INTRODUCTION

Menisci are fibrocartilaginous structures of the knee joint, located between femoral condyles and tibial plateau. It is a specialized tissue that plays a role in the transmission of loads, shock absorption, stability of the knee joint and distribution of synovial fluid and nutrients [1]. It is primarily made of a terwoven network of collagen fibers, while the cells and the extracellular matrix are inserted between the fibers. The collagen components of menisci have an important functional role in the provision of tissue extensibility [2]. The concentration of collagen in meniscus is higher than in articular cartilage [3]. Collagen type I is dominant in the meniscus (90% of the total collagen content), while the rest of collagen content is type II, III, V, VI, and X [4].

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Summary

Introduction. Knee osteoarthritis is a progressive degenerative disease which affects meniscal tissue. The aim of this study was to determine the differences in collagen type I expression in macroscopically unaltered and osteoarthritic menisci, and correlate the expression with the grade of macroscopic damage, age and body mass index of patients, preoperative condition of anterior cruciate ligament, angulation and knee contracture. Material and Methods. The control group consisted of 10 macroscopically unaltered menisci, while the experimental group had 35 osteoarthritic menisci. Besides macroscopic grading of meniscal damage, the analysis of collagen type I expression was determined by immunohistochemical staining with the corresponding antibody using semiquantitative scale scores and quantitative parameters: intensity of expression and stained area size. Results. The results of semiquantitative evaluation showed a statistically significant decrease in collagen type I expression in osteoarthritic menisci, which correlated with an increase in macroscopic damage grade. The results of quantitative evaluation did not show a statistically significant decrease in the expression. In posterior meniscal horns, a more intense collagen type I expression was seen in the women, as well as a positive correlation of quantitatively evaluated expression with body mass index. Collagen type I expression in the anterior horns was significantly lower in varus alignment. Conclusion. In the semiquantitative evaluation, collagen type I expression in osteoarthritic menisci was significantly lower compared to macroscopically unchanged menisci. The decrease in the expression level correlates with the increase in the grade of macroscopic meniscal damage. There was no statistically significant difference in the quantitative evaluation of expression. Key words: Knee Joint; Menisci; Tibial; Osteoarthritis, Knee; Collagen Type I; Pathological Conditions, Anatomical; Immunohistochemistry

Sažetak

Abbreviations

OA – osteoarthritis
BMI – body mass index
ACL – anterior cruciate ligament

made of collagen types II, III, IV, V, VI, and X [4]. Collagen types III and V are found in trace amounts (<1%). The proportion of different types of collagen in the meniscus varies depending on the localization – the peripheral two thirds of the meniscus are made mostly of collagen type I, while the inner third of the meniscus has collagen types II and I in 60%: 40% ratio [5]. In addition to the distribution, the orientation of collagen fibers within the meniscus (circumferential and radial) directly affects the function of the tissue, and it has been observed that the network of collagen fibers in the meniscus changes in the content and structure from one region to another [6, 7].

Osteoarthritis (OA) is a chronic degenerative joint disease that affects millions of people worldwide [8]. Due to the expected longer life expectancy, it is estimated that the two-thirds of people over 60 years of age have OA [9]. The knee joint is most frequently affected by osteoartritic changes [10]. Nuclear magnetic resonance (NMR) images of the knee joint have proven that meniscal destruction is one of the characteristics of the late stage of OA of the knee joint [11]. The loss of meniscal function is a potent factor for the development of knee osteoarthritis and cartilage loss in the course of OA [12].

The macroscopic appearance of the meniscus in knee OA depends on the grade of degeneration. Menisci marked as grade 0 have smooth, whitish shiny surface without signs of degeneration, while those with grade 4 have pale color and rough surface with fissures and clefts. In sites of degenerative changes of meniscal tissue, the amount of collagen and noncollagenous proteins is reduced, which decreases the mechanical quality of this tissue [13]. Menisci with a higher degree of degeneration have been found to have significant structural changes, which includes rifts and cysts within the collagen bundles with the formation of cell nests [14]. Collagen fibers in OA menisci become coarser and less organized, and the amount of individual circumferential and radial fibers is significantly reduced [15]. As for the collagen type I, it was found that there is a reduction of its expression in OA menisci, predominantly in the deeper tissue zones [16], and that the expression significantly decreases with the severity of degeneration [17]. A thorough survey of relevant literature has failed to reveal either an analysis of the variability of the expression of collagen type I in relation to the anatomical parts (body, horns) in unaltered and degenerated menisci or any investigations on a possible correlation between the expression and general characteristics of patients and associated changes in joint structure. The aim of this research was to investigate the immunohistochemical expression of collagen type I in unaltered and osteoarthritic menisci of human knee joint, to determine the differences in the expression among the three parts of the meniscus (anterior horn, body, posterior horn) as well as to establish the connection of expression with the gender, age, body mass index (BMI), preoperative contracture, angulation of the knee joint and the condition of anterior cruciate ligament (ACL).

Material and Methods

The research was conducted with the permission of the Ethics committee of the Department of Physical Medicine and Rehabilitation “Dr Miroslav Žotović” in Banja Luka. The informed consent in writing was obtained from every patient or a family member before taking the material. Each participant in the study was given written information with all the details related to the research.

The samples for the study were collected at the Department of Orthopedic Surgery, Department of Physical Medicine and Rehabilitation “Dr Miroslav Žotović” in Banja Luka from 35 consecutive patients who had total knee arthroplasty due to clinically advanced osteoarthritis; thus, 70 menisci (35 medial and 35 lateral) were included in the study sample of which 10 macroscopically unaltered and 35 degenerated menisci were analyzed. The following data were taken from the patients’ histories: gender, patient’s age, BMI, preoperative contracture, angulation of the knee joint (varus or valgus), preoperative condition of ACL (intact or ruptured) and medications used by the patients.

The study sample did not include patients who had one of the following diagnoses: rheumatoid arthritis, ankylopoetic spondylitis, psoriatic arthritis or reactive arthropathy, as well as those patients who used any chondroprotective drugs, such as corticosteroids, sulfate polysaccharides, chemically modified tetracyclines, diacetylrhein or glucosamine [18], or who had a history of trauma of the knee in which the prosthesis was implanted, and the patients with general systemic weakness or acute contagious disease.

All meniscus samples were placed in the buffered solution of 10% formaldehyde immediately after extraction. After fixation, the obtained meniscus samples were macroscopically analyzed and graded according to the scale of Sun et al. [19]: 0 = normal surface appearance; 1 = minimal fibrillation and degeneration; 2 = moderate fibrillation and degeneration; 3 = severe fibrillation and degeneration, without cleft; 4 = severe fibrillation and degeneration, multiple cleavages, incomplete or complete clefts. Having been graded, the menisci were cut in the frontal plane into 3 levels (the anterior horn, body and posterior horn) and three tissue sections per meniscus were made, each 5 mm thick. The tissue sample processing was done according to the ordinary procedure at the Department of Pathology, Clinical Center, Banja Luka. After molding in paraffin, the samples were cut and stained with hematoxylin eosin.

For immunohistochemical analysis of collagen type I, the paraffin tissue blocks were sliced into the semi-
rial sections that were mounted on glass slides (5 sections per a slide). After the standard tissue processing, immunohistochemical procedure was continued by blocking endogenous peroxidase by incubating the tissue in a 3% hydrogen peroxide solution for 10 minutes at room temperature and antigen unmasking in a commercial solution of Proteinase K (Proteinase K ready-to-use, DAKO Corporation, USA) at room temperature for 10 minutes. The incubation was performed immediately prior to the application of primary antibody with commercial Ultra V Block (TA-125-UB, Lab Vision Corporation, Fremont, USA), and then with the corresponding mouse monoclonal anti-collagen I [COL-1] antibody (ab90395, Abcam plc, Cambridge, UK) at a 1: 400 dilution of concentrated antibody. The diluted antibody was poured onto the slides and incubated overnight in a humidified chamber at 4 °C. Antigen unmasking was performed with the detection system made of anti-mouse and anti-rabbit IgG conjugated with horseradish peroxidase (Lab Vision UltraVision LP Detection System: HRP Polymer (Ready-To-Use), Lab Vision Corporation, Fremont, USA) in the first step and DAB chromogen (DAKO liquid DAB + Substrate Chromogen System, DAKO Corporation, USA) in the second step of immunodetection. After washing of chromogen, the tissue sections were contrasted with Maier's hematoxylin.

The evaluation of extracellular expression of the investigated antibody was carried out:

1. according to the following semiquantitative scale: score 0 (absence of staining, the entire extracellular space without staining); score 1 (weak or moderate, focal extracellular positivity/weak diffuse positivity); score 2 (high focal extracellular positivity/moderate diffuse positivity) and score 3 (high diffuse positivity).

2. quantitatively by determining two parameters: the staining intensity score and the surface of cross-sectional area (in μm²) with the present extracellular expression. This quantification was performed using the software TissueQuant (Manipal Centre for Information Science, Manipal University, India), version 1.0.1.

In order to determine whether a certain knee angulation and the state of ACL together affected collagen type I expression, four subgroups were formed out of the experimental group:

1. Varus angulation with preserved ACL
2. Varus angulation without preserved ACL
3. Valgus angulation with preserved ACL
4. Valgus angulation without preserved ACL, which were used to compare semiquantitatively and quantitatively determined collagen type I expression.

The statistical analysis was performed with SPSS software (SPSS Inc, Chicago, USA) version 16.0 using methods of descriptive statistics, Mann-Whitney test, Kruskal-Wallis test, independent t test and one-way ANOVA with post hoc Tukey’s HSD test. Spearman’s and Pearson’s correlation coefficients were applied for correlation analyses.

**Results**

This study used the samples obtained during total knee arthroplasty performed in 30 women and 5 men. Initially, the samples from 39 consecutive patients were collected, but the samples of four patients were excluded because three patients used chondroprotective drugs and one had infectious arthritis. All patients were operated with the diagnosis of idiopathic arthrosis. This is the first operative intervention on the knee in all patients except one, who had undergone synovectomy before total knee arthroplasty. In all patients, the anatomic graduated component (AGC) monoblock knee endoprosthesis was implanted. The youngest patient was 56 and the oldest was 78 years old (the average age being 69.31 years). The average BMI of our patients was 30.8, 60% of the patients were obese, 28.57% were overweight and 11.43% were physiologically nourished.

Anterior cruciate ligament (ACL) was preoperatively intact in most patients (60%), and preoperative angulation of knee joint was predominantly varus (80%). Further on, regarding the preoperative contracture of knee joint, there was a flexion contracture in more than half of patients (65.71%), the mixed flexion/extension contracture was found in 22.86% of patients, while 5.71% of patients had extension contracture and another 5.71% of patients had no contracture at all. Due to the small number of patients without contractures and with extension contracture, the expression of collagen type I in these patients could not be statistically compared with the patients who had other two types of contractures.

Seventy obtained menisci were classified using macroscopic classification according to Sun et al. [19]. Most of them had grade 4 of damage (30%), 27.14% had grade 3, while the other degrees were significantly less present: grade 2, grade 1 and 0 were found in 15.71%, 14.29% and 12.86% of the menisci, respectively. In all analyzed menisci, varus angulation led to larger damage to the medial meniscus, while lateral menisci were more damaged in valgus angulation.

The control group was formed based on these results (10 menisci- 9 lateral and 1 medial). The samples from this group were used to compare the structural changes in the degenerated meniscus. Out of 35 analyzed menisci in the experimental group, 27 were medial (77.14%) and 8 were lateral (22.86%) menisci; 45.71% of samples had grade 3, and a slightly larger number of samples (54.29%) had grade 4.

The semiquantitative scale was used for the first estimation of semiquantitative expression of collagen type I. The percentages of medians of four expression scores show the preservation of this type of collagen in the control samples and a decreased expression in the samples from the experimental group (Graph 1).

In the control samples, the expression of collagen type I was highest in the body of meniscus, whereas it was lower in both horns. In the experimental group, there was a significant decrease in the expression (score 1 in all three parts of the meniscus). The comparison of the semiquantitatively determined expression of collagen type I between the control and experimental group showed that the
The expression of collagen type I differed statistically significantly between these two groups, the largest decrease in the expression being in the body of meniscus (Table 1).

There were no statistically significant differences in the expression of collagen type I between men and women (U = 58, Z = -0.958, \( p = 0.338 \)). When correlating the expression of collagen type I with the age and BMI, a slight decrease in the expression of this type of collagen was observed in relation to the age (\( \rho = -0.001, p = 0.994 \)). In addition, the obese patients had a lower expression (\( \rho = -0.296, p = 0.085 \)). However, the correlation was weak in both cases.

Among various forms of preoperative contractures of the knee joint there was no difference in the expression of collagen type I (the difference between flexion and flexion/extension contracture: \( U = 91, Z = -0.054, p = 0.957, r = 0.009 \)). No difference was detected between the groups of patients with the intact and absent anterior cruciate ligament and it was \( p = 0.146 \) for the anterior horn, \( p = 0.611 \) for the body and \( p = 0.669 \) for the posterior horn. There was no significant difference in the expression of collagen type I between varus and valgus angulation of the knee joint (for the anterior horn \( p = 0.467 \), for the body \( p = 0.289 \) and for the posterior horn \( p = 0.06 \)).

When the presence of a certain angulation of the knee joint and the integrity of the ACL in those angulations were taken together and compared with the semiquantitative expression of collagen type I, no statistically significant difference was found in the expression in these 4 groups of our study samples (Kruskal-Wallis's test, \( \chi^2(3, n = 35) = 3.359, p = 0.339 \)).

The software quantification of staining intensity score of collagen type I did not show a statistically significant difference among the samples of control and experimental group (Table 2).

More intense staining of collagen type I was observed in the posterior meniscal horns in the female subjects (\( x = 23.663, SD=15.043 \)) than in the male patients (\( x = 5.345, SD=2.541 \)), the difference being statistically significant (\( t(32.947) = -5.301, p = 0.000, \eta^2 = 0.459 \)). More intense staining of this part of the meniscus was noted in the patients with higher BMI (\( r = 0.385, p = 0.022 \)). No difference between the genders and the correlation with BMI, which was seen in the posterior horn, was found in the body and the anterior horn of the meniscus.

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**Table 1.** Expression of collagen type I on semiquantitative scale

<table>
<thead>
<tr>
<th></th>
<th>Control group (median)</th>
<th>Experimental group (median)</th>
<th>( U )</th>
<th>( Z )</th>
<th>( p )</th>
<th>Effect size (( r ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior horn</td>
<td>2</td>
<td>1</td>
<td>71</td>
<td>-3.097</td>
<td>0.002†</td>
<td>0.46‡</td>
</tr>
<tr>
<td>Body/Telo</td>
<td>3</td>
<td>1</td>
<td>11</td>
<td>-4.724</td>
<td>0.000†</td>
<td>0.7§</td>
</tr>
<tr>
<td>Posterior horn</td>
<td>2</td>
<td>1</td>
<td>35</td>
<td>-4.182</td>
<td>0.000†</td>
<td>0.62§</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test; † statistically significant difference; ‡ medium effect size; § large effect size

**Graph 1.** Percentage ratio of semiquantitatively determined expression of collagen type I in both study groups

**Table 2.** Staining intensity scores of collagen type I in relation to study groups and anatomical part of meniscus

<table>
<thead>
<tr>
<th></th>
<th>Control group (( x \pm SD ))</th>
<th>Experimental group (( x \pm SD ))</th>
<th>( t )</th>
<th>( p )</th>
<th>95% Confidence interval</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior horn</td>
<td>19.278 ± 6.003</td>
<td>20.219 ± 14.736</td>
<td>-0.3</td>
<td>(df=37.354) 0.766</td>
<td>-0.94 (-10.62 to 8.74)</td>
<td>0.002</td>
</tr>
<tr>
<td>Body/Telo</td>
<td>20.364 ± 10.043</td>
<td>19.983 ± 15.085</td>
<td>0.075</td>
<td>(df=43) 0.941 0.381 (-9.872 to 10.634)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Posterior horn</td>
<td>18.608 ± 14.81</td>
<td>21.047 ± 17.763</td>
<td>-0.396 (df=43) 0.694</td>
<td>-2.438 (-14.867 to 9.99)</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

* independent samples t-test/#nezavisni t-test
As for the preoperative angulation, the quantitative analysis showed a greater preservation of collagen type I in the anterior horn of valgus knees compared to varus ($t(33) = -2.935$, $p = 0.006$, eta squared = 0.2). These differences were not seen in other parts of the meniscus.

More intense staining of collagen type I in one part of the meniscus was not related to more intense staining in another part (the correlation of expression being the anterior horn - body: $r = 0.194$, $p = 0.263$; the body-posterior horn: $r = 0.133$, $p = 0.446$; the anterior horn-posterior horn: $r = 0.102$, $p = 0.558$). The presence of preoperative contracture and integrity of ACL did not affect the staining intensity score of collagen type I. Neither was there an apparent correlation between the age and this score (Table 3).

Regarding the surface stained on collagen type I, the largest area in both studied groups was in the posterior meniscal horns. The comparison of samples between the control and experimental group showed a reduction of stained surface in the meniscal body, which was not observed in the meniscal horns. The comparison of samples between the control and experimental group showed a reduction of stained surface in the meniscal body, which was not observed in the meniscal horns. However, this decrease was not statistically significant.

Similar to the staining intensity score, a statistically significant difference in the surface stained on collagen type I was observed only in the posterior meniscal horns of male and female patients. The menisci of women had a larger stained surface for this type of collagen ($t = 39.913$, $SD = 18.84$) than of the men ($t = 39.13$, $SD = 18.84$), and this difference was highly statistically significant ($t(32.991) = -2.142$, $p = 0.000$, eta squared = 0.122). In addition, a higher BMI meant a larger stained surface of this part of the meniscus (Pearson’s correlation, $r = 0.419$, $p = 0.012$). The comparisons of staining surface in other parts of the meniscus with the gender and BMI revealed no statistically significant differences.

A larger surface stained on collagen type I in one part of the meniscus did not mean a larger stained surface in another part (the correlation of expression being the anterior horn - body: $r = 0.101$, $p = 0.565$; the body-posterior horn: $r = 0.138$, $p = 0.429$; the anterior horn - posterior horn: $r = 0.05$, $p = 0.777$). Different forms of preoperative contracture, condition of ACL, preoperative angulation and age of the patient did not lead to differences in the surface stained on collagen type I in different parts of the meniscus (Table 5).

The comparison of the grade of macroscopic damage and the semiquantitatively determined expression of collagen type I showed that the damaged menisci had a lower expression of this type of collagen.

### Table 3. Differences of staining intensity scores of collagen type I in relation to anatomical part of meniscus and three examined characteristics of patients and joint changes*†

<table>
<thead>
<tr>
<th>Staining intensity score of collagen type I and:</th>
<th>Anterior horn</th>
<th>Body</th>
<th>Posterior horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skor intenziteta bojenja kolagena tipa I i:</td>
<td>Prednji rog</td>
<td>Telo</td>
<td>Zadnji rog</td>
</tr>
<tr>
<td>1. preoperative contracture*</td>
<td>$t(29)= 0.274$, $p=0.786$</td>
<td>$t(29)= -0.469$, $p=0.643$</td>
<td>$t(29)= 0.296$, $p=0.769$</td>
</tr>
<tr>
<td>2. age of patients†</td>
<td>$r=0.004$, $p=0.148$</td>
<td>$r=0.004$, $p=0.148$</td>
<td>$r=0.119$, $p=0.404$</td>
</tr>
<tr>
<td>3. condition of anterior cruciate ligament*</td>
<td>$t(33)= -1.599$, $p=0.119$</td>
<td>$t(33)= -0.983$, $p=0.333$</td>
<td>$t(33)= -0.853$, $p=0.404$</td>
</tr>
</tbody>
</table>

* independent samples t-test; † Pearson’s correlation

### Table 4. Differences in surface of visual field (in square micrometers) stained for collagen type I in relation to study groups and anatomical part of meniscus*

<table>
<thead>
<tr>
<th>Control group</th>
<th>Experimental group</th>
<th>t (43)</th>
<th>p</th>
<th>95% Confidence interval</th>
<th>Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior horn</td>
<td>Prednji rog</td>
<td>132.71 ± 64.57</td>
<td>136.97 ± 110.94</td>
<td>-0.115</td>
<td>0.909</td>
</tr>
<tr>
<td>Body</td>
<td>Telo</td>
<td>140.32 ± 62.02</td>
<td>132.33 ± 105.44</td>
<td>0.227</td>
<td>0.821</td>
</tr>
<tr>
<td>Posterior horn</td>
<td>Zadnji rog</td>
<td>140.93 ± 120.57</td>
<td>140.796 ± 119.612</td>
<td>0.03</td>
<td>0.998</td>
</tr>
</tbody>
</table>

* independent samples t-test
were differences between the menisci in the knees of the control group. Mine et al. examined whether there were differences in the expression of collagen type I in OA menisci, in comparison to the experimental, OA menisci, in semiquantitative analysis showed that there was a decrease in the expression of collagen type I in the OA menisci, which was less than in the degenerated menisci, and this type of collagen was totally absent in some cases of very severe degeneration [17]. Sun et al. [16] observed a higher content of collagen in the normal menisci compared to the OA menisci. The loss of collagen in the OA menisci was expressed in all areas of the meniscus, more so in the medium and deeper zones than at the surface. In addition, there was a statistically significant reduction of collagen type I expression in OA menisci in their samples. Using the method of collagen densitometry by converting the image to the average levels of gray, Mine et al. got the mean expression of collagen type I of 20.13 in the degenerated menisci, which was less than in the menisci without degeneration, where this value was 151.08. Such a big difference was not observed for type II collagen [17]. These authors believe that the limit of reparation was exceeded in their samples, which led to such a large decrease in the density of collagen type I and loss of tissue structure. The reduction of expression of collagen, observed in our study and cited literature, illustrates the contrast between the meniscus and articular cartilage of the knee joint during OA, because the expression of both types of collagen increases with the severity of degeneration in the articular cartilage [20].

Discussion

Menisci of the knee joint are part of a complex system that should ensure the stability and weight carrying the body during the movements of the joint. In addition, the medial and lateral menisci are an integral component of normal articular homeostasis. When some pathological changes occur in the meniscus, such as acute clefts or chronic degeneration, the whole cascade of processes activates the meniscus, more so in the medium and deeper zones than at the surface. In addition, there was a statistically significant reduction of collagen type I expression in OA menisci in their samples. Using the method of collagen densitometry by converting the image to the average levels of gray, Mine et al. got the mean expression of collagen type I of 20.13 in the degenerated menisci, which was less than in the menisci without degeneration, where this value was 151.08. Such a big difference was not observed for type II collagen [17]. These authors believe that the limit of reparation was exceeded in their samples, which led to such a large decrease in the density of collagen type I and loss of tissue structure. The reduction of expression of collagen, observed in our study and cited literature, illustrates the contrast between the meniscus and articular cartilage of the knee joint during OA, because the expression of both types of collagen increases with the severity of degeneration in the articular cartilage [20].

Table 5. Relationship among surface stained for collagen type I and four examined characteristics of patients*†

<table>
<thead>
<tr>
<th>Surface stained on collagen type I and:</th>
<th>Anterior horn</th>
<th>Body</th>
<th>Posterior horn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front infective contracture</td>
<td>t(29) = 0.398, p = 0.694</td>
<td>t(29) = -0.408, p = 0.687</td>
<td>t(29) = 0.119, p = 0.906</td>
</tr>
<tr>
<td>Age of patients</td>
<td>r = -0.104, p = 0.551</td>
<td>r = 0.200, p = 0.249</td>
<td>r = -0.146, p = 0.403</td>
</tr>
<tr>
<td>Condition of anterior cruciate ligament</td>
<td>t(33) = -1.891, p = 0.067</td>
<td>t(18.906) = -1.395, p = 0.197</td>
<td>t(33) = 0.924, p = 0.366</td>
</tr>
<tr>
<td>Preoperative axis</td>
<td>t(33) = -1.893, p = 0.067</td>
<td>t(33) = 0.909, p = 0.37</td>
<td>t(33) = 0.507, p = 0.616</td>
</tr>
</tbody>
</table>

*Independent samples t-test; † Pearson’s correlation

Tabela 5. Poredenje površine obojene na kolagen tipa I sa četiri karakteristike pacijenata*†

Površina obojena na kolagen tipa I i:

1. preoperativna* kontrakture
2. doba pacijenata†
3. stanja prednje ukrštene veze*
4. preoperativna osnovin*

Discussion

Menisci of the knee joint are part of a complex system that should ensure the stability and weight carrying the body during the movements of the joint. In addition, the medial and lateral menisci are an integral component of normal articular homeostasis. When some pathological changes occur in the meniscus, such as acute clefts or chronic degeneration, the whole cascade of processes activates the meniscus, more so in the medium and deeper zones than at the surface. In addition, there was a statistically significant reduction of collagen type I expression in OA menisci in their samples. Using the method of collagen densitometry by converting the image to the average levels of gray, Mine et al. got the mean expression of collagen type I of 20.13 in the degenerated menisci, which was less than in the menisci without degeneration, where this value was 151.08. Such a big difference was not observed for type II collagen [17]. These authors believe that the limit of reparation was exceeded in their samples, which led to such a large decrease in the density of collagen type I and loss of tissue structure. The reduction of expression of collagen, observed in our study and cited literature, illustrates the contrast between the meniscus and articular cartilage of the knee joint during OA, because the expression of these two types of collagen increases with the severity of degeneration in the articular cartilage [20].

A decrease in the expression of collagen type I in the semiquantitative analysis was most observed in the body of meniscus, while such a loss was less in the meniscal horns. The intactness of the horns of the meniscus, in particular of the anterior one, was observed when correlating the macroscopic damage with the expression of collagen type I. According to our results, a higher grade of macroscopic damage is associated with a lower expression of collagen type I in the meniscus, wherein the level...
of such negative correlation was high for the body and the posterior horn and moderate for the anterior horn. In our samples, the anterior horn was more preserved in valgus knees. Literature data show that the changes are more noticeable in the body and the posterior horn in the OA menisci, while the anterior horn remains well preserved, with only a few signs of matrix loss or degeneration [21]. This was also confirmed by Pauli et al. [22], who concluded that the anterior horns of both menisci were less affected, macroscopically and microscopically. However, an increase in gene expression of procollagen I was observed in these horns, which is considered to be a result of reparative response that occurs in the OA menisci [21]. During the healing of fibrous tissue, such as ligaments and tendons, the fibroblasts exhibit an increased expression of type I procollagen [23], so it is assumed that the same mechanism exists in the tissue of the meniscus.

In order to analyze the expression of collagen type I more precisely, the quantitative analysis was performed in addition to the semiquantitative analysis. The previous ways of quantifying the expression of certain substances did not take into account the possibility that the substance may be present in varying concentrations. To overcome this problem, an algorithm has been developed, which assigns different color scores to different shades of a particular color representing positive staining, so that precise values are assigned to certain shades of color. Therefore, we decided to perform the quantification using software TissueQuant. This form of quantifying the expression of collagen type I did not show statistically significant differences between the control and experimental group. In the control group, the highest value of staining intensity score was seen in the meniscal body, and the lowest one in the posterior horn. The samples in the experimental group differed because the highest expression was observed in the horns, some more in the posterior one. Statistically significant quantitative differences in the expression of collagen type I were observed only in the menisci of the experimental group, and only in the posterior horn. Higher staining intensity scores, as well as the size of stained surface in this horn, were observed in women, and there was a positive correlation between these scores and BMI. These associations were not found in the body and the anterior horn of the meniscus. On the basis of NMR images in T2 sequences, Chiang et al. [24] found that the collagen content in the posterior horn of meniscus in asymptomatic volunteers from all age groups was higher in women and increased with the age of female subjects.

Although the staining intensity score was the same in both groups, certain differences were observed on the surface stained for collagen type I. A decrease in the stained surface was seen in the body of meniscus from 140.32 μm² in control to 132.33 μm² in the experimental group; however, it was not statistically significant. This reduction was not observed in the horns. The results of quantitative studies of Katsuragawa et al. [21] showed that in the OA menisci there was a significant reduction in the average diameter of collagen fibrils and the percentage of area where these fibrils were placed, but at the same time there was an increase in the number of fibrils per unit of area. Wen et al. [25] found that collagen fibers became thicker in the beginning and during the progression of OA, which could explain the decrease in the area stained for collagen type I. Sun et al. [16] observed that the collagen network of OA meniscus was less organized and less compact than the normal menisci, that being in accordance with the differences found between our two study groups.

**Conclusion**

In the semiquantitative evaluation of osteoarthritic menisci, the expression of collagen type I marked by immunohistochemical staining of anti-collagen I antibody was significantly lower compared to the macroscopically unaltered menisci. A decrease in the level of expression correlates with an increase in the grade of macroscopic damage of the meniscus. A decrease in the expression is larger in the body of the meniscus than in the horns. In the quantitative evaluation of expression of collagen type I there was a mild, statistically non-significant decrease in the stained surface in the experimental group compared with the control group, but only in the body of the meniscus, while there were no differences in the horns. No changes were found in the immunohistochemical staining intensity score between the two study groups. A more intense expression of collagen type I was seen in the posterior horn of the meniscus of women than of men. There was also a positive correlation of quantitatively estimated expression with body mass index. The expression of collagen type I in the anterior horn was significantly lower in varus than in valgus knees.

**References**

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