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Complex evaluation in patients with knee osteoarthritis

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Abstract

Knee osteoarthritis (KOA) is a very common, slowly progressive and incurable articular disease resulting in the breakdown of cartilage and bone in the joint, which causes significant discomfort, pain and disability, with a significant socio-economic impact. The aim of our observational study for patients with symptomatic KOA was to investigate the changes of C-terminal telopeptide of type II collagen (CTX-II) in serum pre and post a complex rehabilitation program and to establish the correlation between all studied parameters [clinical, functional, serum CTX-II (sCTX-II) and histological aspects obtained through arthroscopy]. During 2016–2017, we performed an observational study including 24 patients, between 59 and 76 years old, diagnosed with KOA. Studied patients were completely assessed before (T1) and four months after a complex rehabilitation program (T2). The measured parameters were stiffness, pain, and physical function and we used the Visual Analogue Scale (VAS) for pain, with Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), sCTX-II value obtained by enzyme-linked immunosorbent assay (ELISA) test, histological assessment of knee cartilage after arthroscopy. In the histological assessment of our patients, we observed that the cells of the superficial areas were round and hypertrophic, the cartilage tissue had few proteoaminoglycans and glycosaminoglycans, with an airy aspect of the matrix and degenerate cells, numerically reduced. After positive diagnosis, the complete treatment, including the rehabilitation program, seems to be the ideal option for improving the CTX-II values, as well as the quality of life in KOA patients.

Keywords: osteoarthritis, knee, serum CTX-II, histology.

Introduction

Defined as an increasingly important problem for the health system [1], with an increasingly high prevalence around the world [2], osteoarthritis (OA) is a very common, slowly progressive and incurable articular disease resulting in the breakdown of cartilage and bone joint condition, which causes significant discomfort, pain and disability, with a significant socio-economic impact [3, 4].

Although OA is one of the major health issues in middle-aged patients [5] and one of the most prevailing joint afflictions worldwide [6], this problem becomes more pronounced with increasing age. Because its prevalence increases with age (it is found in almost 70% of the population over 60 years old), OA will have an increasingly higher prevalence from this time forth [4]. Today, 8–10% of both genders suffers from OA (single or multiple joints), which leads to disability and a decrease in quality of life – functional and social activities, body image, and emotional well-being [7]. Commonly considered an organ disease, OA is now regarded as a relentless joint condition altering all articular tissues (meniscus, ligaments, synovial membrane and cartilage) and periarticular tissues such as muscles, tendons and adipose tissue [8].

Particular attention was paid to articular cartilage degeneration as a primary event in the disease development. A progressive destruction of joint in OA starts in the articular cartilage, mainly in the weight-bearing joints [5], combined with osteophyte formation, and pathological changes in the synovial membrane and the subchondral bone at the joint margin with the endochondral process of bone formation [9]. The gradual degradation and loss of joint cartilage with the associated structural and functional changes in the whole joint defines this complex inflammatory process [10]. The latter can be a remedial reaction to joint damage and modified mechanical loading and an adaptive reaction of joint instability [11]. However, the etiology and precise pathogenetic mechanisms (biomechanical, biochemical, or other) of OA are presently uncertain and still has no known cure [12]. In the last decade, OA is more and more seen as the dynamic metabolism process involving both the destruction of cartilage and the repair of cartilage. Both biochemical and mechanical changes can cause OA and all tissues in the articulation become part of an adaptive response [13]. Though the condition exists in different joints, it commonly affects the weight-bearing joints of the knees and hips [14, 15].

Knee osteoarthritis (KOA) is the most frequent type (6% of adults). OA has a higher probability of occurrence that increases with age [6]. It is a prevalent joint disease with a worldwide prevalence of about 4% [16]. The

incidence of KOA has increased significantly in the last decade in population under the age of 65 and has an increased debilitating potential, leading to significant care costs and a significant decrease in quality of life [17]. A chronic disease with a long "silent" period, KOA has severe financial and emotional consequences as one of the most weakening diseases that affect humanity [18]. The degenerative process of the knee involves loss of hyaline articular cartilage, bony remodeling, bone marrow lesions, laxity of ligaments, capsular stretching, and weakness of periarticular muscles [19]. Often malalignment and further mechanical imbalance develop. These are accompanied by intermittent synovitis and local inflammation. Inflammation of the periosteum as a result of reshuffling, denuded bone, effusion, bursitis and spasm of surrounding muscles contribute to pain in OA. Hyaline joint cartilage probably will not cause pain because it does not contain any nociceptive fibers. Obviously, osteoarthritic pain has multifaceted etiologies from within and outside the joint [20]. KOA, complex multifactorial, local inflammatory joint disease, remodeling of subchondral bone [21], loss of function and biochemical integrity of joint cartilage, has highly variable natural history. The disease improves in some patients, remaining stable, or gradually worsening in others. Moreover, there is a poor correlation of clinical symptoms with radiological appearance [20]. How inflammation is an initiator against the result of the destructive joint process is still unclear [22].

Many researchers are studying patients with OA to find the ideal fluid biological markers (BMs) to reflect articular cartilage metabolism, and to reveal disease activity or prognosis [23]. The assessment of type II collagen synthesis and degradation is considered relevant in the study of OA progression [11]. Among the degradation products of type II collagen, C-terminal telopeptide of type II collagen (CTX-II) is the marker that is most studied. In OA patients, high levels compared to asymptomatic individuals or radiological OA signs have been demonstrated. Some papers have shown an important link between OA and CTX-II radiographical progression [24].

The aim of our observational study in patients with symptomatic KOA was to investigate the changing of CTX-II values in serum pre and post a complex rehabilitation program and to establish the correlation between all studied parameters (clinical, functional, serum CTX-II [sCTX-II] and histological aspects obtained through arthroscopy).

Patients, Materials and Methods

During the period 2016–2017, we performed our observational study on 24 patients diagnosed with KOA. They were examined (clinical, functional and imagistic evaluation – knee ultrasound and radiographic exam) in the Department of Physical and Rehabilitation Medicine, "Filantropia" Municipal Hospital, Craiova, Romania. All the procedures used in our clinical and paraclinical trial were first approved by "Filantropia" Municipal Hospital Ethics Committee and an informed consent form was signed by all patients. The first part of our observational study comprises of a histological examination of knee cartilage, with a correlation between histological aspects and initial level of sCTX-II. The second part is comprised of an intricate assessment regarding the symptoms, clinical and functional status and serum level of sCTX-II in KOA patients, pre and post a complete rehabilitation program.

Studied patients were completely assessed before (T1) and after rehabilitation program (T2). The parameters we used were: stiffness, pain and physical function (self-reported disability) – Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and we used the Visual Analogue Scale (VAS) for pain; CTX-II [ng/mL] was measured with the commercially available enzyme-linked immunosorbent assay (ELISA) test; we analyzed serum samples for each patient, pre-rehabilitation program (T1) and after four months (T2); cartilage histological assessment after knee arthroscopy performed to solve the pieces of torn cartilage that were loose in the joint; the histological examination was performed initially and after four months, to evaluate the consequences of the complex rehabilitation program in knee cartilage.

The biological material, represented by femoral and patellar cartilage fragments, was processed for the use of conventional histology technique for paraffin inclusion resulting in a series of 4–5 μm samples, which were stained and examined using the optical microscope. Using classical histological methods, Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome stainings, we were able to view microscopic aspects of the cartilage lesions and to evaluate its extension.

All patients diagnosed with symptomatic KOA performed a complete rehabilitation program, three weeks, 12 sessions (we applied conservative treatment: glucosamine and chondroitin sulfate substances – 1500 mg daily, three months, pain medication – in accordance with international guidelines, physical methods – ice, iontophoresis, massage and kinetic measures – range of motion and strengthening exercises).

Statistical analysis

Since the study involves the dynamic evaluation of numerical parameters (VAS, WOMAC and sCTX-II, at baseline and reassessment) for the same patients, and the data are not Gaussian distributed, we used the Wilcoxon test. Because the VAS can also be interpreted as a qualitative scale, we used the χ^2 (*chi*-square) test to check its relationships with the other qualitative variables.

Results

We included 24 patients in our study (12 females, 12 males), older than 59 years (average age 72.4 years, between 59 and 76 years), with a positive diagnosis of KOA based on the *American College of Rheumatology* (ACR) criteria for OA.

After the four months rehabilitation program, we found significant changes of sCTX-II level values [ng/mL] in our patients' serum, the range of values decreasing from 0.07–1.49 to 0.07–0.58, with the Q1–Q3 interval (interquartile or 25–75% interval) reduced from 0.163–0.92 to 0.151–0.422). As such, for sCTX-II the differences were significant with a 99% confidence (p=0.0013 <0.01) in T2 evaluation comparing to T1 assessment.

In all the patients included in our study, we found

that for VAS there were highly significant values (99.9% confidence), just like for the WOMAC scale, *p* being less than 0.001. VAS values dropped from range of values of 7–9 to an interval of 4–6, with a decrease of the Q1–Q3 interval from 8–9 to 4.75–5, while for WOMAC the value range decreased from 58–78 to 48–67, with a Q1–Q3 interval reduction from 60.75–71.25 to 51–61.25 (Table 1).

Table 1 – ***Mean values of studied parameters***

Parameter	VAS		WOMAC		sCTX-II [ng/mL]	
Moment	*T1*	*T2*	*T1*	*T2*	*T1*	*T2*
Mean ±SD	8.167 ±0.637	4.917 ±0.654	66.417 ±6.049	56.083 ±5.904	0.524 ±0.481	0.297 ±0.166
Min.	7	4	58	48	0.07	0.07
Q1	8	4.75	60.75	51	0.16375	0.1515
Median	8	5	67.5	55	0.295	0.291
Q3	9	5	71.25	61.25	0.92	0.4225
Max.	9	6	78	67	1.49	0.68

VAS: Visual Analogue Scale; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; sCTX-II: Serum C-terminal telopeptide of type II collagen; SD: Standard deviation. Wilcoxon test, $p<0.001$ – highly significant (HS) for VAS and WOMAC; Wilcoxon test, $p<0.0013$ – significant (S) for sCTX-II; T1: Initial evaluation; T2: Reevaluation – after four months.

All patients were diagnosed with other conditions as well, diabetes mellitus type 2, dyslipidemia, and hypertension. When we compared the numeric parameter values, initial and final, using univariate analysis, taking into consideration the important qualitative variables (gender, residence, metabolic disorders and hypertension), we did not find significant differences, the mean values being sensible equal.

There were no significant differences between groups, divided by gender or residence, but we noticed that VAS values were both greater for women, almost reaching the significance level (0.05), and for urban inhabitants, WOMAC values were both greater in men. sCTX-II values were both greater in women and rural inhabitants, all of them not surpassing the limit for statistical significance.

Only at the initial moment we have highlighted the influence of the distribution of VAS values in the association with diabetes mellitus (*chi*-square $p=0.015 <0.05$), the influence that was not found in the final moment at revaluation (*chi*-square $p=0.407 >0.05$) (Table 2).

Table 2 – ***Mean values of studied parameters: gender and residence variables***

Parameter	Women (12)	Men (12)	*p* Mann–Whitney	Urban (14)	Rural (14)	*p* Mann–Whitney
VAS1	8.42 ±0.51	7.92 ±0.67	0.0665	8.29 ±0.47	8 ±0.82	0.3696
VAS2	5.17 ±0.58	4.58 ±0.79	0.0595	4.93 ±0.62	4.8 ±0.92	0.8432
WOMAC1	66.25 ±6.97	66.58 ±5.28	0.6432	66.29 ±5.92	66.6 ±6.55	0.8372
WOMAC2	55.92 ±5.57	56.25 ±6.47	0.8846	56.14 ±6.16	56 ±5.85	0.7461
sCTX-II1 [ng/mL]	0.62 ±0.57	0.43 ±0.38	0.3708	0.52 ±0.49	0.52 ±0.49	0.93
sCTX-II2 [ng/mL]	0.31 ±0.18	0.29 ±0.16	0.8625	0.26 ±0.15	0.35 ±0.18	0.1687

T1: Initial evaluation; T2: Reevaluation – after four months; VAS: Visual Analogue Scale; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; sCTX-II: Serum C-terminal telopeptide of type II collagen.

We also performed a multivariate analysis for VAS, WOMAC and sCTX-II, taking as a factor assessment time, gender, diabetes and dyslipidemia, but the only factor that had an influence on the change of values was just the time of the evaluation, the interactions between the factors being insignificant.

Histological assessment

During arthroscopic surgery and limited arthrotomy under regional anesthesia, an orthopedic surgeon removed the 24 partially hemorrhagic soft tissue specimens. The microscopic aspects varied between the initial and final assessment. In T1 evaluation of histological samples, we observed: hypertrophic low-cell number, lysed cartilage, mature cartilage cells. Cartilaginous tissue at which the decrease in the tincture of the fundamental substance is observed. Protein and glycosaminoglycans poor tissues, airy cell matrix appearance, degenerated cells – anucleate cells or hyperchromatic nuclei (Figures 1–3).

In T2, the microscopic aspect of cartilage revealed an appearance such as: whole cartilaginous tissue with numerous mature, clustered cells, rich in young cells clustered in isogenic series, some in the form of an articular filamentous cell-rich areas, with dense basic substance (Figures 4–6).

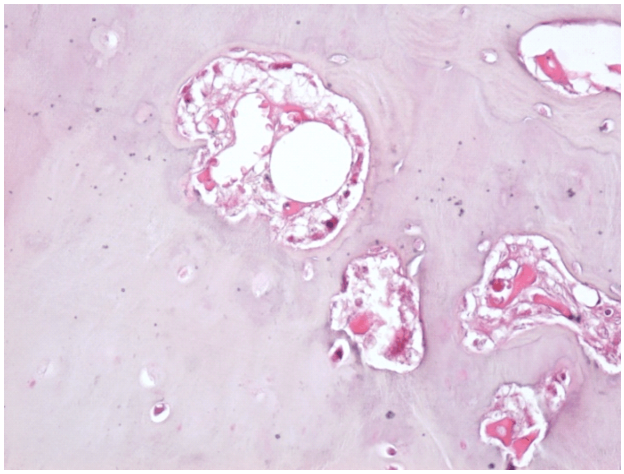

Figure 1 – ***Low-cell hypertrophic, lysed cartilage, prior to treatment (HE staining, ×100).***

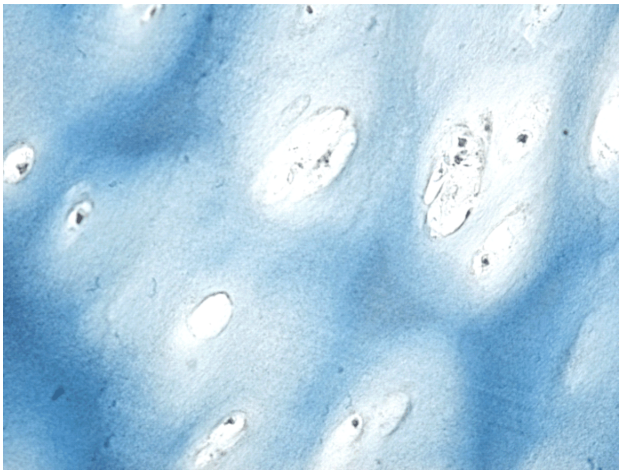

Figure 2 – ***Fuzzy cellular matrix appearance, degenerated cells – anucleate cells or hyperchromatic nuclei, before treatment (GS trichrome staining, ×400).***

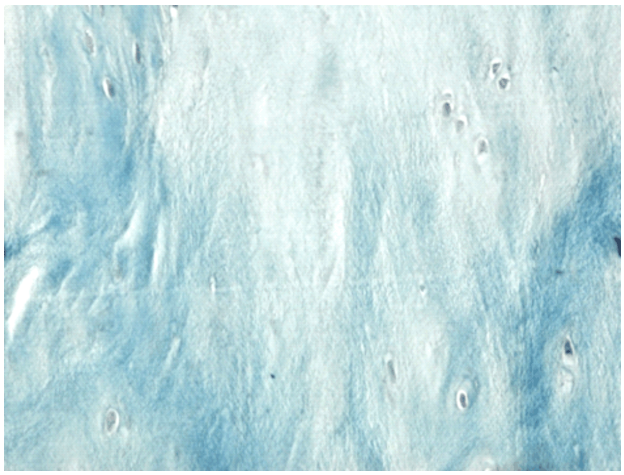

Figure 3 – ***Low cell count cartilage tissue, with a marked decrease in staining intensity, before treatment (GS trichrome staining, ×100).***

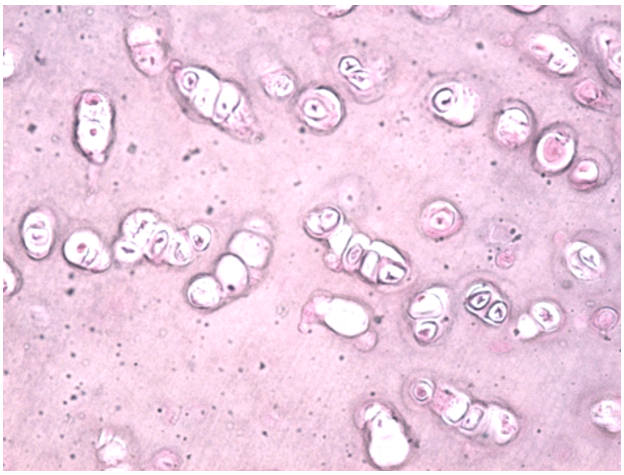

Figure 4 – ***Cartilaginous tissue sample rich in young cells connected in series, after treatment (HE staining, ×200).***

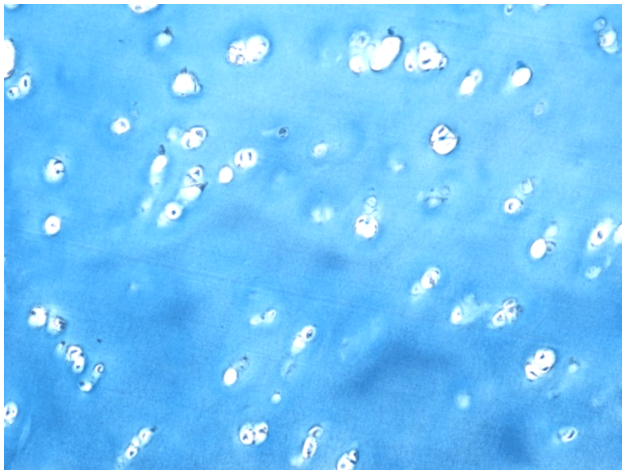

Figure 5 – ***Young cartilage cells, grouped in isogenic series, after treatment (GS trichrome staining, ×100).***

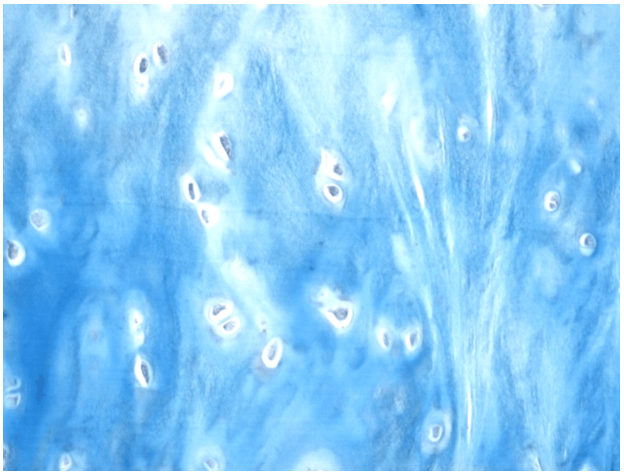

Figure 6 – ***Cell-rich cartilage area with dense cellular matrix, after treatment (GS trichrome staining, ×100).***

Discussions

In our study, we conducted a complicated evaluation of KOA patients (clinical, paraclinical, functional, imagistic and histological evaluation), and tried to established correlations between functional and lab parameters. We applied a complete rehabilitation program in accordance with the *Osteoarthritis Research Society International* (OARSI) guidelines. Non-surgical KOA treatment should be the first choice and can involve both physical exercise and pharmaceutical methods [21].

In medical literature, there are many studies concerning KOA (epidemiological data, risks factors, pathophysiology, symptoms and clinical features, diagnosis and treatments) [25, 26]. Since 2015, OA is defined as a complex disease which occurs primarily through molecular disorder (a change in the cartilaginous tissue metabolism) followed by anatomical and/or physiological changes (initial such as degradation of cartilage tissue, bone remodeling, osteophyte development, articular inflammation, and in the end the loss of the normal joint by a massive reduction of articular space) [27].

The mean age of our studied patients is in accordance with other similar studies. KOA can develop at any age, but it most frequently develops in people aged over 50 years, and the number of patients with painful knee OA is significantly increasing due to the significant growth of the aging society [1].

All patients presented risk factors (obesity, genetic predisposition, overuse of the joint, perturbations in existing alignment in the affected joints, weak thigh muscles, genetic factors) which are important to mention in KOA diagnosis [17]. Like in other studies [16], the results of complex assessment of our patients proved that OA affects all parts of the knee, this complex joint of lower limb – the underlying bones (radiographic knee exam Kellgren–Lawrence grade 2), the synovium (ultrasound exam), and the cartilage (histological and lab assessments).

The functional and pain status of our patients has significantly improved and maintained after the complex rehabilitation program. This aspect is very important because people with symptomatic KOA have a substantially reduced quality of life [5], and the cost for medical procedures for these disorders has a high consequence on health system. The WOMAC index (24 items – two items for stiffness, five components for pain and 17 items for the physical function) is a well-certified scale designed to convey the issues experienced by patients

with lower limb OA; a higher scale score indicating an increase in disability [28] and VAS scale is the most used tool in the assessment of pain in musculoskeletal diseases [29].

The pathogenesis of OA is multi-factorial and it affects several tissues starting with the articular cartilage. The mature articular cartilage is a highly specialized structure, created and maintained by the chondrocytes, embedded within extracellular matrix (ECM) containing up to 85% of water. The ECM mechanical properties are determined by the presence of highly organized macromolecular structures involving proteoglycans, type II collagen and hyaluronic acid (HA) [30]. Adult cartilage has low repair capabilities, which can also lead to gradual damage and arthritic joint degeneration, leading to considerable pain and disability [31]. The consequent articular cartilage degradation is frequently associated with some concurrent factors are age, abnormal chronic joint stresses, metabolic diseases, immune system disorders and inflammation leading to biochemical changes that also favor cartilage degradation [30]. The pathogenesis of OA has inflammation as a major factor, promoting synovitis concurrently while bone and cartilage get destroyed by chemokines secretion, cytokines and other molecules. All these items can be found in the synovial fluids and [26, 27] through angiogenesis and chemotaxis they bring their contribution to OA pathogenesis [32].

We evaluated the serum level of CTX-II, one of the fundamental results of type II collagen degeneration [33]. Our reason for assessing this lab parameter is sustained by the multitude of studies in this domain. The degeneration of articular cartilage includes matrix degeneration that mainly results in the losses of proteoglycans and type II collagen. CTX-II is discharged into the synovial fluid and absorbed by serum when type II collagen is degraded [12]. Through the metabolism of type II collagen fibers and early diagnosis of OA and the effect of clinical treatment predictions, dynamic detection of the CTX-II concentration can reflect therefore the severity of articular OA cartilage lesions [34]. Our dynamic evolution of sCTX-II levels expressed the consequence of the complex rehabilitation program applied in KOA patients.

When we prescribed pharmacological, physical and kinetic measures to our patients, we took into consideration that KOA is a disorder that begins as a disease of a single tissue (cartilage) and progresses to become a disease of an organ (knee joint) in which cartilage is primarily affected but which involves many other tissues and structures [6]. Therefore, physical and kinetic measures could optimally condition the medication actions. We found only one study in rehabilitation literature correlated with our purpose. Pascarelli *et al.* (2016) mentioned in their paper the effects of balneotherapy (mud baths) for patients with knee OA on serum biomarkers; more exactly, a significant increase in serum levels of CTX-II is associated with cartilage turnover induced by the balneotherapy [35].

Our pioneer study presented two limitations. The first limit is that we measured only one biochemical marker of type II collagen. CTX-II alone only partially reflects overall cartilage collagen degradation, also [36] at very early stages in OA development, type II collagen fibers are destroyed. Some studies have shown that a coalescence of a biochemical marker of type II collagen synthesis (serum type IIA collagen *N*-propeptide) and deterioration [two BM cartilage oligomeric matrix protein (COMP) and cross-linked CTX-II] [12] was superior than one of these two markers in predicting the destruction of articular cartilage and the disease progression in knee OA [23, 37]. In samples of patients with different severities of OA during the previous year, several biomarkers have been tested [22, 38]. In patients diagnosed with early-stage KOA involving minimal destruction of cartilage tissue, the level of COMP and HA showed significant increases [39]. Type I collagen degradation and type II collagen should be studied because type I reflects bone degradation and CTX-II reflects degradation of the cartilage [11]. CTX-II may indicate, in some cases, the connection between bone and cartilage in articular diseases, leads to the conclusion that CTX-II is not a biomarker for bone turnover, while being a valid biomarker of cartilage breakdown [40]. Over the past year, a series of recommendations were published by *OARSI* regarding the use of soluble biomarkers during clinical trials, the diverse contexts for which OA biomarkers may be used and also which key steps should be taken in order to qualify a biomarker as a tool for drug development [39].

The second limit is that we followed our parameters in all patients after a complex rehabilitation program, without organizing many subgroups for each type of measure – pharmacological treatment – glucosamine, physical and kinetic measures. Regarding CTX-II levels in other studies, an increased probability for a response after glucosamine treatment is linked with a greater cartilage component turnover [41, 42].

We established, in our study, an elevated level of CTX-II in patients with important histological cartilage modification. Bai & Li studied the connection present between COMP and CTX-II concentrations and morphological transformation in articular cartilage during the pathological process in adult rabbits with OA. They concluded that the extent of OA joint cartilage lesions, early diagnosis and prognosis of clinical treatment effectiveness could be detected by dynamic concentration of the sCTX-II [12].

After histological examination, literature data concerning microscopic aspects of the joint cartilage in KOA could be confirmed. Several animal models of OA have been promoted to histologically examine the premature aspects of cartilage degeneration, because early stage OA cartilage tissue is not easily available as the disease is not yet clinically obvious [43, 44], such as the knee joint, and it is found in the growth plate of the metaphysis [45]. Our study was performed on human patients with KOA. The histological and staining methods used in our study, were also used to detect changes in cartilage morphology in other musculoskeletal disorders [46, 47]. The osteochondral plugs were removed from the distal lateral femoral condyle and the center of the medial patella facet. Almost all patients had presented advanced disease stages, so the hypertrophic villi and full-thickness defect

areas with missing cartilage and bare subchondral bone could be observed in arthroscopic assessment. The thickness and density are greater in the subchondral plate itself. Cartilage adjacent to severe lesions is also affected, presenting a thickening in line with hypertrophy. Healthy joint cartilage has a smooth surface without fissures; the ECM provides compressive and unique viscoelastic properties, composed in part by large proteoglycan aggrecan and type II collagen [48].

The OA process is a continuous degeneration process. At the early stage, minimal changes are detected in the cartilage surface, which is no longer smooth. The superficial area is populated with mild fibrillations, while the distribution of glycosaminoglycans remains homogenous. After, changes in the cellular structure appear. The cells of the superficial zone, usually flat in the first place become round and hypertrophic before disappearing from the tissue. Mild-to-moderate hypercellularity is displayed in the cells of the middle and deep areas. In the superficial area, multicellular chondrocyte clusters were found, with large nuclei, and in the intermediate and radial regions, the necrotic chondrocytes were described with pyknotic nuclei. The cartilage shows signs of full rupture in advanced OA stages. Fissures appear at the surface of the cartilage, which is rough and broken [49, 50].

The present experiment demonstrated that the histological aspect of knee and patellar cartilage improved after the rehabilitation program in all our patients. We did not use the histological–histochemical grading Mankin or *OARSI* systems for the evaluation of cartilage degeneration because it is used to establish end-stage cartilage degeneration. The Mankin system has severe limitations in the evaluation of mild to moderate OA. In the fundamental research, histological modifications of degenerating cartilage are evaluated using the Mankin score or the Sakkara modified score. Tissue structure along with cell morphology, appearance of tidemark and matrix staining are evaluated with these scores. In the Mankin scale, 14 is the highest score correlating with the highest damage and 32 when applying the modified Mankin score [51].

OARSI presented a new OA cartilage histopathology assessment system since 2006, validated for animal and human articular cartilage. The *OARSI* system consists of a grading element (0 to 5), a higher grade indicates a biological progression that is more aggressive and a higher phase that reveals a greater extent of illness. The most important aspect of the *OARSI* system is its ability to identify early or mild OA differences [52, 53].

The lack of using one of the two histological classification systems for the patients studied is justified by the fact that fragments for histological examination were obtained by arthroscopic exploration, not by direct joint approach. Studies state the importance of correctly harvesting the four fragments with a thickness of at least 8 mm, so that the histological examination allows for a definite assessment of changes in all layers of cartilage [54]. Our experiment had included histological assessment in patients who did not require a classic knee intervention, following the association of these histological changes with those of a much-used OA biomarker – sCTX-II.

Conclusions

Clinical, paraclinical, imagistic and histopathological evaluation should always be used in the assessment of mild to advanced KOA in order to obtain the best therapeutic outcome. After diagnosis, a complete treatment including a complex rehabilitation program, represents the ideal option for improving pain and functional status as well as improving articular cartilage morphology in patients with KOA. The biochemical assessment has demonstrated its usefulness in detecting early changes occurring in KOA patients and should be used as prognostic tools for future cartilage alterations.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Magdalena Rodica Trăistaru, Mirela Radu and Daniela Teodora Maria equally contributed to this article.

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