in the serum and synovial fluid of patients with ACL rupture (Graph 2) points a statistically significant increase in the synovial fluid when compared to the serum (SF=1.8±1.76 S=0.06±0.04). This finding indicates lipid damage caused by FRs in the synovial fluid. Implication of this is that production and oxidative damage can extend to all underlying tissues bathed in the fluid of the joint and may amplify the damage, although from the systemic point of view, it spreads in a very limited way.



Graph 2. Comparative analysis of TBARS concentration in the blood and synovial fluid of patients with ACL rupture. Results expressed as mean±standard error. *p<0.01 non-parametric data analysis through the Wilcoxon-Mann-Whitney test

Catalase is an antioxidant enzyme involved in neutralization of endoperoxides. Determination of catalase activity in the serum and synovial fluid of patients with ACL rupture (Graph 3) shows a greater enzyme activity in the synovial fluid when compared to the serum (SF=3.4±2.17 S=3.3±2.9). However, this difference was not statistically significant.



Graph 3. Comparative analysis of catalase activity in the blood and synovial fluid of patients with ACL rupture. Results expressed as mean±standard error

DISCUSSION

Increasing interest in practice of physical activities nowadays raises number of associated complications such as ACL rupture. ACL rupture is the most prevalent knee injury, which in addition to cause instability in the knee joint, it may trigger changes in a systemic level[18].

The knee is one of the most vulnerable joints of the body during exercises, since at the same time it develops complex movements, it has to associate them with maintenance of body weight, which makes the activity even more difficult[19,20]. Among the parts involved in the articulation, the ACL is the most attacked[21], and as described by Neto[22], when it ruptures, brings several consequences to this joint, since permanent instability is higher than any other joint injury.

According to Sebben et al.[23], the inflammatory process (as verified after an ACL injury) causes generation of FRs, which are incessantly produced during metabolic processes and trigger an OS[24] mechanism, which is an imbalance between oxidant systems and antioxidant defenses, being favorable to the first ones[24,25]. According to Pereira[26], generation of FRs and consequent formation of reactive oxygen species (ROS) is what will involve the whole problem.

The established relationship when there is an oxidative process in the body after a joint injury in the knee, has not yet been well founded regarding comparisons between samples of synovial fluid and serum. However, according to Baccarin[27], it is possible to state in general that this process triggers an imbalance in the relationship between oxidants and antioxidants. FRs are involved in inflammatory damage amplification of synovial fluid via OS[28,29]. Most studies reviewed evaluated degree of oxidative stress in the plasma of patients and not in the fluid itself, as performed in our study. Differences mentioned here show that oxidative damage is greater at the inflammation location and that this intensity does not directly reflect in a systemic way (serum).

Analysis of the results for flavonoids, which was selected as a water-soluble antioxidant biomarker in this study (Graph 1), shows a statistically significant decrease in the concentration of these compounds in the synovial fluid, probably induced by its consumption via FRs of the inflammatory process, which did not reflect in the serum of patients.

The half-life of FRs is extremely short, being almost impossible to determine them in normal clinical conditions. Nevertheless, oxidative damage can be analytically determined. Lipid damage via free radicals can be determined through lipid peroxidation analysi[16,25,26]. TBARS dosage (substances reactive to thiobarbiturate acid) is a product of lipid oxidation via FRs, also named lipoperoxidation[30].

Lipid damage analysis through TBARS determination demonstrates a significantly higher oxidative damage in the synovial fluid when compared to the serum (Graph 2). Correlation of the results listed on Graphs 1 and 2 points that ACL injury causes a consumption of antioxidants combined with a greater lipid damage induced by FRs more intensely at the inflammation location, and that did not reflect in a systemic way. Impact of this imbalance culminates in the OS that can be amplified by the degree and time of injury of the patient. Besides, the synovial fluid in an OS status can expand the oxidative damage to subjacent tissues bathed in synovial fluid, aggravating the oxidative and inflammatory situation.

Rodrigues and Barboni[31] mention that the catalase function is implicated in the decomposition of endoperoxides originated from the normal cellular metabolism. This conversion is a body protection way against OS[32], otherwise, endoperoxides would carry out degradative and toxic actions on some cellular types, such as erythrocytes[31]. Statistical analysis of the results pointed out that there was no significant difference in the activity of this enzyme in the synovial fluid when compared to the serum. Whereas catalase is an antioxidant enzyme produced by the body in OS conditions and the ACL injury is usually acute, it is possible that this encounter means the body has not adapted yet to the inflammatory condition caused by the injury. In a chronification stage, activity of this enzyme would possibly be increased.

All evaluated patients were treated during the acute phase of the injury, which could justify the more intensely OS at the inflammation location when compared to the serum. This indicates that studies related to ACL injury that use serum as biological matrix may have serious limitations when carried out during the acute phase, once during chronification, biomarkers may spread to the blood and change in a systemic way.

The study is limited due to the low number of samples since the collection procedure of both biological samples are invasive. Future studies evaluating patients during the acute phase and the chronic phase may clarify whether OS systemic changes can be used with confidence in ACL injury studies, since the results obtained here allow to conclude that during the acute phase, serum is a sample that does not reflect the real intensity of the process of local OS.

OS in the synovial fluid can be amplified by the damage to healthy tissues bathed in the same fluid, which can worsen damage and clinical condition of patients. In this sense, use of antioxidants is a promising alternative for the clinical and postoperative conduction of these patients.

CONCLUSION

Analysis of the results obtained in this study allows to conclude that the oxidative profile of serum and synovial fluid samples of patients with ACL rupture, presented a higher consumption of antioxidants, associated with increased oxidative damage caused by FRs, which indicates an OS condition induced by ACL injury.

Comparison of OS measure between the serum and the synovial fluid points out that studies of these biomarkers in the serum during the acute phase may underestimate the real intensity of the local inflammatory damage.

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