Current Research in Translational Medicine xxx (2016) xxx–xxx

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Original article

Quantitative study on morphology of calcified cartilage zone in OARSI 0∼4 cartilage from osteoarthritic knees

B. Deng [a,b], F. Wang [a], L. Yin [a], C. Chen [a], L. Guo [a], H. Chen [a], X. Gong [a], Y. Li [c], L. Yang [a,*]

[a] Center for Joint Surgery, Southwest Hospital, The Third Military Medical University, 400038 Chongqing, China
[b] Department of Orthopaedics, General Hospital of Chengdu Military Region, 610083 Chengdu, Sichuan province, China
[c] Department of Information, General Hospital of Chengdu Military Region, 610083 Chengdu, Sichuan province, China

ARTICLE INFO

Article history:
Received 8 November 2015
Accepted 19 January 2016
Available online xxx

Keywords:
Calcified cartilage
Angiogenesis
Osteoarthritis
Histopathology
Human

ABSTRACT

Objective. – To observe the morphological feature of calcified cartilage zone (CCZ) in mild to moderate degeneration of cartilages from patients with knee osteoarthritis (OA), revealing the pattern of CCZ during OA progression and its correlation to the surrounding structures.
Methods. – Osteochondral specimens were collected from the center of the lateral tibial plateau of 42 OA patients undergoing total knee replacement. Sections were stained with hematoxylin-eosin and Safranin-O/Fast green. Morphological parameters (thickness of CCZ, hyaline cartilage, and subchondral bone, roughness of tidemark and cement line, number of tidemarks and chondrocytes in CCZ, area and number of vascular channels in CCZ) of OARSI grades 0∼4 cartilages were measured.
Results. – The thickness of CCZ increased with grading except in grade 2. This changing trend of CCZ was in accordance with chondrocyte number and area of vascular channel. The roughness of cement lines increased with the grading, and was correlated with the thickness of subchondral bone. The roughness of tidemarks was associated with thickness of hyaline cartilage in grade 0 to grade 3.
Conclusions. – In mild OA, the thickness of CCZ was increased at first and then decreased, the roughness of tidemark and cement line was nearly unchanged, which suggests that the pathological change of CCZ is reversible. However, in moderate OA, the thickness of CCZ, the roughness of tidemark and cement line were progressively increased, which suggests that the pathological change of CCZ is irreversible.

1. Introduction

Osteoarthritis (OA), the most common form of arthritis, induces pathological changes including cartilage lesion, tidemark duplication, angiogenesis, sclerotic bone, and osteophyte in the degenerated joint. The pathogenesis of OA is yet not fully understood. Some researchers ascribe apoptosis of chondrocytes and degradation of hyaline cartilage matrix to the main pathogenesis of OA [1–3], suggesting a predominant role of articular cartilage; while others argue that the fate of articular cartilage is dependent on the remodeling of subchondral bone [4–7], indicating the leading role of subchondral bone. In either way, the interaction between cartilage and subchondral bone has to take effect through the calcified cartilage zone (CCZ), which is both the end stage of hyaline cartilage and the frontier of subchondral bone. Therefore, it is of significance to investigate the characteristics of CCZ within the osteoarthritic cartilage, and its relationship to the pathogenesis of OA.

CCZ is a highly mineralized region in the articular cartilage, linking the hyaline cartilage by tidemark, and joining the subchondral bone by cement line; it supplies the connection, biomechanics transfer, and crosstalk between bone and cartilage [8]. It has been shown that CCZ thickens along with cartilage degeneration, but the age of the patients or animals was not specified, and the cartilage regions for taking samples were not restricted in these studies, both of which might influence the morphology of CCZ [9]. Moreover, the collected samples of human in the research are limited [10]. Our group previously studied the morphology and component of human normal CCZ [8], but the morphological feature of osteoarthritic CCZ remains unclear.

The objective of this study was to analyze the morphological changes of CCZ in human knee cartilage graded 0–4 by Osteoarthritis Research Society International (OARSI) grading system. The osteochondral specimens were collected from the center of lateral tibial plateau in osteoarthritic knees with varus deformity undergoing total knee replacement (TKR), and graded with OARSI

* Corresponding author.
E-mail address: yangliujoint@126.com (L. Yang).

http://dx.doi.org/10.1016/j.retram.2016.01.009
2452-3186/

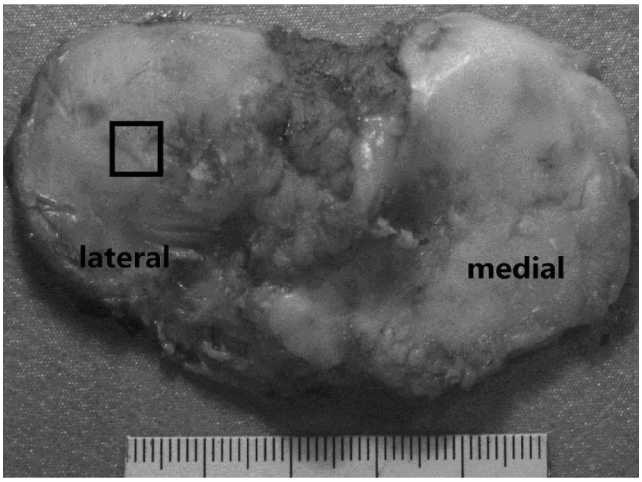

Fig. 1. Tibial plateau of OA. Sites of sampling (black frame).

system using Safranin-O/Fast green stained sections [11]. The parameters of CCZ were measured using hematoxylin-eosin stained sections.

2. Methods

2.1. Sample collection and processing

This study was conducted in compliance with regulations of the ethics committee of Third Military Medical University. To minimize the effect of age variance on CCZ, we only recruited patients at the age between 55 and 59. During January 2013 to January 2014, forty-two patients (body mass index: range 23∼33, 26.85 ± 2.81; age: range 55∼59, 56.81 ± 1.34) undergoing TKR were included in this study, and these OA patients were confirmed with no other joint diseases. To avoid the difference of location, all the samples (0.5 × 0.5 × 1 cm) were collected from the center of lateral tibial plateau (Fig. 1). The samples were then fixed in neutral-buffered formalin and decalcified in 10% ethylenediaminetetraacetic acid and 0.01 M PBS (pH 7.2) at 37 °C prior to wax embedding.

2.2. Histology and image analysis

Perpendicular to the articular surface, twelve serial sections (5 μm) were cut from each paraffin block with a Vibratome-1500 automatic microtome (Vibratome Company, USA). The 1st, 6th, and 11th sections were stained with Safranin-O/Fast green, and scored by the OARSI grade system. The remaining 9 sections of each sample were stained with HE. A colored CCD camera (DP26, Olympus) mounted on a binocular light microscope (Olympus BX51-PMS, Olympus) and a life science documentation software (cellSens, Olympus) were utilized for digital image evaluation. The polarized light component was used to observe the cement line. The projection length of CCZ was defined as L_0 and the following parameters were calculated as per millimeter L_0:

- mean thickness of CCZ was defined as T_{mean}, which was measured as follows: $T_{mean} = S_{cc}/L_0$, where S_{cc} is the true area of CCZ observed at the image of longitudinal section[8];
- mean thickness of hyaline cartilage was defined as H_{mean}, which was measured as follows: $H_{mean} = S_{hc}/L_0$ where S_{hc} is the true area of hyaline cartilage observed at the image of longitudinal section;
- mean thickness of subchondral bone was defined as B_{mean}, which was measured as follows: $B_{mean} = S_{sb}/L_0$ where S_{sb} is the true area of subchondral bone observed at the image of longitudinal section;
- NCC: the number of the chondrocytes in CCZ;
- N_{TM}: the number of tidemarks;
- NVC: the number of the vascular channel in CCZ;
- AVC: the area of the vascular channel in CCZ;
- the surface roughness parameter was defined as $R = L/L_0$ [8], where L is the true length of line.

The roughness of the tidemark (R_{TM}) and the cement line (R_{CL}) could be figured from the length of them. Then, the mean values were calculated. Blind detection of pathology was finished by three associate professors. For reliability assessment, all parameters' measurements were repeated after 4 weeks in 10 randomly selected samples.

Table 1
General condition of donors (grade 0–4).

Grade	*n*	Gender (M/F)	Sides (L/R)	Age (years)	BMI (kg/m^2)
0	5	1/4	3/2	56.8 ± 1.7	27.49 ± 4.24
1	10	2/8	4/6	56.7 ± 1.1	26.76 ± 3.35
2	11	4/7	3/8	57.0 ± 1.4	25.81 ± 2.04
3	9	4/5	3/6	56.7 ± 1.4	27.80 ± 1.80
4	7	3/4	4/4	56.5 ± 1.2	26.95 ± 3.20
F/χ^2		3.481[a]	3.451[a]	0.177[b]	0.684[b]
P		0.481	0.485	0.949	0.608

P: values for differences among OARSI subgroups; BMI: body mass index.
[a] Pearson's χ2 test statistic.
[b] ANOVA statistic.

2.3. Statistical analysis

All statistical analyses were performed using SPSS version 16.0 software (IBM Corporation, Somers, NY, USA). The categorical variables were reported as absolute value and percentage value, continuous variables as average ± standard deviation. Comparison between the frequencies of the categorical variables was assessed by Pearson's χ^2 test, corrected for continuity. Kolmogorov-Smirnov test was used to test the normality of the distribution for continuous variables. One-way analysis of variance followed by least significant difference (LSD) test was used to compare the different study groups for normally distributed continuous variables. If data were not normally distributed, Kruskal-Wallis H test was performed, followed by the Mann-Whitney U-test and Bonferroni correction. The Spearman rank correlation analysis was used to identify a correlation between two parameters. Differences with *P* value of 0.05 or less were considered to be statistically significant.

3. Results

3.1. General condition

In this study, age, gender, side and body mass index of 42 donors (grade 0∼4) were evaluated (Table 1). All of these general conditions had no statistical difference in different grades ($P > 0.05$).

3.2. Overall view of pathology

OARSI grade of all three sections from each sample was consistent. In mild to moderate degeneration, hyaline cartilage was thickened and fibrosis occurred. Subchondral bone was thickened and fiber of subchondral bone was more disordered. CCZ was also thickened. In severe degeneration, hyaline cartilage and CCZ were worn and replaced by fibrous cartilage. No CCZ was observed between fibrous cartilage and subchondral bone (Fig. 2).

3.3. Morphological parameters

3.3.1. Thickness of CCZ

In this study, we found that the thickness of CCZ was correlated to the degree of OARSI ($r = 0.777$, $P < 0.001$). From mild to moderate degeneration, the thickness of CCZ in general increased. The growth pattern of CCZ was different to the hyaline cartilage and subchondral bone (Fig. 3A). CCZ in grade 2 was thinner than in grade 1 ($P < 0.05$) (Table 2).

3.3.2. Tidemark

Tidemark duplication appeared in each grade, even in grade 0. The number of tidemarks was unrelated to the grade ($r = 0.151$, $P = 0.341$) or the thickness of CCZ ($r = 0.130$, $P = 0.412$). Nevertheless, the roughness of tidemark was correlated to the grade ($r = 0.742$, $P < 0.001$) and the thickness of CCZ ($r = 0.676$, $P < 0.001$), which became more volatile in grade 4 compared to grade 0–3 ($P < 0.05$) (Table 2).

Please cite this article in press as: Deng B, et al. Quantitative study on morphology of calcified cartilage zone in OARSI 0∼4 cartilage from osteoarthritic knees. Curr Res Transl Med (2016), http://dx.doi.org/10.1016/j.retram.2016.01.009