Bone 77 (2015) 91–97

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Influence of age and gender on microarchitecture and bone remodeling in subchondral bone of the osteoarthritic femoral head

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ARTICLE INFO

Article history:
Received 19 August 2014
Revised 9 April 2015
Accepted 12 April 2015
Available online 17 April 2015

Edited by: Sharmila Majumdar

Keywords:
Osteoarthritis
Subchondral bone
Microarchitecture
Age
Gender

ABSTRACT

Introduction: Age and gender have been reported to have a remarkable impact on bone homeostasis. However, subchondral bone, which plays a pivotal role in the initiation and progression of OA, has been poorly investigated. This study was to investigate age- and gender-related changes of microarchitecture and bone remodeling in subchondral bone in OA.
Methods: Subchondral trabecular bone (STB) and deeper trabecular bone (DTB) specimens were extracted in the load-bearing region of femoral heads from 110 patients with OA. Micro-CT and histomorphometry were performed to analyze microarchitectural and bone remodeling changes of all specimens.
Results: Compared to DTB, STB showed more sclerotic microarchitecture, more active bone remodeling and higher frequency of bone cysts. There were no gender differences for both microarchitecture and bone remodeling in STB. However, gender differences were found in DTB, with thinner Tb.Th, higher Tb.N, higher OS/BV and ES/BV in males. In both STB and DTB, no correlation between microarchitecture and age was found in both genders. However, bone remodeling of STB increased significantly with age in males, while bone remodeling of DTB increased significantly with age in females. No age or gender preference was found in subchondral bone cyst (SBC) frequency. The cyst volume fraction was correlated with neither age nor gender.
Conclusions: There were differences in microarchitecture and bone remodeling between STB and DTB, which may be due to the distinct biomechanical and biochemical functions of these two bone structures in maintaining joint homeostasis. OA changed the normal age- and gender-dependence of bone homeostasis in joints, in a site-specific manner.

Introduction

Bone is an organ substantially influenced by age and gender [1–3]. Aging often leads to compromised bone quality, characterized by low bone mass and mineral density, microarchitectural deterioration, accumulated microdamages and decreased strength [1,4,5]. With aging, bone remodeling continues at a slower rate and shifts bone balance in a direction of bone loss, with the predominance of bone resorption over bone formation [6]. Gender also exerts a profound effect on the bone phenotype and remodeling, evidenced by higher rates of bone loss observed in women than men with aging [7,8]. The gender divergence has been assumed to be related primarily to different sex steroid actions [9].

Osteoarthritis (OA), which is characterized by cartilage degeneration and subchondral bone sclerosis, is a classic age- and gender-related disorder [10]. Interestingly, the influence of age and gender on bone homeostasis in OA has been reported to be different from that in normal subjects. Several studies have shown that individuals with OA have higher bone mineral density (BMD) compared with controls [11–13]. Fazzalari *et al.* [14] reported that in trabecular bone from the intertrochanteric region of the femur of OA, neither the bone mineral density nor bone volume fraction was correlated with age. OA patients also showed no age dependency for bone volume fraction in the trabecular bone taken infero-medial to the fovea in the femoral head [15]. Another study by Perilli *et al.* showed that, in patients with OA, structural and mechanical properties of trabecular bone in an inferior level of the femoral head were independent of age and gender [16]. Collectively, these studies have suggested that OA may alter the normal age and gender dependence in bone homeostasis. However, these studies had several limitations, including small sample size and lack of investigation

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http://dx.doi.org/10.1016/j.bone.2015.04.019
8756-3282/

on bone remodeling. More importantly, they neglected an important structure—subchondral bone, which plays a pivotal role in the initiation and progression of OA [17].

To understand the influence of age and gender on subchondral bone homeostasis in OA, we recruited a cohort of patients with primary OA and analyzed microarchitectural and bone remodeling changes of subchondral trabecular bone (STB) and deeper trabecular bone (DTB), in the load-bearing region of femoral heads. The age- and gender-related changes of microarchitecture and bone remodeling in both STB and DTB regions were investigated.

Materials and methods

Study subjects

110 patients who underwent total hip replacement for late-stage primary OA were recruited for the present study (60 males, mean age 64.63 ± 11.72 years, range 40–90 years; and 50 females, mean age 69.16 ± 12.33 years, range 37–95 years). There was no significant difference in age between males and females ($p = 0.084$). All patients had radiographic evidence of moderate or severe OA, according to the Kellgren and Lawrence criteria [18]. 22 specimens were identified with moderate OA (12 males and 10 females, mean age 63.95 ± 12.15 years) and 88 specimens were identified with severe OA (48 males and 40 females, mean age 67.38 ± 12.14 years). Patients with moderate and severe OA did not differ significantly in age ($p = 0.246$) or male/female ratio ($p = 1.000$). Exclusion criteria were as follows: 1) known metabolic or bone disorders other than primary OA, which could affect bone metabolism, such as severe renal impairment, thyroid or parathyroid disease, and malignancy; 2) other known joint diseases, which could affect bone architecture and quality, such as rheumatoid arthritis, Paget disease, congenital hip dysplasia and other secondary OA; 3) receiving treatments that affect bone metabolism such as anti-resorptive drugs, calcitonin, thyroid or parathyroid hormone therapy, or hormonal replacement therapy; or 4) hip osteotomy history. Informed consent was obtained from each patient. The study protocol was approved by the Human Research Ethics committee of The University of Western Australia and complied with the Declaration of Helsinki.

Specimen preparation

A cylindrical specimen of peri-articular bone was extracted from the center of superior load-bearing region where the overlying cartilage was severely eroded (Fig. 1) [19–21]. Before extraction, the femoral head was immobilized by a holder, with the load-bearing region placed in the center. Then, the cylindrical specimen with the size of 15 mm in height and 9 mm in diameter was obtained under continuous water irrigation using a precision bone trephine, with the trephine axis perpendicular to the surface. All specimens comprised the subchondral trabecular bone (STB) and deeper trabecular bone (DTB). STB is defined as the most superficial 5 mm of the trabecular cylinder immediately under cartilage and subchondral cortical bone [22–24], while DTB is defined as the deepest 5 mm of the cylindrical specimen. Specimens were fixed in 4% paraformaldehyde in PBS for 5 days and stored in 70% ethanol.

Micro-CT examination

Within an average of one week after fixation, specimens were scanned by a micro-CT scanner (Skyscan 1174, Bruker, Kontich, Belgium). Prior to scanning, specimens were all rehydrated by overnight storage in 0.9% physiological saline. Then each specimen was placed inside a saline-filled acrylic case for image acquisition. The device worked at a voltage of 50 kV, current of 800 μA, an isotropic pixel size of 14.4 μm (1024 × 1024 pixel image matrix), and with a 0.75-mm-thick aluminum filter for beam hardening reduction. After scanning and reconstruction, the original images were binarized (Fig. 2). The mineralized tissue was separated from soft tissue by a consistent global threshold range (90 to 255), which was established based on the grayscale histogram analysis and empirical observations [25–27]. Bone microarchitecture parameters were then measured, using the built-in software. The measurement region was 8 mm in diameter and 5 mm in height in a columnar region for both STB and DTB. The diameter of the measurement region was chosen 1 mm smaller than the diameter of the specimen, in order to avoid the inclusion of bone debris due to the cutting procedure. Subchondral bone cyst (SBC) and cysts in DTB were also screened. Bone cysts are radiologically defined as radiolucent osteolytic cavitary lesions, with diverse shapes [10,28,29]. The cyst was extracted by a semi-automatic method, with tools provided by the built-in software: The rim of the cyst was established by hand drawing on each cross section involved, and then 3D reconstruction and analysis of the cyst were automatically implemented. The ratio of the cyst volume to the whole specimen volume (CV/TV; %) was measured. In specimens with bone cysts, measurement for bone microarchitecture was only conducted in the trabecular region surrounding the cyst, rather than the whole specimen.

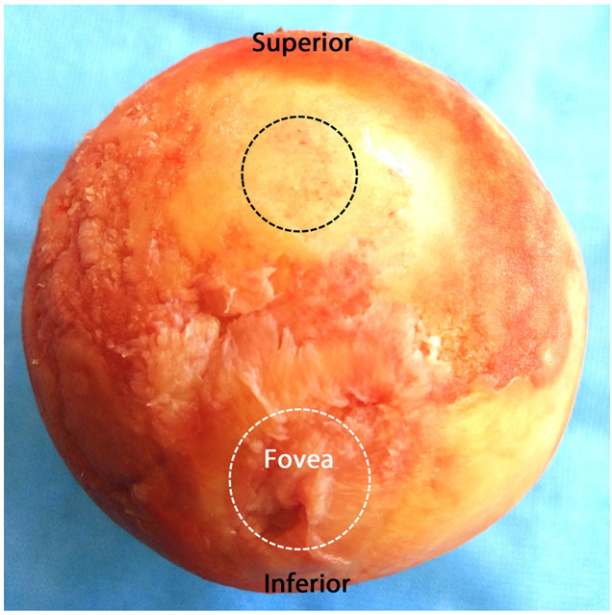

Fig. 1. Location of the cylindrical peri-articular bone specimen: the bone cylinder was extracted from the center of superior load-bearing region where the overlying cartilage was severely eroded (indicated by the black dotted circle).

The following microarchitectural parameters were calculated: bone volume fraction (BV/TV; %), trabecular thickness (Tb.Th; μm), trabecular separation (Tb.Sp; μm), trabecular number (Tb.N; 1/mm), structure model index (SMI), degree of anisotropy (DA), and connectivity density (Conn.D; $1/mm^3$). SMI is a topological index for estimating the characteristic form in terms of plates and rods composing the 3D structure, and it is sensitive to bone volume. An ideal plate and rod have SMI values of 0 and 3 respectively [30]. SMI could also be negative if trabecular bone is very dense and trabecular surfaces are concave. DA is a measure of how highly oriented substructures are within a volume, which is determined by the mean intercept length method. A higher value indicates higher anisotropy [31]. Conn.D is a parameter of the degree of trabecular connectivity, where a higher value means greater connectivity [32]. BMD (mg/cm^3) was obtained by conversion of X-ray attenuation coefficient (Fig. 2), using a calibration curve obtained from two BMD phantoms [33–35].

Histological process and histomorphometry

Each specimen was fixed, infiltrated and embedded in methylmethacrylate. All bone blocks were trimmed and sectioned on a microtome