Differences in the Microarchitectural and Histomorphologic Characteristics Between Glucocorticoid-induced Osteonecrosis of Femoral Head and Alcohol-induced Osteonecrosis of Femoral Head

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Research article

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Abstract

Aims

To analyze microarchitecture and histomorphology characteristics of different regions in femoral heads from patients with glucocorticoid-induced osteonecrosis of femoral head (GIONFH) and alcohol-induced osteonecrosis of femoral head (AIONFH).

Methods

Patients diagnosed with GIONFH and AIONFH were recruited. Femoral heads were obtained after total hip replacement. Micro-CT was applied to evaluate the microstructure of 9 regions of interest (ROIs) in the femoral head. Along the supero-inferior orientation, the femoral head was divided into necrotic region, reactive interface, and normal region; along the medio-lateral orientation, the femoral head was divided into medial region, central region and lateral region. Decalcified and undecalcified bone histology were then performed to assess histopathological alterations and bone remodeling levels.

Results

42 GIONFH patients (50 hips) and 43 AIONFH patients (50 hips) anticipated in the study. In the necrotic region, most of the microarchitectural parameters did not differ significantly between GIONFH and AIONFH, whereas both the reactive interface and normal region illustrated significant differences in the microstructure and histomorphometry. The reactive interface and normal region exhibited a less sclerotic microarchitecture, but a higher bone remodeling level in GIONFH as compared with AIONFH. Despite similar necrotic pathological manifestations, subchondral trabecular microfracture in the necrotic region was more severe and vasculature of the reactive interface was more abundant in GIONFH.

Conclusions

Although these two subtypes of ONFH shared similar microarchitecture and pathological features in the necrotic region, GIONFH exhibited a less sclerotic microarchitecture and a more active bone metabolic status in both the reactive interface and normal region.

Introduction

Osteonecrosis of femoral head (ONFH) is known as avascular necrosis or aseptic necrosis of the femoral head, associated with an interruption of the blood supply¹. One of the pathological features of ONFH is the damage of subchondral vascular microcirculation, which leads to osteonecrosis, eventually resulting in repeated microfractures in the femoral head and articular collapse². In the United States, about 10,000 to 20,000 patients are newly diagnosed with the disease annually³. In a recent nationally representative survey, the cumulative number of patients with non-traumatic ONFH was estimated to be around 8.12 million among the Chinese population⁴. As a devastating disease frequently leading to secondary hip

arthritis, ONFH imposes a heavy economic burden on the whole society ^{5,6}. Most patients end up having to choose total hip replacement (THA) ⁷, and younger patients undergoing THA may require further revision of hip arthroplasty, which will lead to increased morbidity⁸.

The etiology and pathogenesis of nontraumatic ONFH are not well understood ⁹. Long-term use of glucocorticoid, alcohol abuse, genetic mutations, and thrombotic/fibrinolytic diseases have been identified as possible risk factors for ONFH ^{10,11}. Glucocorticoid usage and alcohol abuse are the most widely reported risk factors for nontraumatic ONFH ¹². Glucocorticoid is the most common cause of nontraumatic osteonecrosis (51%)¹¹, and this was followed by alcohol, which is more than one in five ¹³. Glucocorticoid may have a complex relationship with lipid metabolism and vascular endothelium in the pathogenesis of glucocorticoid-induced osteonecrosis of femoral head (GIONFH) ¹⁴, possibly affecting the number and function of circulating endothelial progenitor cells, which are necessary for vascular repair and new angiogenesis ¹⁵. In addition, glucocorticoid has the potential effect on directing precursor cells towards an adipogenic or osteogenic pathway ¹⁶. There is a hypothesis that abnormal alcohol metabolism may lead to the deterioration of bone tissue in the femoral head through toxic by-products such as acetaldehyde, free radicals and acetaldehyde adducts ¹⁷, with the potential mechanism of intravascular coagulation and coagulation cascade regulation ¹⁸. Alcohol could also disrupt osteogenic differentiation and promotes lipogenesis of bone marrow mesenchymal stem cells, in the pathogenesis of alcohol-induced osteonecrosis of femoral head (AIONFH) ^{19,20}.

Using histological analysis, Kim et al. found that patients with idiopathic ONFH or AIONFH have normal or nearly normal cancellous bone in the acetabulum and proximal femur, whereas GIONFH might be associated with more extensive osteonecrosis ²¹. Patients with ONFH secondary to sickle-cell disease or steroid use have been found to have poor results of total hip arthroplasty when compared with age-matched controls ²²⁻²⁶. This heterogeneity may be related to the different biological effects on the bone tissue induced by different risk factors. However, in most histopathological studies on ONFH, only regular decalcified sections and hematoxylin-eosin (H&E) staining were applied, in which mineralization and bone remodeling status cannot be assessed using histology or imaging mass spectrometry. Undecalcified bone histology shows tremendous importance in a variety of bone research areas, including metabolic bone diseases and disorders of mineral metabolism²⁷. Undecalcified sections and related trichrome stains could demonstrate both the mineralized and cellular components of bone tissue, and provide pivotal information on bone turnover²⁸, which have not been widely applied in the histopathological analysis of ONFH ^{29,30}.

The morphology of the femoral head is unique, and the structure of bone trabecula is complicated and heterogeneous ³¹. Bone microstructure plays an important role in the pathogenesis and development of ONFH^{32,33}. Micro-computed tomography (micro-CT) has been introduced to perform a three-dimensional evaluation of the detailed microstructure in the femoral head with osteonecrosis. However, most of these previous studies only focused on selective specimens with small diameters, which were extracted from

specific regions of the proximal femur³⁴. The overall bone microstructure of the whole femoral head with osteonecrosis, which is of great clinical significance, is not frequently evaluated in early studies. The microarchitectural analysis will deepen our understanding of the detailed overall morphological alterations of the whole femoral head in the development of ONFH, and help clinicians reevaluate the severity level of the disease, which might modify the indication for joint-preservation surgery for ONFH.

Despite distinct etiologies between GIONFH and AIONFH, the differences of the microarchitectural and histomorphologic characteristics between these subtypes of ONFH are rarely investigated. Accordingly, we hypothesized that the microstructural and histomorphometric characteristics of the femoral head in GIONFH and AIONFH are different. To address this, we assessed microarchitecture, bone remodeling activity and histopathological alterations of trabecular bone in different regions of the femoral head from patients with GIONFH and AIONFH, using micro-CT, decalcified and undecalcified bone histology.

Methods

Subjects

Between September 2015 and October 2019, 85 patients diagnosed with ONFH who underwent THA surgery, were recruited in the study. These patients who participated in this study were divided into two groups based upon which etiology they belonged to: (1) glucocorticoid-induced osteonecrosis of femoral head (GIONFH) or (2) alcohol-induced osteonecrosis of femoral head (AIONFH). Patients were recruited to the GIONFH group if they met the classification criteria of glucocorticoid-induced osteonecrosis of femoral head included the following: (1) Patients should have a history of glucocorticoid use more than 2 g of prednisolone or its equivalent within 3 months;(2) ONFH was diagnosed within 2 years after glucocorticoid administration;(3) There were no other risk factors except glucocorticoids^{35,36}. Regarding AIONFH, we collected information about age at starting and cessation of alcohol consumption, usual frequency of drinking, and the usual volume of alcohol intake by beverage type. This volume was consequently converted to grams of ethanol and values for each beverage type were added. The ethanol content for calculation was as follows: 4.5% for beer, 12% for wine, 43% for whisky, 15.5% for rice wine and 50% for baijiu (a distilled alcoholic beverage made in China)³⁷. We described habitual drinking in patients with alcohol-associated ONFH as more than 3032 drink years of the cumulative alcohol intake according to the previous report³⁷. The cumulative drinking amount termed as 'drink-years' was calculated by multiplying the weekly ethanol consumption (gram) by the total number of years of drinking. Patients recruited to the AIONFH group should also meet classification criteria of alcohol-associated ONFH: (1) patients should have a history of alcohol intake of more than 400ml/week of pure ethanol (320g/week of any type of alcoholic beverage) for more than 6 months;(2) ONFH was diagnosed within 1 year after this dose of alcohol intake;(3) There should be no other risk factors³⁸.

Exclusion criteria for ONFH patients were as follows: (1) other known metabolic or bone disorders which could affect bone metabolism, such as thyroid or parathyroid disease, and malignancy; (2) receiving

other treatments that affect bone metabolism such as anti-resorptive drugs, calcitonin, thyroid or parathyroid hormone therapy, or hormonal replacement therapy; or (3) history of hip fracture and osteotomy. Information about glucocorticoid usage, alcohol consumption, smoking in two groups is shown in Table 1. All cases were classified with different clinical stages recommended by the Association Research Circulation Osseous (ARCO)³⁹. The cases in this study were all ARCO stage II, IIIA, IIIB or IV, with severe pain or severe hip dysfunction (Fig. 1). Given that location of the necrosis may affect topographical observations, the Japanese classification is important to understand changes, particularly addressing lateral extension. Therefore, we use the Japanese classification to classify all cases as well. Type A lesions account for only a third or less of the weight-bearing portion. Type B lesions accounted for two-thirds or less of the weight-bearing portion. Both type C1 and C2 lesions account for more than two thirds of the medial weight bearing portion of the acetabulum, but C2 lesions extend laterally to the acetabulum margin, while C1 lesions do not⁴⁰. Informed consent was obtained from each patient. The study protocol was approved by the Human Research Ethics committee of our hospital and complied with the Declaration of Helsinki.

Micro-CT examination

Femoral head specimens were obtained after THA surgery and stored in a -80°C freezer. Then, they were scanned using the high resolution micro-CT system (μ CT100, Scanco Medical, Bassersdorf, Switzerland), with the following scan settings: X-ray tube voltage, 70 kVp; tube current, 200 μ As; exposure time, 300 ms ; projection number, 1000; and voxel size, 73.6 μ m⁴¹. The scan length was approximately 49 mm, resulting in 1200 slices and a 45-minute scan time. Orientation adjustment of the femoral head, segmentation of the regions of interest (ROIs), and measurement of bone microstructural parameters in each region were performed by the built-in software. Orientations of all femoral heads were adjusted based on anatomical landmarks using the fovea capitis femoris and the main trabeculae direction (MTD), where the principal compressive trabeculae were located (Fig. 2A)^{31,42}. 9 ROIs were set in the central coronal plane of the femoral head (Fig. 2B). Specifically, along the supero-inferior orientation, the femoral head was divided into three main regions with reference to the boundary of the necrotic lesion: superior region (necrotic region), central region (i.e. reactive interface or necrotic-normal junction region), and inferior region (normal region); along the medio-lateral orientation, the femoral head was divided into another three main regions with reference to MTD: medial region, central region (principal compressive trabeculae region) and lateral region. Thus, a total of nine ROIs (Sup–Med, Sup–Cen, Sup–Lat, Cen–Med, Cen–Cen, Cen–Lat, Inf–Med, Inf–Cen, and Inf–Lat) were included in the microstructure analysis. In each ROI, a cylindrical bone specimen (5 mm in height and 5 mm in diameter) was set for analysis (Fig. 2C).

After scanning and reconstruction, the images were transferred with a fixed threshold to binary images; color images were also produced based on X-ray attenuation coefficient values, representing mineralization distribution in the trabeculae (Fig. 3-4). Bone microarchitecture parameters were calculated. In specimens with cystic lesions and patchy necrotic debris, the measurement for bone

microarchitecture was only conducted in the trabecular region in which the cysts and debris were excluded.

The following microarchitectural parameters were measured: bone volume fraction (BV/TV) (%), bone surface/volume ratio (BS/BV) (1/mm), trabecular thickness (Tb.Th) (μm), trabecular separation (Tb.Sp) (μm), trabecular number (Tb.N) (1/mm), structure model index (SMI), degree of anisotropy (DA), connectivity density (Conn.D) (1/mm³)⁴³.

Specimen preparation

After micro-CT scanning, all femoral heads were cut in half with a diamond saw, coronally separating the anterior hemisphere from the posterior hemisphere (Fig. 3A-C). A 10-mm-thick coronal bone slice was then sectioned from each femoral head hemisphere. One slice was prepared for decalcified bone section, and the other slice was used for undecalcified bone histology. Each slice was divided into nine ROIs as aforementioned. All these specimens were fixed in paraformaldehyde for 2 weeks, which was followed by the related process for undecalcified or decalcified histology. Decalcified samples were embedded in paraffin wax, sectioned into 5- μm -thick slices and stained with H&E. Undecalcified samples were embedded in methyl-methacrylate, sectioned into 5- μm -thick slices, and were stained by Goldner's Trichrome method.

Histopathological assessment and histomorphometry

The decalcified sections were examined for the evidence of osteonecrosis and secondary pathologic changes. For the undecalcified sections, histomorphometry was performed using Bioquant Osteo Histomorphometry software (Bioquant Osteo, Nashville, TN, USA). The following bone formation and resorption parameters were measured in each ROI: thickness of osteoid (O.Th, μm), percentage osteoid volume (OV/BV) (%), percentage osteoid surface (OS/BS) (%), specific osteoid surface (OS/BV) (mm²/mm³), percentage eroded surface (ES/BS) (%), specific eroded surface (ES/BV) (mm²/mm³) and eroded surface in bone tissue volume (ES/TV) (mm²/mm³)⁴⁴.

Statistical analysis

The Kolmogorov–Smirnov test was used to determine if parameters followed a normal (Gaussian-shaped) distribution and no significant departures were identified. The unpaired Student's t test was used to compare all microarchitecture and bone remodeling parameters between groups. A conservative two-tailed $P < 0.05$ was chosen a priori to declare a statistically significant result to account for multiple comparisons and to avoid type I errors⁴⁵. Analysis of data was performed using the Statistics Package for Social Sciences (SPSS for Windows, version 17.0; SPSS Inc, Chicago, IL, USA). Continuous data are presented as mean $\pm$ standard deviation (SD). Gender, smoker, diabetes, hypertension, side of ONFH, ARCO stage⁴⁶, Japanese Classification System⁴⁰ were compared between GIONFH and AIONFH groups using Fisher's exact test, Chi square or Chi square test with Yates correction, as appropriate.

Table 1 Characteristics of the study participants

Data are the median (interquartile range) for quantitative variables and no. (%) of patients for qualitative variables. Bold indicates statistically significant difference.

Results

Subject characteristics

A total of 85 patients (100 hips) diagnosed with ONFH were recruited in the study. 42 GIONFH patients (50 hips) had various indications for glucocorticoid treatment, including autoimmune diseases [systemic lupus erythematosus (n=15), **rheumatoid arthritis** (n=2)], renal diseases [nephritic syndrome (n=5), pyelonephritis (n=1), IgA nephropathy (n=2), chronic nephritis (n=2) and renal transplantation (n=1)], dermatologic diseases [psoriasis (n=1)], skin pruritus (n=1), eczema (n=2), urticaria (n=1), and neurodermatitis (n=2)], thrombocytopenic purpura (n=2), chronic pulmonary fibrosis (n=1), **craniopharyngioma** (n=1), **myocarditis** (n=1), bronchial asthma (n=2). 43 participants (50 hips) with alcohol-associated ONFH were classified into the AIONFH group. Patients who were diagnosed with bilateral ONFH are all at different stages.

GIONFH and AIONFH subjects were well matched on demographic factors and medical conditions (Table 1), with the exception being the proportion of males, which was significantly higher in the AIONFH group than that in the GIONFH group ($P<0.0001$).

Comparative analysis of microarchitecture in different regions between GIONFH and AIONFH

In Sup-Med region, none of the microarchitecture parameters differed significantly between GIONFH and AIONFH, with the exception of DA. There were significantly lower values of DA in GIONFH, compared to AIONFH. Similarly, in Sup-Cen and Sup-Lat regions, none of the microarchitecture parameters differed significantly between GIONFH and AIONFH, with the exception of SMI (Fig. 4-5). There were significantly higher values of SMI in GIONFH, compared to AIONFH.

In Cen-Med region (Fig. 4-5), BS/BV, Tb.Th, Conn.D and DA were not significantly different between GIONFH and AIONFH. However, there were significantly lower values of BV/TV and Tb.N, higher values of Tb.Sp and SMI in GIONFH, compared to AIONFH.

In Cen-Cen region, all the microarchitecture parameters were significantly different between GIONFH and AIONFH, with the exception of DA (Fig. 4-5). Compared to AIONFH, there were significantly higher values of BS/BV, Tb.Sp, Conn.D and SMI in GIONFH, with significantly lower values of BV/TV, Tb.Th, Tb.N.

In Cen-Lat region, Tb.Th, Conn.D, SMI and DA were not significantly different between GIONFH and AIONFH (Fig. 4-5). Nevertheless, there were significantly lower values of BV/TV and Tb.N, higher values of BS/BV and Tb.Sp in GIONFH than in AIONFH.

	GIONFH	AIONFH	P-value
Characteristics of study participants			
Number of patients	42	43	
Gender (male/female)	22/20	42/1	<0.0001
Age (years)	47.29(34.75-45)	51.44(43-58)	0.1610
BMI (kg/m ²)	24.17(22.42-26.51)	24.83(22.65-27.56)	0.5187
Smoker n (%)	19[45.23]	26[60.47]	0.1597
Diabetes	4	4	0.9721
Hypertension	8	9	0.8283
Characteristics of osteonecrosis			
Number of hips	50	50	
Side (right: left)	24:26	20:30	0.5459
Timing since the onset of the ONFH, months	27.05(8-18)	24.12(8-12)	0.8168
Glucocorticoid dosage			
Glucocorticoid pulse therapy, n (%)	14(33)	NA	NA
Duration of glucocorticoid administration, years	9.12(7-10)	NA	NA
Glucocorticoid treatment			
Drug species	Prednisone	NA	NA
	Methylprednisolone	NA	NA
	Dexamethasone	NA	NA
Alcohol consumption			
Duration of alcohol consumption	NA	23(20-30)	NA
Weekly ethanol consumption (g/week)	NA	587.3(525-542.5)	NA
Ethanol drink-years ((g/week) × years)	NA	13142(7875-16275)	NA
ARCO STAGE			0.9382
STAGE II	8	9	
STAGE IIIA	12	14	
STAGE IIIB	17	16	

STAGE IV	13	11
Japanese classification system		0.8863
Type A	1	2
Type B	6	6
Type C1	22	19
Type C2	21	23

In Inf-Med region, there were lower values of BV/TV, higher values of BS/BV and SMI in GIONFH than in AIONFH, while the other parameters showed no significant difference. In Inf-Cen region, BS/BV, Tb.Th, Conn.D and DA were not significantly different between GIONFH and AIONFH. However, there were significantly lower values of BV/TV and Tb.N, higher values of Tb.Sp and SMI in GIONFH, compared to AIONFH. In Inf-Lat region, none of the microarchitecture parameters differed significantly between GIONFH and AIONFH, except for Tb.N (Fig. 4-5).

Histological assessment of osteonecrosis in both groups

In the necrotic region of the femoral head, bone marrow necrosis and osteocytic death were observed in both groups. Hematopoietic cells disappeared and adipose nucleus staining was absent in the bone marrow, indicating the necrosis of bone marrow (Fig. 7). The necrotic lesions were not scattered and patchy in the bone marrow space, but exhibiting as a large continuous area with different shapes. In addition, most bone trabeculae represented empty osteocyte lacunae, seldom encompassing some pycnotic nuclei of osteocytes. In the subchondral trabecular bone of the necrotic region, microfracture and patchy debris was frequently observed, which was more obvious and severe in GIONFH.

In the necrotic-normal junction region, reparative process, such as vascular-rich granulation tissue, was observed. Histiocytes and giant cells aggregated in the reactive fibrous interface, especially in the lateral aspect. Although vessels penetrated into the fibrotic capsule, the angiogenesis was blocked at the boundary of the sequestrum and reparative fibrovascular tissue could not penetrate into the bone marrow space of the necrotic region. Constantly, the reparative fibrovascular tissue would convert to a sclerotic rim in the late stages of the disease, which was attributed to the bone formation by osteoblasts. Compared to AIONFH, the angiogenesis was more abundant in the reactive interface in GIONFH, bringing about a more active bone remodeling status, despite the failure of thorough reparative substitution of the sequestrum in both groups (Fig. 7).

Comparative analysis of bone remodeling levels between GIONFH and AIONFH

Due to the devitalization status of the necrotic region, the Sup-Med, Sup-Cen and Sup-Lat regions were not analyzed in the aspect of bone remodeling levels.

In the necrotic-normal junction region, including Cen-Med, Cen-Cen and Cen-Lat regions, all the bone remodeling parameters were significantly higher in GIONFH, compared with AIONFH (Fig. 6, Fig. 8). There were higher values of O.Th, OV/BV, OS/BS and OS/BV, indicating a more active bone formation status. Bone resorption activity was also higher, as suggested by higher values of erosion parameters including ES/BS, ES/BV and ES/TV.

In Inf-Med region, most bone remodeling parameters were significantly higher in GIONFH than AIONFH, with the exception of ES/BV (Fig. 6, Fig. 8). In Inf-Cen region, the bone formation parameters O.Th, OV/BV, OS/BS and OS/BV were significantly elevated in GIONFH samples than those in AIONFH (Fig. 6, Fig. 8). The bone resorption parameter ES/BS was significantly elevated in GIONFH. No significant differences were observed in ES/BV and ES/TV. In Inf-Lat region, there were higher values of O.Th, OV/BV, OS/BS in GIONFH, indicating a more active bone formation status (Fig. 6, Fig. 8). OS/BV were also higher in GIONFH, although no significant difference was observed. The bone resorption parameter ES/BS was significantly elevated in GIONFH. No significant differences were observed in ES/BV and ES/TV.

Discussion

In this study, we analyzed simultaneously the microarchitecture, histopathologic alterations and bone remodeling levels in different regions of the involved femoral head between GIONFH and AIONFH. The two groups shared similar microarchitectural characteristics in the necrotic region, whereas both the reactive interface and normal regions illustrated significant differences in the microstructure and histomorphometry. The reactive interface and normal regions exhibited a less sclerotic microarchitecture, but a higher bone remodeling level in GIONFH as compared with AIONFH. Despite similar necrotic pathological manifestations, subchondral trabecular microfracture and debris was more severe in the necrotic region, and the reactive interface was more vascular in GIONFH.

Microstructure is an important component of bone quality, and its integrity contributes to the maintenance of bone strength^{47,48}. The microarchitectural patterns in different regions of the joint may differ, reflecting different types and levels of biomechanical loads^{49,50}. Most of the stress on the femoral head is borne by the principal compressive trabeculae in daily activities, exhibiting compact microarchitecture and high biomechanical strength⁵¹. Our results revealed that the reactive interface and normal region displayed a less sclerotic microarchitecture in GIONFH as compared with AIONFH, exhibiting a lower bone volume fraction, less trabecular number, and wider trabecular space, especially in the Cen-Cen region. The deteriorated microarchitecture has been reported to play a pivotal role in the failure of mechanical stabilization in the femoral head, and eventually leads to the progression of collapse⁵². The alterations of microstructure may be due to the distinct bone remodeling status⁵³, which could be influenced by different reagents that affect bone metabolism such as glucocorticoid and alcohol.

Our study demonstrated that the reactive interface and normal region exhibited higher bone remodeling levels in GIONFH, despite a less sclerotic microarchitecture observed. This phenomenon might be due to

the possibility that the activity of osteoclast-mediated bone resorption in GIONFH was much higher, outweighing the effect of the elevated level of bone formation activity. This variation might be attributed to the different biological responses of relevant bone cells trying to repair necrotic tissue⁵⁴, reflecting distinct effects of these two etiologies. It has been proposed that glucocorticoid and alcohol reduce femoral artery perfusion through mechanisms such as vascular endothelial injury and microvascular thrombosis^{55,56}. They can also induce the formation of intramedullary fat, which increases the pressure in the bones, leading to venous congestion and blocked arteries^{19,57,58}. Glucocorticoid has been reported to reduce the production of osteoblasts, increase the apoptosis of osteoblasts and prolong the life of osteoclasts^{16,59}. Accumulating evidence has also shown that heavy alcohol consumption impairs bone homeostasis, which disturbs the osteoblast formation and proliferation, and promotes osteoclastogenesis^{60,61}. Despite the adverse effect on bone metabolism by both glucocorticoid and alcohol, glucocorticoid might illustrate a more negative interference on osteogenesis and reparative process in the condition of ONFH. Chernetsky et al. found that histologic alterations of necrosis and repair evolved in a given sequence after bone death, the rate of which was faster in the GIONFH⁶². In a study by Wei et al.⁶³, GIONFH was characterized by multiple “osteolytic bone destruction”, while AIONFH was manifested by some kinds of “coagulative destruction”.

Both GIONFH and AIONFH showed a large continuous area of bone marrow necrosis, osteocytic death and disordered subchondral trabecular structure in the necrotic region, with a fibrous reactive interface at the boundary of the sequester. The similar histologic features of necrosis between these two subtypes of ONFH was consistent the previous study by Chernetsky et al.⁶². The difference between the two groups was that the microstructure and arrangement of subchondral trabeculae in the necrotic region of GIONFH were more seriously damaged, with a more vascularized fibrous interface. The more deteriorated and chaotic subchondral microarchitecture might be attributed to the biomechanically weak strength of trabeculae and excessive osteoclast activity, induced by prolonged overdose usage of glucocorticoid in the condition of weight-bearing. Angiogenesis is an important part of the reparative response following necrosis⁶⁴. Despite detrimental effects on bone angiogenesis by glucocorticoid in the pathogenesis of osteonecrosis, introduction of glucocorticoid could also promote angiogenesis under certain conditions⁶⁵, which might be the explanation of the highly vascularized fibrous interface in GIONFH. The underlying mechanism needs further investigation in the future. High concentration of alcohol has been reported to show an adverse effect on neovascularization^{66,67}, explaining the relatively less evident vascularization in the reactive interface in AIONFH.

There are several limitations in this study. One limitation of the present study was the absence of normal subjects. All of these bone specimens were obtained from patients with ONFH, without healthy controls. The relatively young age of these patients undergoing THA makes the acquisition and age matching of control samples difficult. But for all that, we cannot reveal whether there was a specific degree of heterogeneity in the microstructure of the femoral head in ONFH patients, even with the same trend in healthy controls. Second, this was a cross-sectional study using specimens of the femoral head, lacking

dynamic bone remodeling parameters. Micro-CT scanning of the hip joint cannot be performed *in vivo*. Future advances in CT and MRI with improved spatial resolution may help solve this problem⁶⁸⁻⁷¹.

In conclusion, GIONFH and AIONFH showed similar microarchitecture characteristics in the necrotic region, while the reactive interface and normal region exhibited a less sclerotic microarchitecture and a more active remodeling status in GIONFH than that in AIONFH. In the aspect of histopathology, microfracture of subchondral trabeculae in the necrotic region was more severe and vasculature of the reactive interface was more abundant in GIONFH. These phenomena might be due to the distinct biological effects of glucocorticoid and alcohol on bone metabolism and angiogenesis. This study provides a better understanding of microstructural, pathological and histomorphologic alterations in the whole femoral head with osteonecrosis induced by different risk factors, which might further contribute to the development of disease-modifying prevention strategies and therapeutics for ONFH, taking into etiologies.

Abbreviations

ONFH: Osteonecrosis of the femoral head; GIONFH: Glucocorticoid-induced osteonecrosis of femoral head; AIONFH: Alcohol-induced osteonecrosis of femoral head; ARCO: Association research circulation osseous; ROI: Regions of interest; THA: total hip replacement; micro-CT: Micro-computed tomography; H&E: Hematoxylin-eosin; MTD: Main trabeculae direction; BV/TV: Bone volume fraction; BS/BV: Bone surface/volume ratio; Tb.Th: Trabecular thickness; Tb.Sp: Trabecular separation; Tb.N: Trabecular number; SMI: Structure model index; DA: Degree of anisotropy; Conn.D: Connectivity density; O.Th: Thickness of osteoid; OV/BV: Percentage osteoid volume; OS/BS: Percentage osteoid surface; OS/BV: Specific osteoid surface; OS/BS: Percentage eroded surface; ES/BV: Specific eroded surface; ES/TV: Eroded surface in bone tissue volume; SD: Standard deviation.

Declarations

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Authors' contributions

All authors listed have read and approved all versions of the manuscript YWC and GYL had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy. Critical revision of the article for important intellectual content: YWC, KXL, YM, BZ, FX, JHY, MHZ, GYL, CQZ. All authors approved the final version to be submitted for publication.

Authors' information

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Availability of data and materials

The authors are committed to sharing their data, publishing the data, and making available the resources described in this publication to the scientific community.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice. The protocol and patient's informed consent received institutional review board/independent ethics committee approval prior to initiation of the study.

Consent for publication

Patient consent for publication was not required.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

The cases in this study were all ARCO stage II, IIIA, IIIB or IV, with severe pain or severe hip dysfunction

Figure 2

Orientations of all femoral heads were adjusted based on anatomical landmarks using the fovea capitis femoris and the main trabeculae direction (MTD), where the principal compressive trabeculae were located

Figure 3

After micro-CT scanning, all femoral heads were cut in half with a diamond saw, coronally separating the anterior hemisphere from the posterior hemisphere

Figure 4

There were significantly lower values of DA in GIONFH, compared to AIONFH. Similarly, in Sup-Cen and Sup-Lat regions, none of the microarchitecture parameters differed significantly between GIONFH and AIONFH, with the exception of SMI

Figure 5

There were significantly lower values of BV/TV and Tb.N, higher values of Tb.Sp and SMI in GIONFH, compared to AIONFH. In Inf-Lat region, none of the microarchitecture parameters differed significantly between GIONFH and AIONFH, except for Tb.N

Figure 6

In the necrotic-normal junction region, including Cen-Med, Cen-Cen and Cen-Lat regions, all the bone remodeling parameters were significantly higher in GIONFH, compared with AIONFH

Figure 7

Hematopoietic cells disappeared and adipose nucleus staining was absent in the bone marrow, indicating the necrosis of bone marrow

Figure 8

The bone resorption parameter ES/BS was significantly elevated in GIONFH. No significant differences were observed in ES/BV and ES/TV. In Inf-Lat region, there were higher values of O.Th, OV/BV, OS/BS in GIONFH, indicating a more active bone formation status