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RESEARCH ARTICLE

Correlation of Serum and Synovial Osteocalcin, Osteoprotegerin and Tumor Necrosis Factor-Alpha with the Disease Severity Score in Knee Osteoarthritis

Csifo Enikő[1], Katona Tímea[2], Arseni Juliánna[2], Nagy E[2], Gergely I[3], Nagy Ö[3]

[1] Department of Analytical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
[2] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Tîrgu Mureş, Romania
[3] Department of Orthopaedics and Traumatology I, Faculty of Medicine, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

Objectives: Study of circulating and synovial levels of osteocalcin, osteoprotegerin and tumor necrosis factor-alpha (TNF-α) in patients with different stages of knee osteoarthritis and correlation analysis of these parameters with disease severity.
Methods: We enrolled 20 patients with different stages of knee osteoarthritis. The IKDC score (International Knee Documentation Comittee, 2000) was determined for each patient. Based on these data patients were divided into two groups: group I (advanced osteoarthritis) and group II (early osteoarthritis). Serum and synovial fluid levels of osteocalcin, osteoprotegerin, TNF-α were determined.
Results: For the entire group the level of osteocalcin in the serum showed higher values than in the synovial fluid. We found statistically significant differences in the serum levels of osteocalcin between the two groups (group I: 2.18 ± 0.54 ng/ml, group II: 6.07 ± 1.98 ng/ml, $p = 0.019$). Serum and synovial osteocalcin in the whole study lot could not be correlated with the disease score, however we observed a tendency towards significant negative correlation between the serum osteocalcin and IKDC score for group I and between synovial osteocalcin and IKDC score in group II. In the entire group, synovial osteoprotegerin concentration was six times higher than the serum osteoprotegerin level ($p < 0.0001$) and TNF-α showed higher circulating levels than local concentrations.
Conclusions: In the advanced osteoarthritis group the serum and synovial osteocalcin show lower values than in the early osteoarthritis group, which means that as the disease progresses, bone anabolism decreases. In the case of osteoprotegerin, no significant difference between the two groups was detected.

Keywords: knee osteoarthritis, IKDC score, bone metabolism, osteocalcin, osteoprotegerin

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Introduction

Osteoarthritis is the most common musculoskeletal disorder, which involves the degenerative alteration of articular cartilage, synovial inflammation and consecutive changes of subchondral bone. Synovitis can be detected in both early and advanced stages of osteoarthritis and it is characterized by synovial membrane hiperplasia, infiltration of inflammatory cells, such as B and T lymphocytes and macrophages in the synovium. The inflammatory process is initiated and maintained by pro-inflammatory cytokines, among which interleukine-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) are the most important [1–3].

The involvement of subchondral bone in the pathology of osteoarthritis is poorly understood, but it is proved that in the evolution of the disease there is an upraising metabolic interaction between cartilage and the underlying bone. Osteoblasts induce the production of matrix-metalloproteinases: MMP-3 and MMP-13 by chondrocytes, leading to cartilage degradation. Beside this, subchondral bone osteoblasts produce cytokines (IL-1, IL-6, TNF-α), which promote osteoclast activity, thereby increasing bone resorption. Bone metabolism suffers an imbalance, anabolic processes decreasing in favour of catabolism [4].

Osteocalcin and osteoprotegerin are two important factors in bone metabolism. Osteocalcin is the most abundant non-collagenous bone protein, produced by osteoblasts and chondrocytes too. This protein is a marker of bone formation and turnover. Its role in the pathology of osteoarthritis is still not fully known, but former studies reported an increased release of osteocalcin by subchondral bone [5–7].

Osteoprotegerin and receptor activator for nuclear factor k B ligand (RANKL), two cytokines of the TNF superfamily, are described as key elements in the differentiation and function of osteoclasts. Secreted by the subchondral bone and cartilage chondrocytes, osteoprotegerin plays an important role in bone remodelling.

Correlation of osteocalcin and osteoprotegerin with the radiographically assessed severity of osteoarthritis is still an issue, when trying to establish the role of these biomarkers in osteoarthritis activity [6,8–10].

The golden standard for the evaluation of the severity of osteoarthritis is plain radiography, but multiple grading

Correspondence to: Előd Nagy
E-mail: elod.nagy@umftgm.ro

systems were developed, designed to assess the activity of the disease. One of these is the IKDC (International Knee Documentation Comittee) score, which is a subjective evaluating system for symptoms, function and physical activity among patients with knee injuries [11,12].

The aim of this paper is to study circulating and synovial levels of osteocalcin, osteoprotegerin and TNF-α in patients with different stages of knee osteoarthritis, assessed by the IKDC score, and to correlate these parameters with disease severity.

Methods

In this study 20 patients with different stages of knee osteoarthritits were recruited (mean age 41.15 ± 3.15 years; 4 female, 16 male), who underwent arthroscopic investigation at The Orthopaedics and Traumatology Clinic of the County Emergency Clinical Hospital of Tîrgu Mureș in 2010–2011. The study was approved by the Ethics Committee of the Hospital.

Cartilage surface has been studied trough arthroscopy and synovial liquid was sampled from the articular joints. At the same time blood was sampled, sera and synovial fluid supernates were separated by centrifugation at 3000 rpm/min for 10 minutes. All these samples have been stored frozen at –50°C until the determination of biological parameters.

The markers of bone metabolism: osteocalcin, osteoprotegerin and the inflammatory cytokine TNF-α were assayed by sandwhich ELISA method, using the following reagent kits: OC- Osteocalcin Intact ELISA, Alpco (USA); OPG- human OPG/ TNFRSF11B, R&D Systems (USA); TNF-α – BD OptEIA human TNF-α, BD Biosciences (USA).

The IKDC score was determined for each patient and based on the results, the subjects were divided into two groups: group I with the IKDC score between 18–33 (advanced osteoarthritis) and group II with the IKDC score between 35–58 (early osteoarthritis).

The statistical processing of the experimental data was performed using GraphPad Instat 3 and Microsoft Excel softwares. The normality of distribution of the data was analyzed in each case applying the Kolmogorov-Smirnov test. The multiple comparisons between the groups were performed with Kruskal-Wallis-Anova test and Mann-Whitney U test was applied for comparison between the two groups. Spearman test was used for correlation analysis of the studied parameters with the IKDC score. Statistical significance was set at a p value of 0.05.

Results

Osteocalcin and TNF-α presented higher values in the serum than in the synovial fluid, when the entire group was studied. Analyzing osteoprotegerin concentrations, these showed six times higher values in the local synovial liquid than in the serum, the difference being highly statistically significant ($p < 0.0001$).

Separately, for the two groups the ratio of concentrations of serum and synovial osteocalcin and osteoprotegerin was approximately the same, as it was for the entire group. However, TNF-α for group II showed equal values for serum and synovial fluid (Table I).

Comparing group I to group II, both serum and synovial osteocalcin showed higher concentrations in group II with early osteoarthritis, with a statistically significant difference regarding the circulating levels. Contrary to osteocalcin, serum and synovial osteoprotegerin showed minor differences between the two groups. A statistically significant difference was observed in the synovial levels of TNF-α, when comparing the two groups (Table I).

Analyzing the IKDC score correlations with the studied markers, one could notice that TNF-α for the whole group

Table I. Serum and synovial levels of biological markers studied

Biological marker	All patients (n = 20)	Group I (n = 10)	Group II (n = 10)	p values Group I vs Group II
Ser OC (ng/ml)	4.12 ± 1.09	2.18 ± 0.54	6.07 ± 1.98	0.019*
Syn OC (ng/ml)	2.17 ± 0.36	1.70 ± 0.43	2.64 ± 0.56	0.123
Ser OPG (pg/ml)	761.81 ± 98.95	795.24 ± 157.45	724.67 ± 123.26	0.780
Syn OPG (pg/ml)	4797.38 ± 527.75	4658.04 ± 723.94	4952.20 ± 813.73	0.842
Ser TNF-α (pg/ml)	11.15 ±3.90	8.64 ± 5.75	13.66 ± 5.45	0.089
Syn TNF-α (pg/ml)	7.35 ± 2.32	1.05 ± 0.53	13.64 ± 3.70	0.029*

Values represent mean levels ± standard error. Ser= serum; Syn= synovial fluid; OC= osteocalcin; OPG= osteoprotegerin; TNF-α= tumor necrosis factor alpha. p values calculated with Mann-Whitney U test, *- statistic significance

Table II. Correlation between IKDC score and biomarkers studied

Correlations	All patients	Group I	Group II
IKDC vs. Ser OC	r= 0.28 (p= 0.223)	r= -0.462 (p= 0.179)	r= -0.345 (p= 0.328)
IKDC vs. Syn OC	r= 0.126 (p= 0.595)	r= -0.207 (p= 0.565)	r= -0.479 (p= 0.162)
IKDC vs. Ser OPG	r= -0.215 (p= 0.374)	r= -0.407 (p= 0.247)	r= -0.150 (p= 0.708)
IKDC vs. Syn OPG	r= -0.100 (p= 0.683)	r= 0.224 (p= 0.536)	r= -0.433 (p= 0.249)
IKDC vs. Ser TNF-α	r= 0.390 (p= 0.090)	r= -0.037 (p= 0.918)	r= 0.389 (p= 0.266)
IKDC vs. Syn TNF-α	r= 0.386 (p= 0.093)	r= -0.191 (p= 0.598)	r= -0.079 (p=0.828)

IKDC= IKDC score; r= correlation coefficient; Ser= serum; Syn= synovial fluid; OC= osteocalcin; OPG= osteoprotegerin; TNF-α= tumor necrosis factor alpha

showed a tendency towards positive correlation with the activity score, both in serum and synovial fluid (Table II).

In the advanced stages of osteoarthritis (group I) there was a tendency towards negative correlations between serum osteocalcin levels and IKDC score. In group II with early osteoathritis, synovial osteocalcin showed a tendency towards negative correlation with the disease activity score.

In case of osteoprotegerin, no significant correlation was observed with IKDC score, concentrations of this bone marker showing only low negative correlation with activity score.

Regarding the correlation of studied parameter with the age of the patients, a statistically significant negative correlation was revealed between serum osteocalcin and age, when the entire group was tested ($r = –0.647$, $p = 0.002$). Serum osteoprotegerin showed a slightly positive correlation with the age of the subjects ($r = 0.328$, $p = 0.170$). A significant negative correlation was observed between the IKDC score and the age of the patients ($r = –0.503$, $p = 0.002$).

Discussions

The aim of this study was to explore the relationship between the markers of bone metabolism: osteocalcin, osteoprotegerin and the severity of osteoarthritis, characterized by the IKDC score. Currently the diagnostic gold standard of osteoarthritis is plain radiography, but this can not detect early stages of the disease and it can not monitor the its progression over short periods of time [6,9]. Given that the pathological mechanism of osteoarthritis involves changes in the subchondral bone too, cutting edge molecules for bone metabolism may play a role in diagnosing and monitoring the disease.

Based on our results osteocalcin, the bone turnover marker, showed higher serum levels than synovial concentrations. This finding is in agreement with the results of Salisbury and Sharif's research and suggests that synovial osteocalcin is derived from blood [7]. Correlations between the serum and local osteocalcin levels could not be found. In the early stage of the disease osteocalcin showed significantly higher levels than in the advanced stages, especially in the serum of the patients. This fact indicates an increased bone cell activity in the incipient stages of osteoarthritis, with intense bone remodelling processes which decrease during the progression of the disease. The amplification of bone formation and turnover in early osteoarthritis may suggest a compensatory action of subchondral bone, which tries to prevent the destabilization of cartilage and progression of the disease.

The significant negative correlation between serum osteocalcin and the age of the patients has not been reported in earlier studies [6].

Regarding osteoprotegerin, this other marker molecule of bone metabolism, our results indicate that, unlike osteocalcin, this showed higher values in the synovia than in the serum. This result is similar with those reported by Pilichou et al. [8], who showed synovial osteoprotegerin levels four times higher than serum ones. Therefore, synovial osteoprotegerin is involved in the pathology of knee osteoarthritis, suggesting along with osteocalcin the dynamism of subchondral bone remodelling. Regarding osteoprotegerin, depending on the stage of the disease, a minor change of synovial and serum concentrations was observed while a correlation between the circulating levels and severity of the disease could not be described.

Our results regarding TNF-α showed that in the early stage of osteoarthritis, this inflammatory cytokine presents higher values, indicating a more intense inflammatory reaction, which decrease with progression of the disease.

Conclusions

The results of this study emphasize the involvement of subchondral bone in the pathology of osteoarthritis. Increased concentrations of osteocalcin in early stages of the disease suggest an intense bone remodelling process, which decreases as osteoarthritis worsens. Although osteoprotegerin, along with osteocalcin, is a bone turnover marker, we could not evidence its association with the severity of disease. The studied parameters showed no correlation with disease severity, however this could be caused by the fact that only a reduced number of patients have been enrolled. Further research is needed to establish the role of these biomarkers in assessing the progression and severity of osteoarthritis.

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