



Original Article

Analysis on the serum levels of the biomarker CTX-II in professional indoor soccer players over the course of one season[☆]



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ABSTRACT

Objective: The aim of this study was to analyze the blood serum levels of CTX-II in professional indoor soccer players, at three different times during one season: at the start of the pre-season, four months later (a time that marks the middle of the season) and at the end of the season.

Methods: Fourteen male soccer players of mean age 19 years were included. Blood samples of 3 mL were collected from each individual. The samples were analyzed by means of Elisa tests.

Results: There was a significant increase in the serum level of CTX-II in the indoor soccer players, from the beginning to the end of the season ($p < 0.01$).

Conclusion: These data suggest that joint degradation had occurred in these soccer players, by the end of this period. It is evident that further studies are needed, with methodological rigor, so as to make an effective contribution toward precise elucidation of the etiology of this osteoarthritis and its relationship with the biomarkers, as a tool for early diagnosis.

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Análise dos níveis séricos do biomarcador CTX-II em atletas profissionais de futebol de salão durante uma temporada

RESUMO

Objetivo: Analisar os níveis séricos sanguíneos de CTX-II em atletas profissionais de futebol de salão, em três momentos distintos durante uma temporada: no início da pré-temporada, quatro meses após (período que marca o meio da temporada) e no fim da temporada.

Métodos: Foram incluídos 14 atletas do gênero masculino e média de idade de 19 anos. Foram coletados 3 mL de sangue de cada indivíduo. As amostras foram analisadas pelo teste do tipo Elisa.

Palavras-chave:

Osteoartrite

Cartilagem articular

Biomarcadores farmacológicos

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Resultados: Houve aumento significativo dos níveis séricos de CTX-II nos atletas de futebol de salão, comparando-se o início e o fim de uma temporada ($p < 0.01$).

Conclusão: Esses dados sugerem a ocorrência de degradação articular nos atletas, ao término desse período. Fica evidente a necessidade de futuros estudos, com rigor metodológico, que possam contribuir efetivamente para a elucidação precisa da etiologia da OA e sua relação com os biomarcadores como instrumento de diagnóstico precoce.

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Introduction

Primary osteoarthritis (OA) is a multifactorial disease characterized by irreversible joint degeneration, with formation of osteophytes and reduction of the joint space. It presents the following main symptoms: progressive increase in pain, loss of function, limitations on day-to-day activities and restrictions on sports practice.¹⁻⁷

Some theories have correlated continual intense practice of physical activity with development of primary OA in elite athletes, caused by joint overloading. Moreover, there has been speculation regarding how the joint cartilage responds to this overloading. However, the relationship between joint cartilage damage and intense physical activity still seems to be a matter of controversy in the literature.^{8,9}

Some studies have demonstrated that athletes who practice sports that include rapid acceleration with instant deceleration or continuous training with a high impact on joints, or who compete at elite level for prolonged periods of time, present greater likelihood of developing OA. However, the types and intensities of exercises that are harmful to joint cartilage remain unknown.⁸⁻¹¹

OA presents an initial asymptomatic phase, which may be influenced by the level of overloading to which the joint is exposed in a physical activity. However, identification of this joint damage is difficult, given the limitations of the assessment instruments available. In this light, recent studies have demonstrated that biochemical biomarkers are a potential option for early detection of asymptomatic OA.^{9,12,13}

Under physiological conditions, the metabolism of type II collagen is slow. Its fibrils have a half-life measured in years. In the initial stages of cartilage degeneration, degradation of these collagen fibrils is observed. Enzymes named metalloproteinases are released, and these contribute toward this degradation, especially collagenases and aggrecanases. Collagenases are responsible for cleaving type II collagen and produce fragments of this collagen. Specific antibodies for these fragments can be detected in synovial fluid, blood or urine and have been studied as potential biomarkers for the onset of joint degradation.^{2,5,7}

When a joint component is degraded and ejected out of its original tissue, measuring this in the joint fluid would be the most precise method. One biomarker of joint degradation is the C-telopeptide of type II collagen (CTX-II). Use of CTX-II as a marker for progression of cartilaginous lesions and its direct relationship with radiological grades and clinical scores for OA have been proven in the literature. Therefore, assaying of

CTX-II levels seems to be an effective manner for ascertaining type II collagen turnover.¹⁴⁻²¹

Biomarkers are instruments for measuring the progression of diseases or the effects of treatment on disease progression. Thus, they can serve as tools for elucidating the effects of exercise on joint cartilage and the possible development of primary OA.^{12,13} In this light, the objective of the present study was to analyze and compare blood serum assay levels of the biomarker CTX-II in professional indoor soccer players, at three different times during one season.

Methods

This was a prospective longitudinal study that was approved by the Research Ethics Committee of Santa Casa de Misericórdia de São Paulo.

The study included 14 players in a professional indoor soccer team (under-21 category). The players were male, of mean age 19 years, all in the same team, and were subjected to the same training and match load.

Players with previous knee surgery (two players) or who were using chondroprotectant medications (three players) were excluded.

In addition, four players who were undergoing physiotherapy treatment for femoropatellar overload due to muscle imbalance in the pelvic belt were excluded because they presented pain of patellar origin that limited their sports practice at the beginning of the season.

CTX-II levels were assayed at three times: A – at the beginning of the pre-season; B – four months later (a time that marked the middle of the season); and C – at the end of the season.

Blood samples (3 mL) were collected from each individual by means of simple puncture in the non-dominant arm, using vacuum collection kits. The blood samples were centrifuged and stored at a temperature of -80 degrees, until all the samples were ready to be tested.

Each sample was analyzed by means of an ELISA test for detecting human CTX-II (Hu CTX-II kit, Cusabio Biotech, catalog number CSB – E09323h, batch S20045731, produced in the United States). This kit presents 100% specificity for human CTX-II alone, without cross-reactions, and with a minimum detectable level of lower than 0.3 ng/mL. This test was performed in a private laboratory in the city of São Paulo, with costs entirely borne by the researchers.

To compare the assayed levels between the three evaluation times, the nonparametric paired Wilcoxon statistical test and Bonferroni correction for multiple comparisons were

Table 1 – Data on individuals and their CTX-II assay levels (in ng/mL), observed for each player at each time during the season.

	Age	Intensity of pain	Beginning of season	Middle of season	End of season
Player 1	19	No pain	0.321	0.328	0.328
Player 2	18	No pain	0.201	0.278	0.401
Player 3	19	Occasional non-limiting pain	0.182	0.331	0.551
Player 4	20	No pain	0.825	1.121	2.537
Player 5	20	Occasional non-limiting pain	0.312	0.298	0.399
Player 6	19	Occasional non-limiting pain	0.311	0.315	0.324
Player 7	19	Limiting pain	0.299	0.311	0.324
Player 8	20	Occasional non-limiting pain	0.282	0.301	0.309
Player 9	20	No pain	0.311	0.315	0.497
Player 10	20	Occasional non-limiting pain	0.892	2.012	2.349
Player 11	17	No pain	0.312	0.299	0.395
Player 12	19	No pain	0.287	0.277	0.268
Player 13	17	Occasional non-limiting pain	0.347	0.521	0.577
Player 14	19	No pain	0.247	0.317	0.378
Mean (SD)	19		0.366 ± 0.214	0.5 ± 0.485	0.685 ± 0.751

applied (95% confidence interval and p value <0.05). This non-parametric test was the best option because it was impossible to assume that the sample had normal distribution.

Results

Table 1 presents the data on each individual and the marker levels observed for each of them at each time during the season. It could be seen that two players (4 and 10) presented discrepant data, with much higher assayed levels than those of the other players evaluated in this study, in all the evaluations. Nevertheless, these participants did not report any decline in physical performance, or any presence of symptoms such as pain, edema or instability.

Individuals 3, 5 and 10 presented pain at the origin of the patellar tendon in one knee, which became worse at the end of the sports practice, improved with physiotherapy and did not limit their participation in training and matches. Individual 13 presented a condition of non-limiting pain above the insertion of the pes anserinus. Also, individuals 6 and 8 complained of lateral pain in their knees, above the lateral epicondyle, during sports practice, which was also non-limiting.

Because of the tendinous origin of the pain in the individuals included in this study (patellar tendinopathy, anserine tendinopathy and friction of the iliotibial tract), it was decided not to include them separately in statistical comparisons regarding degradation of type II collagen exclusively of the cartilaginous tissue.

Comparison of CTX-II assay levels between different times during the season

Because of the limited size of the sample, which was selected in a non-randomized manner because this was a closed indoor soccer team, the distribution of the individuals and results from the sample cannot be considered to be normal. When this occurs, nonparametric statistical tests need to be used. In the graphic distribution of the data, two individuals had values that were very different from those of the

remainder of the team and which were considered to be outliers.

To avoid the risk of statistical error, two separate analyses were performed: 1 – comparisons using the complete data; and 2 – comparisons using reduced data (without the information on players 4 and 10) (Table 2).

In comparing the values from the first two analyses, i.e. beginning of the season versus middle of the season, no statistically significant differences between the samples could be seen, in either of the comparisons (1 and 2).

In the analysis between the middle and the end of the season, there was a significant difference in assayed levels, in the comparison among the 14 participants ($p < 0.02$). However, when players 4 and 10 were taken out of the analysis, no significant difference could be seen (analysis with reduced data).

In comparing the beginning and end of the season, both analyses (comparisons 1 and 2) indicated that there was a statistically significant increase in the CTX-II biomarker ($p < 0.003$ and $p < 0.01$, respectively). In other words, between the beginning and the end of the season, there was a significant increase in the level of the CTX-II joint degradation biomarker, irrespective of whether the extreme results were taken into consideration.

Table 2 – Comparison of assayed levels for the biomarker CTX-II (in ng/mL) observed between the different times during the season. Complete data (14 players) and reduced data (12 players: players 4 and 10 were disregarded).

Comparison	Mean difference	p
<i>Complete data</i>		
Beginning vs. middle	0.134	0.06
Middle vs. end	0.186	0.02
Beginning vs. end	0.320	0.003
<i>Reduced data</i>		
Beginning vs. middle	0.041	0.19
Middle vs. end	0.069	0.06
Beginning vs. end	0.109	0.01

Discussion

This study aimed to analyze and compare blood serum assay levels for the biomarker CTX-II among professional indoor soccer players, at three different times during one season. From the results observed, it could be seen that there was a statistically significant increase in CTX-II between the beginning and end of the season. It has been well established in the literature that increased levels of the biomarker CTX-II are a predictive factor for joint degradation. Thus, it is believed that early identification of CTX-II levels would be a useful tool for making equally early diagnoses of primary osteoarthritis (OA) and taking preventive action.^{22,23}

Corroborating the findings of the present study, O'Kane et al.²² evaluated different sports categories and demonstrated through urine analyses that samples from marathon runners presented higher CTX-II levels than those of swimmers or rowers. According to these authors, runners expose their lower-limb joints to repetitive axial overloading, which may cause early damage to their joint cartilage.

The pure and simple increase in CTX-II in the players studied here does not necessarily mean that they are suffering or will suffer osteoarthritis. It is known that patients with arthrosis have high levels of this serum biomarker and increased levels are directly related to radiographic worsening, according to the Kelgren-Laurence classification.¹⁶ What we can be sure of from these data is that, at least during competitions, chondral degradation was higher in the patients studied, since the biomarker CTX-II comes from destruction of type II collagen, exclusively in joint cartilage.

It is possible that changes to training, intensification of anaerobic exercises with muscle strengthening, compulsory physiotherapeutic follow-up and even use of chondroprotectant medications might be viable solutions for joint protection. In order to protect the joint health of soccer players, teams should start to think about this type of preventive strategy.

After all, the aim today should be prevention before treatment. Injury avoidance through practicing preventive medicine should be the focus of exercise and sports medicine, since careers can certainly be prolonged in this manner.

The possible limitations of this study are the small sample size ($n = 14$) and the absence of a control group for comparisons between findings. The sample size is explained by the fact that this study investigated a single team within this category, whose members were all subjected to the same training and competition loads. It was decided not to use a control group, because our aim was only to ascertain whether there would be any increase in joint collagen degradation over the course of a single season of training and competition. In other words, the individuals became their own controls.

Other studies underway within the Sports Trauma Group of Santa Casa de São Paulo are comparing types of sports and control groups, including indoor soccer. Preliminary studies indicate that there are large differences regarding type II collagen degradation.

The discrepancies encountered in our sample, in two of the individuals analyzed, can be explained by a variety of theories. These include the notions that the markers may have been influenced by the individuals' hormonal state, diet

or genetic factors,²² given that neither of these individuals reported any reduction in physical performance, or any symptoms such as pain, edema or instability. According to Dam et al.,²⁴ the amount of cartilage degradation estimated from the biomarker CTX-II is related to the presence of pain.

Conclusion

There was a significant increase in the serum levels of CTX-II in the indoor soccer players, comparing the beginning and end of the season. These data suggest that increased degradation of type II joint collagen was occurring at the end of this period. It is clear that further studies are needed, with appropriate methodological rigor, so as to make effective contributions toward precise elucidation of the etiology of OA and its relationship with biomarkers as a tool for early diagnosis.

Conflicts of interest

The authors declare no conflicts of interest.

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