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Histomorphometric Analysis of Subchondral Bone of the Femoral Head in Osteoarthritis and Osteoporosis

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A B S T R A C T

There have been reports both supporting and refuting an inverse relationship between hip fracture and hip osteoarthritis (OA). We have investigated this relationship using histomorphometric study of femoral head subchondral bone[1–5]. We studied 74 subjects with hip fracture (74% females) and 24 subjects with osteoarthritis (45% females). By histomorphometric analysis of parafined sections, we analysed followed subhondral trabecular bone parameters bone volume(BV), bone volume/tissue volume (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N.) and trabecular separation (Tb.S.). The subjects with osteoarthritis and subjects with hip fracture had BV/TV 31.3% and 19.6% respectively. BV/TV of osteoarthritis group was rather uniform whereas BV/TV of hip fracture group was greatly ranged and we devided it into three subgroups, 13.2%, 19.8% and 25.9% recpectively. The OA group and hip fracture groups had Tb.Th. as followed 0.205 mm, 0.148 mm, 0.170 mm and 0.183 mm respectively. The OA group and hip fracture three subgroups had Tb.N. as followed 1.454/mm, 0.897/mm, 1.170/mm and 1.425/mm respectively. The OA group and hip fracture three subgroups had Tb.S. as followed 0.518 mm, 0.681 mm, 0.620 mm and 0.550 mm respectively. The results of our study support an inverse relationship between hip fracture and hip osteoarthritis.

***Key words**: cortical plate, microCT, osteoarthritis, osteoporosis, subchondral bone*

Introduction

Osteoarthritis (OA) and osteoporosis (OP) are both common disorders which affect quality of life in the elderly. Although OA is characterized mainly by the progressive destruction of articular cartilage, the subchondral bone might play an important role in pathogenesis of OA[1]. OA is considered as a disorder of cartilage degeneration with secondary bone changes, such as marginal osteophytes and subchondral bone sclerosis. Contrary, OP is considered primary as a bone disorder characterized by a reduced bone mass and mineral content what leads to diminished physical strength of the bone and increased the risk of fracture, particularly, femoral neck fracture. So in OA increases bone formation what leads toward bone sclerosis while in OP increases bone resorption what leads to the reduction of bone mass. These alterations are not only characterized by an augmentation or reduction of bone mass but also by structural changes in the microarchitecture of bone. Interestingly, these two diseases are rarely observed in the same patient by most clinical and epidemiological studies[2,3]. So the patients with hip fracture very rarely have hip osteoarthritis[4]. Evenmore, there are reports showing that OA protects against or retards the development of OP[5]. Some studies have reported that hip OA is only protective against intracapsular fractures but not against extracapsular or pertrochanter fractures[6,7]. Contrary, one study has found that patients with hip OA have even an increased risk for fracture[4]. Commonly there are an inverse relationship between hip OA and hip fracture[8,9]. Some authors have investigated cortical subchondral plate and have observed a thickening of the subchondral cortical bone with advancing OA while others, doing on animal models have found the both thickening and reduction in cortical subchondral plate[10,11]. A problem of the human studies is that already established and severe OA is studied and longitudinal data showing the changes from the very be-

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ginning until full clinical osteoarthritis signs do not exist. The aim of the present study was to evaluate alterations of subchondral bone on human femoral heads with hip OA and after hip fracture using micro-CT. Evalution was done for subchondral cortical plate and cancelous bone. We hypothesized that all hip fractures must not be treated exclusively as OP.

Material and Methods

The bone subchondral specimens were sampled from femoral heads removed during hip arthroplasty caused by hip osteoarthritis and hip fracture (Department of Traumatology, Clinical Hospital Rijeka). Cylindrical bone samples were taken from 97 persons with ages ranging from 42 to 94 years (mean 76, SD 13,5 years). The mean age was 66, SD 15,9 years for OA patients (45% females, 55% males) and 79, SD 13 years for OP patients (74% females, 26% males). All bone samples were always taken perpendicular to the surface of the femoral head in the superior area near the fovea. All samples comprised the subchondral cortical bone immediately under cartilage and subchondral trabecular bone. Each sample with size of 20 mm height and 8 mm in diameter was prepared by using bone trephine. They were stored in 70% ethanol until they were scanned in a micro-CT scanner which is commercially available under the name μCT 20 (Skyscan 1076, Skyscan Antwerp Belgium) with isotropic voxel size of 18 μm (Figure 1). The trabecular bone and subchondral plate were separated automatically using software. For the trabecular bone, bone volume fraction (BV/TV), which describes the ratio of bone volume over tissue volume, trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), structure model index (SMI), which estimates of how rod-like or plate-like the bone structure is, the geometrical degree of anisotropy (DA) is a measure of how highly oriented substructures are within a volume, connectivity density (Conn.D) describing the number of connections per volume, total porosity (Po(tot)) and fractal dimension (FD) were calculated. SMI is nonmetric indices and for an ideal plate and rod-like structure the SMI value is 0 and 3, respectively. For a structure with both plates and rods of equal thickness, the value is between 0 and 3, depending on the volume ratio between plates and rods. In the osteoarthritic bone samples prevail plate-like structures while in osteoporotic samples prevail rod-like structures. For the subchondral plate, the cortical thickness (Cor. Th) and porosity (Cor.Por), which describes the ratio of the volume of the pores in the cortical plate over the total volume of the plate, were calculated. The data were averaged.

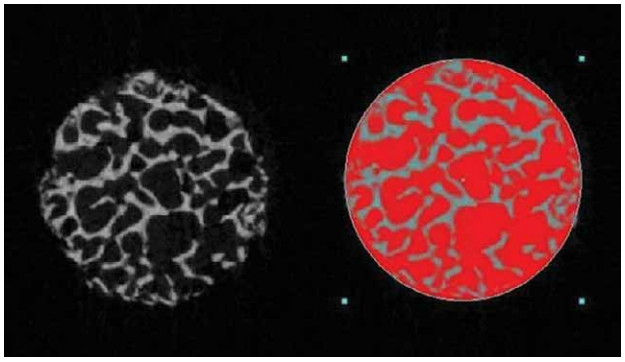

Fig. 1. Transversal section image through bone cylinder after reconstruction with Nrecon software. Right image shows marked region of interest (ROI) from witch bone structural parameters will be calculated by CTAn software.

Data analysis

The distributional properties of the data were analyzed using Kurtosis and Shapiro-Wilk tests for normality. The hypotheses were tested using both the parametric t-test on log-transformed WTP estimates and the non-parametric Mann-Whitney u-test. The hypothesis of equality of population means was be resolutely rejected at the 10% level (critical p-value of 0.1) instead of conventional 5% (critical p-value of 0.05), due to relatively small sample size and considerable sample heterogeneity. Statistical analyzes were performed in STATA version 11.

Results

Table 1 shows the basic descriptive statistics of the calculated variables for OA and OP patients. Bone volume fraction (BV/TV) was statistically higher in OA femoral head. All parameters such as metric indices, Conn.D,

TABLE 1
STRUCTURAL PARAMETERS OF OSTEOPOROTIC (OP) AND OSTEORATRHRITIC (OA) SAMPLES. MEAN VALUES AND STANDARD DEVIATION (SD). STATISTICAL SIGNIFICANCE BETWEEN TWO GROUPS IS DENOTED BY*.

Structural parameters	OP (n=74) $\overline{X}$±SD	OA (n=23) $\overline{X}$±SD
BV/TV Tr. (%)	20.80*±6.71	31.87±9.38
Tb.Th (mm)	0.17*±0.03	0.21±0.04
Tb.N (1/mm)	1.20*±0.29	1.54±0.27
Tb.Sp (mm)	0.61*±0.09	0.50±0.08
Conn.D (1/mm^3)	11.54*±3.33	16.12±3.88
Po(tot) (%)	79.13*±6.68	68.13±9.38
FD	2.21*±0.06	2.24±0.03
SMI	0.89*±0.69	0.26±0.87
DA	1.72**±0.27	1.61±0.24
BV/TV Cor. (%)	91.62±5.46	88.16±8.01
Cor.Th (mm)	236.18±174.80	240.95±159.85

BV/TV Tr. – bone volume fraction, trabecular bone; Tb.Th – trabecular thickness; Tb.N – trabecular number; Tb.Sp. – trabecular separation; Conn.D – connectivity density; Po(tot) – total porosity; FD – fractal dimension; SMI – structure model indeks; DA – degree of anisotropy; BV/TV Cor. – bone volume fraction; cortical bone; Cor.Th – cortical thickness

* denotes statistical significance between groups at p>0,05

TABLE 2.
STRUCTURAL PARAMETERS OF BONE SAMPLES GROUPED BY BONE VOLUME FRACTION OF TRABECULAR BONE (BV/TV).

	Group 1 (BV/TV <16%)	Group 2 (BV/TV 16–22%)	Group 3 (BV/TV 22–30%)	Group 4 (BV/TV >30%)
	$\overline{X}$±SD	$\overline{X}$±SD	$\overline{X}$±SD	$\overline{X}$±SD
BV/TV (%)	13.23±2.06	19.77±1.44	25.92±2.33	38.21±5.28
Tb.Th (mm)	0.15±0.02	0.17±0.02	0.18±0.2	0.22±0.03
Tb.N (1/mm)	0.90±0.14	1.17±0.13	1.43 ±0.16	1.72±0.23
Tb.Sp (mm)	0.68±0.09	0.62±0.08	0.55±0.06	0.46±0.07
Conn.D (1/mm^3)	9.68±2.45	10.99±2.63	13.85±2.99	17.31±3.87
Po(tot) (%)	86.51±2.53	80.23±1.44	74.08±2.33	61.79±5.28
FD	2.14±0.04	2.21±0.03	2.25±0.03	2.27±0.05
SMI	1.52±0.28	1.04±0.39	0.50±0.46	–0.34±0.76
DA	1.66±0.23	1.79±0.28	1.69±0.28	1.57±0.19
BV/TV cor (%)	95.04±3.67	90.42±4.98	89.57±6.33	88.97±8.21
Cor.Th (mm)	122.56±66.31	193.46±94.19	343.78±214.97	297.30±189.10

Po(tot), FD, DA and SMI showed statistically significant difference between OA and OP samples (with $p<0.01$ except DA: $p<0.05$). All these data describes significant difference in bone microarchitecture between OA and OP bone samples. Cortical thickness and porosity did not differ among OA and OP samples. To see which parameters might have a potential of giving structural information about bone architecture, correlation coefficients were performed with the bone volume fraction as the independent variable (Table 2). The results revealed quite high correlations for most parameters except for cortical thickness. OP samples showed great heterogeneity and observed data about morphological and structural parameters differed very much. We grouped all data according bone volume fraction in the next groups: BV/TV< 16%, BV/TV from 16 to 22%, BV/TV from 22 to 30% and BV/TV>30%. Data about OA samples were mainly collected in the fourth group (17/18) with the highest values of BV/TV, but some of them took a part of the third group of data (6/25). OP data were dominantly in the first (0/21) and second (0/33) groups with pronouncedly lower BV/TV values. Such distribution of the data according BV/TV values showed that some patients with hip fracture had higher values of bone volume fraction what means higher bone stiffness but less bone quality. Cortical thickness was very significant parameter. In the third group the thickness of subchondral cortical bone was markedly increased. Thickening of the cortical bone in the third group was unexpected finding because the most of the patients in that group were the patients with hip fracture. Obviously, cortical thickness indicated increasing of bone formation what is a sign of osteoarthritic bone changes. Concerning such distribution of data, in the patients with hip fracture that took part of the third group of data the bone changes indicated the signs of osteoarthritis and it could be the sign of early OA. The data in the fourth group (17/18) which was composed mostly of OA bone samples showed that cortical thickness was decreased. Some of the specimens without cartilage showed bone sclerosis while most of them with the residual articular cartilage showed decresing of cortical thickness. Coefficient of correlation for OP and OA bone samples showed high correlation with BV/TV for all morphological and structural parameters except for DA in OP group (Table 3). Cortical thickness showed very good correlation with BV/TV in OP group while in OA group did not correlate probably because of heterogeneity of the data. Visual correlation between OP and OA bone samples in microCT imaging was shown in Figure 2.

TABLE 3.
COEFICIFIENT OF CORRELATION BETWEEN OSTEOPOROTIC (OP) AND OSTEOARTHRITIC (OA) SAMPLES WITH BV/TV AS INDEPENDENT VARIABLE.

with BV/TV (%)	OP	OA
Tb.Th (mm)	0.667*	0.809*
Tb.N (1/mm)	0.896*	0.814*
Tb.Sp (mm)	–0.626*	–0.785*
Conn.D (1/mm^3)	0.599*	0.626**
Po(tot) (%)	–0.996*	–0.997*
FD	0.804*	0.498**
SMI	–0.840*	–0.785*
DA	–0.027	–0.419**
Cor.Th (mm)	0.526	0.196

*$p<0.001$; **$p<0.05$

Discussion

OA and OP are the two main musculoskeletal disorders in the aged population. Generally accepted axiom is that bone mass in osteoarthritic patients is higher than that in osteoporotic patients. According that, to identify

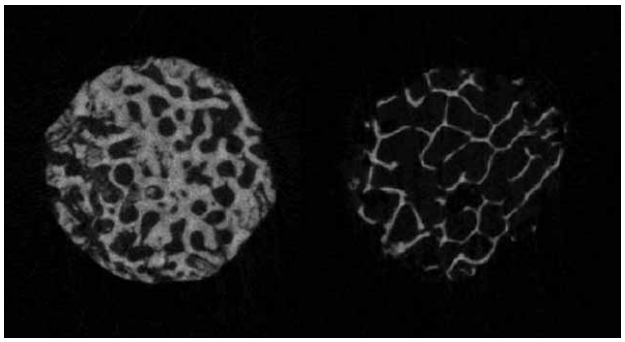

Fig. 2. Comparison between two cylinders from ostearthritic (left) and osteoporotic (right) bone.

the possible inverse relationship between OA and OP, we compared the microstructural characteristics of subchondral trabecular bone and cortical subchondral plate. In this paper, a total of 97 bone biopsies taken from the femoral head of the patients with hip fracture and osteoarthritis were measured using a microCT system. Our results showed that morphometric parameters of subchondral trabecular and cortical bone were affected differently between OP and OA bone samples what was consistent with some reports[1,5]. In our study, bone volume fraction was higher in OA group than in OP group. Ding et al. demonstrated that BV increased in early stage of OA, and decreased with aging in normal and osteoporotic populations[12]. Chappard et al. found that no difference in BV/TV was found between OP and early osteoarthritic male patients, but difference occured between OP and end-stage OA samples without cartilage[13]. Moreover, results from our analysis showed that some bone samples taken from the patients with hip fracture had higher values of BV/TV and there was no difference between OP and OA bone samples. Consistent with other studies, we also found that BV/TV was positively correlated with Tb.Th, Tb.N and negatively correlated with Tb.Sp. Concerning metric parameters such as Tb.Th, Tb.N and Tb.Sp they significantly differ between OP and OA bone samples. Contrary to Zhang et al, we found significantly higher Tb.Sp and lower Tb.N from OP patients[14]. Total porosity values were significantly higher in OP bone samples than in OA samples. SMI is structural parameter that serves for an estimation of the plate-like or rod-like characteristic of the structure. It was reported that SMI increased with age, which meant bone structure changed towards rod-like appearance[15]. Hildebrand et al has found that among iliac crest and lumbar spine, femoral head has more plate-like structure[16]. In our cases femoral head had also very pronounced plate-like structure concerning both OP and OA group although SMI was significantly higher in OP bone samples what indicated something more rod-like structure than in OA bone samples. We found strong correlation for SMI with BV/TV in both OP and OA group. Analyzing SMI data distributed in four groups according BV/TV, we could recognize that specimens in the third group had more pronounced plate-like structure in relation to the first two groups although in that group most of the specimens originated from hip fractured patients. Ding et al (2000) reported that the changes of structure from rods to plates in trabecular bone in early OA does not enhance mechanical strength. That could explain although bone volume fraction is greater and SMI is lower the hip fracture might occurs. Such distribution of the results suggested that patients with hip fracture and osteoporosis have a signs of early osteoarthritis what is described also by Franklin et al.[17]. Important parameter in bone changes is also the thickness of the subchondral cortical plate. The thickness of the cortical plate between OP and OA samples did not show any significant difference. But if we distribute the results according BV/TV the thickness of cortical plate of the samples in the third group was markedly increased what additionally support the suggestion that patients with hip fracture in the third group might have early osteoarthritis. In the most of the patients with diagnosed end-stage osteoarthritis the thickness of cortical plate decreased except in the cases without articular cartilage. Analysis of subchondral cortical bone in animal models of OA gave us opposite results, in canine model they have found that the thickness of the subchondral plate decreased while in rabbit model the thickness increased[10,11]. Evidently, animals differently react on developing of OA. In conclusion our results showed that bone samples from the patients with hip fracture and osteoporosis have morphometric parameters that could be connected with developing of OA. On one hand they have markedly increased cortical thickness what means higher bone formation but on the other hand bone volume fraction was still not increased in the same manner. These results suggest that these patients although suffered of the hip fracture have early osteoarthritis without clinical signs[17]. This group of samples with combination of OA and OP needs to be evaluated additionally concerning the articular cartilage.

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HISTOMORFOMETRIJSKA ANALIZA SUBHONDRALNE KOSTI GLAVE FEMURA KOD OSTEOARTRITISA I OSTEOPOROZE

S A Ž E T A K

Postoje radovi koji podržavaju i oni koji opovrgavaju inverznu vezu između prijeloma kuka i osteoartritisa kuka (OA). Istraživali smo ovu vezu korištenjem histomorfometrijske studije subhondralne kosti glave femura. Istražili smo 74 osobe sa prijelomom kuka (74% žena) i 24 osobe sa osteoartritisom (45% žena). Histomorfometrijskom analizom parafinskih rezova analizirali smo sljedeće parametre subhondralne trabekularne kosti: koštani volumen (BV), koštani volumen/tkivni volumen (BV/TV), debljina trabekula (Tb.Th), broj trabekula (Tb.N.) i odvojenost trabekula (TB.S.). Osobe sa osteoartritisom i osobe sa prijelomom kuka imale su omjer BV/TV 31,3% i 19,6% pojedinačno. BV/TV u grupi osteoartritisa je bio više jednoličan u odnosu na BV/TV u grupi sa prijelomom kuka gdje je bio u većem rasponu. Podijelili smo ga u tri podgrupe sa vrijednostima BV/TV pojedinačno od 13,2%, 19,8% i 25,9%. Grupa OA i grupe sa prijelomom kuka imale su vrijednost Tb.Th pojedinačno kako slijedi: 0,205 mm, 0,148 mm, 0,170 mm i 0,183 mm. Vrijednost Tb.N. kod OA grupe i tri podgrupe sa prijelomom kuka je bila sljedeća: 1,454 mm, 0,897 mm, 1,170 mm i 1,425 mm pojedinačno. Vrijednost Tb.S. bila je kako slijedi: 0,518 mm, 0,681 mm, 0,620 mm i 0,550 mm pojedinačno. Rezultati naše studije podržavaju inverznu vezu izveđu prijeloma kuka i osteoartritisa kuka.