Central Osteophytes in Patients with Osteoarthritis of the Hip Joint Compared to Healthy Subjects: A Histological study

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Abstract

Objective: The objective of this study was to determine the prevalence and size of marginal and central osteophytes in patients with osteoarthritis (OA), and to compare these to that of healthy subjects.

Design: We investigated femoral heads from 25 patients with OA following hip replacement surgery, and 25 femoral heads from healthy subjects obtained *post-mortem*. The area and boundary length of the femoral head, marginalosteophytes, and central osteophytes were determined with histomorphometry. Marginal osteophytes were defined as bony projections at the peripheral margin of the femoral head, while central osteophytes were defined as areas of bone that expanded from the normal curvature of the femoral head up into the articular cartilage.

Results: The median[25th–75th percentile] number of marginal osteophytes was 5[5–6] for patients with OA compared to 0[0–1] for the healthy subjects (P<0.001). The median number of central osteophytes was 3[2–4] for patients with OA, which was significantly higher thanthat of 1[0–2]for the healthy subjects (P < 0.001). The marginal and central osteophytes were significantly larger in patients with OA than in healthy subjects. In healthy subjects, the central osteophytes were more frequent than the marginal osteophytes (P = 0.045).

Conclusion: At the hip, bothmarginal- and central osteophytes were more frequent and larger in patients with OA than in healthy subjects. The higher number of central osteophytes compared to marginal osteophytes in the healthy subjects may suggest that central osteophytes arean early phenomenon of osteoarthritis.

Background

Osteoarthritis (OA) is the most common joint disease, and thus OA of the hip afflicts 3–6% of the population over 50 years[1]. The disease is characterised by loss of articular cartilage, sclerosis of the subchondral bone and formation of osteophytes. Recently, we demonstrated that bone turnover is increased in patients with OA of the hip before the volume of the subchondral bone is increased[2]. Importantly, it has been shown that subchondral bone sclerosis and formation of marginal osteophytes at the knee could be detected before the thickness of the articular cartilage changes and the joint space narrows[3, 4].

Osteophyte formation occurs by the proliferation of periosteal cells, which differentiate into chondrocytes forming cartilage, which through endochondral ossification creates bony projections[5]. However, the formation of marginal osteophytes is likely not the only example of bone growth in OA.

It has been hypothesised, that the loss of articular cartilage in patients with OA might be caused by microcracks in the subchondral bone plate and the calcified cartilage[6]; These microcracks reactivate the secondary ossification centre, and the cartilage is lost due to endochondral ossification. Using quantitative backscattered electron imaging, Ferguson *et al*. found high density mineralised protrusions

(HDMP) in the calcified cartilage mineralising front in femoral heads from patients with OA[7]. These HDMP have also been found in the knee of patients with OA, using magnetic resonance imaging (MRI) and micro-computed tomography[8]. The HDMP emerged from the calcified cartilage and subchondral bone junction and could extend up to two-thirds of the articular cartilage thickness, resulting in cartilage degeneration[9]. However, the mechanism for the cartilage degeneration is currently unknown, but, HDMP could perhaps be associated with endochondral ossification and formation of osteophytes. McCauley *et al*. showed that central osteophytes in the knee visualized with MRI were associated with articular cartilage defects[10]. Similar to HDMP, these central osteophytes were defined using MRI[11]. At present, it is unknown whether HDMP transforms into central osteophytes or whether central osteophytes actually are HDMP. Until now, central osteophytes have not been investigated with quantitative histomorphometric methods.

We hypothesised that central osteophytes exists and forms a part of the early pathological changes in patients with osteoarthritis. Therefore, we quantified the boundary length and the area of the entire femoral head, the marginal osteophytes and the central osteophytes in both patients with OA and sex and age-matched healthy subjects.

Materials & Methods

Study population

Femoral heads obtained from 50 human subjects comprising 25 patients with OA aged 54–77 years (64.4(7.2) years [mean (SD)]) and 25 healthy subjects aged 50–78 (61.5(7.8) years) were included in the study. Both groups included 13 females and 12 males.

The arthritic femoral heads were obtained from patients with primary hip OA, who underwent hip replacement surgery at the Department of Orthopaedics at Farsø Hospital in Denmark. The Western Ontario, and McMaster Universities Arthritis Index (WOMAC) score and the radiographic Kellgren-Lawrence grade was obtained from all patients prior to surgery. However, these data were available for OA patients only. The patients met the combined clinical and radiographic criteria of the American College of Rheumatology for OA[12]. Only patients without known bone metabolic diseases, diabetes mellitus, malignant diseases, secondary OA or other joint diseases were included in the study.

The femoral heads from the control group were obtained at autopsy from previously healthy subjects with macroscopically normal femoral heads at the Department of Forensic Medicine, Aarhus University. All subjects in the healthy group had died suddenly from accidents or acute diseases. Healthy subjects with a history of high-energy pelvic trauma or any signs of hip OA after macroscopic inspection were excluded from the study. Furthermore, previously healthy subjects were excluded if they had any known diagnosis of bone metabolic disease, diabetes mellitus, malignant diseases or other joint diseases. The Ethics Committee of Medical Research in Central Denmark Region (J. no. 10776) and The Danish Data

Protection Agency (J.nr: 2003-41-3447) approved the study. Written and informed consents were obtained from the patients with OA before their hip replacement surgery.

Processing of Tissue

The procedure for preparing the tissue has previously been described in detail[13]. In short: The femoral heads were fixated in 70% ethanol immediately after removal. Afterwards, the femoral heads were processed according to the principles of Vertical Uniform Random sections[14]. The femoral head was randomly rotated and cut with a diamond precision-parallel saw (Exakt Apparatebau, Norderstedt, Germany) parallel to a vertical axis through the top of the femoral head to produce five to eight 7-mm-thick parallel slices, which were halved. Alternating left and right half slices were randomly selected for the following microscopic evaluation. Each of the five to eight 7-mm-thick halved parallel slices were embedded undecalcified in methylmethacrylate and cut into 7-µm-thick histological sections, using a Jung model K microtome (R. Jung GmbH, Heidelberg, Germany) equipped with a tungsten microtome knife. The sections were mounted and stained with Masson-Goldner trichrome as described previously[2] (Fig. 1).

Definition of tissue structures

Marginal osteophytes were defined as bony projections at the peripheral margin of the femoral head[5] (Fig. 1A). Central osteophytes were defined as areas of bone, which expanded from the normal curvature of the femoral head into the cartilage, and which was not located in relation to the peripheral margin of the femoral head or the fovea capitis (Fig. 1). The fovea capitis was excluded in the present study.

Histomorphometry

Data were collected using a light microscope (Nikon Eclipse 80i, Tokyo, Japan) equipped with a motorized specimen stage (Prior Proscan 11 TM, Rockland, MA, USA), a microcator (Heidenhain MT 1201, Traunreut, Germany), and a digital video camera (OlympusDP72, Tokyo, Japan) connected to a PC running the newCAST interactive stereology software (v. 3.4.1.0, Visiopharm, Hørsholm, Denmark). Sampling regions were automatically aligned in newCAST. Test points and test lines were superimposed on the digital images of the tissue sections and viewed on the PC monitor at a total magnification of × 121.64. For each tissue section, the number of sites with central- and marginal osteophytes was counted and added. The average area and boundary length were also estimated. The total area and boundary length of the femoral head was also estimated for each tissue section.

The area (*Ar*) of the femoral head, the central osteophyte and marginal osteophytes were estimated with point counting. For estimations of the area of the central and marginal osteophytes, a grid with an area per point of 0.49 mm^2 was used. For estimations of the area of the femoral head, a grid with an area per point of 2.93 mm^2 was used.

$$Ar = a(p) \times \sum_{i=1}^{n} p$$

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Where *n* is the number of sections, $\sum_{i=1}^{n} p$ is the total number of test points hitting the structure of interest and *a(p)* is the area per test point[15].

The boundary length (Bd) of the total femoral head, the central osteophytes, and the marginal osteophytes were estimated using a line probe with an area per length of 0.297 mm.

$$Bd = \pi/2 \times \sum_{i=1}^{n} l \times a(l)$$

Where π/2 is a constant used for sine-weighted test-lines, *n* is the number of sections, $\sum_{i=1}^{n} l$ is the total number of intersections between the tissue surface and the sine-weighted test-line grid, and *a(l)* is the area per test line length of the line-grid superimposed on the Sect. [15].

Statistics

Data were analysed using STATA 12 (StataCorp LP, College Station, TX, USA). Normal distribution of the data was investigated with Q-Q plots and histograms. Normally distributed data are presented as mean [95% confidence interval], and differences between the two groups were tested for statistical significance using Student's *t*-test. Data, which was not normally distributed, are presented as median [25th – 75th percentile], differences between the two groups were tested for statistical significance using the Mann-Whitney U test. For both groups. Differences in the prevalence of marginal and central osteophytes were tested for statistical significance using Fisher's exact test. The results were considered statistically significant at $P < 0.05$.

Results

The patients with OA had a median [25th – 75th percentile] Kellgren-Lawrence grade of 4 [4 – 4] and a mean [95%CI] WOMAC score for pain of 54 [45 – 63], for stiffness of 58 [46 – 70], and physical activity of 43 [37 – 49].

The area and boundary length of the femoral head did not differ significantly between the OA patients and the healthy subjects (Fig. 2).

The prevalence of marginal osteophytes was significantly higher in the OA patients as all 25 of the 25 (100%) OA patients had marginal osteophytes compared to seven of the 25 (28%) healthy subjects (P <

0.001). Marginal osteophytes were found at a total of 133 sites in the group of patients with OA, which was significantly more frequent than in the healthy subjects were only 16 sites with marginal osteophytes were identified ($P < 0.001$). The median number of marginal osteophyte sites per patient was also significantly higher in the patients with OA (5[5 – 6]) than in the healthy subjects (0 [0 – 1], $P < 0.001$). Moreover, the average size of the marginal osteophyte sites was significantly larger in the patients with OA than in the healthy subjects both with respect to their area and to their boundary length (Fig. 2).

The prevalence of central osteophytes was significantly higher in the patients with OA, where 21 of the 25 (84%) patients had central osteophytes compared to 14 of the 25 (56%) healthy subjects ($P < 0.001$). In addition, 19 of the 25 (79%) patients with OA had more than one site of central osteophytes, which was a significantly larger proportion of the individuals than for the healthy subjects, where multiple sites of central osteophytes were only present in 7 of the 25 (28%) subjects ($P = 0.001$). Central osteophytes were found in a total of 82 sites in the patients with OA, of which 68 (83%) was superficially covered with articular cartilage. The remaining 14 sites (17%) had a denuded bone surface. Central osteophytes were found at 26 sites in the healthy subject, which were significantly fewer than for the patients with OA ($P = 0.014$). Moreover, only one of the 26 sites (4%) of central osteophytes had denuded bone, while the remaining 25 (96%) were superficially covered with articular cartilage. However, the proportion of central osteophytes with denuded bone did not differ between patients with OA and healthy subjects ($P = 0.082$). The median number of central osteophyte sites per individual was significantly higher in patients with OA (3 [2 – 4]) compared to the healthy subjects (1 [0 – 2], $P < 0.001$). Moreover, the average size of the central osteophyte sites was significantly larger in the patients with OA than in the healthy subjects both with respect to their boundary length and their area (Fig. 2).

The proportion of OA patients with central osteophytes did not differ from the proportion of OA patients with marginal osteophytes. For the healthy subject, we observed a significantly higher proportion of the subjects with central osteophytes compared to subjects with marginal osteophytes ($P = 0.045$).

Discussion

In this cross-sectional study, we found that central osteophytes protruding from the normal curvature of the femoral head into the articular cartilage were a common feature of patients with OA. Healthy subjects also had central osteophytes, but they were not as common and were smaller than in patients with OA. Marginal osteophytes are a hallmark of osteoarthritis. This was clearly apparent in the present study as the patients with OA had larger and more frequent marginal osteophytes than the healthy subjects.

To our knowledge, central osteophytes have not previously been quantified using histology. McCauley *et al*. investigated central osteophytes from 200 patients who were referred for MR imaging of the knee[10]. In that study, central osteophytes were defined as focal excrescences that extended from the cortical surface and were surrounded by articular cartilage on all sides. This definition is similar to the definition of central osteophytes used in the present study. McCauley *et al*. found that central osteophytes were associated with more articular cartilage defects and meniscal tears. Moreover, patients with central

osteophytes were significantly older and had a higher BMI than patients without central osteophytes[10]. Increased weight and age are known to predispose to osteoarthritis. Interestingly, in the present study, the central osteophytes were slightly more common than marginal osteophytes in healthy subjects. It has been shown that bone growth, in the form of marginal osteophytes, occurs before a loss of articular cartilage could be detected[3]. Therefore, it is not unreasonable to suggest that central osteophytes could also be an early event of OA, especially as the size and frequency of central osteophytes were greater in patients with OA than in healthy subjects. Furthermore, 17% of the central osteophytes had denuded bone in patients with OA compared to 4% of the healthy subjects. However, this difference was not statistically significant, but indicate that the central osteophytes invade the articular cartilage from the bone-cartilage unit. Currently, the formation of marginal osteophytes, joint space narrowing, and subchondral bone sclerosis are the only features determined in a radiographical OA assessment. However, these features are, nevertheless, not sensitive enough to detect OA at an early stage. Therefore, we suggest that the central osteophytes investigated in the present study may be an early marker for diagnostic imaging of OA.

It is well known that marginal osteophytes grow by a form of endochondral ossification with progressive changes of cellular proliferation, differentiation, and elaboration of intercellular matrix[5]. However, it is currently unknown how central osteophytes form. A possible mechanism for the progression of central osteophytes could be that they form as a consequence of microcracks in the calcified cartilage and subchondral bone. This increases bone turnover[6] and may result in HDMP[11], supposing that HDMP initiates reactivation of the secondary ossification centre. The articular cartilage may be lost due to endochondral ossification as illustrated in Fig. 3, and not as a consequence of fragmentation leading to a process of wear and tear[7]. However, longitudinal studies are needed to investigate this hypothesis.

A weakness of the current study is that it is a cross-sectional study, and we can, therefore, only speculate on how the central osteophytes develop. Another weakness is that some areas of the femoral head from patients with OA were devoid of articular cartilage. Hence, in some areas, it was not possible to define whether central osteophytes were present or not. Thus, our estimates for the number of central osteophytes in patients with OA were likely underestimated, while this was not the case for the healthy subjects. Lastly, the healthy subjects were slightly younger than the OA patients, although this difference was not statistically significant.

Conclusions

In conclusion, the central osteophytes were larger and more frequent in patients with osteoarthritis compared with healthy subjects, suggesting that central osteophytes are a common feature of OA pathology. Furthermore, in the healthy subjects, the frequency and size of central osteophytes suggest that central osteophytes may be an early phenomenon of osteoarthritis. Consequently, central osteophytes could possibly be used as an imaging marker for diagnosis and grading of early OA.

Abbreviations

HDMP – High Density mineralised protrusions

MRI -Magnetic resonance imaging

OA - Osteoarthritis

WOMAC - Western Ontario, and McMaster Universities Arthritis Index (WOMAC)

Declarations

Ethics approval and consent to participate

The Ethics Committee of Medical Research in Central Denmark Region (J. no. 10776) and The Danish Data Protection Agency (J.nr: 2003-41-3447) approved the study. Written and informed consents were obtained from the patients with OA before their hip replacement surgery.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Ellen-Margrethe Hauge reports personal fees from MSD, personal fees from Pfizer, personal fees from UCB, personal fees from Sobi, grants from Roche, grants from Novartis, outside the submitted work. Kresten Krarup Keller reports speaking fee from Pfizer. Rasmus Klose Jensen, Andreas Wiggers Nielsen, Louise Brøndt Hartlev, Lene Warner ThorupBoel, Mogens Berg Laursen and Jesper Skovhus Thomsen have no conflict of interest to declare.

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Author Contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

Study conception and design: Rasmus Klose-Jensen, Kresten Krarup Keller, and Ellen-Margrethe Hauge.
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Analysis and interpretation of data: Rasmus Klose-Jensen, Kresten Krarup Keller, Ellen-Margrethe Hauge and Andreas Wiggers Nielsen.

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Figures

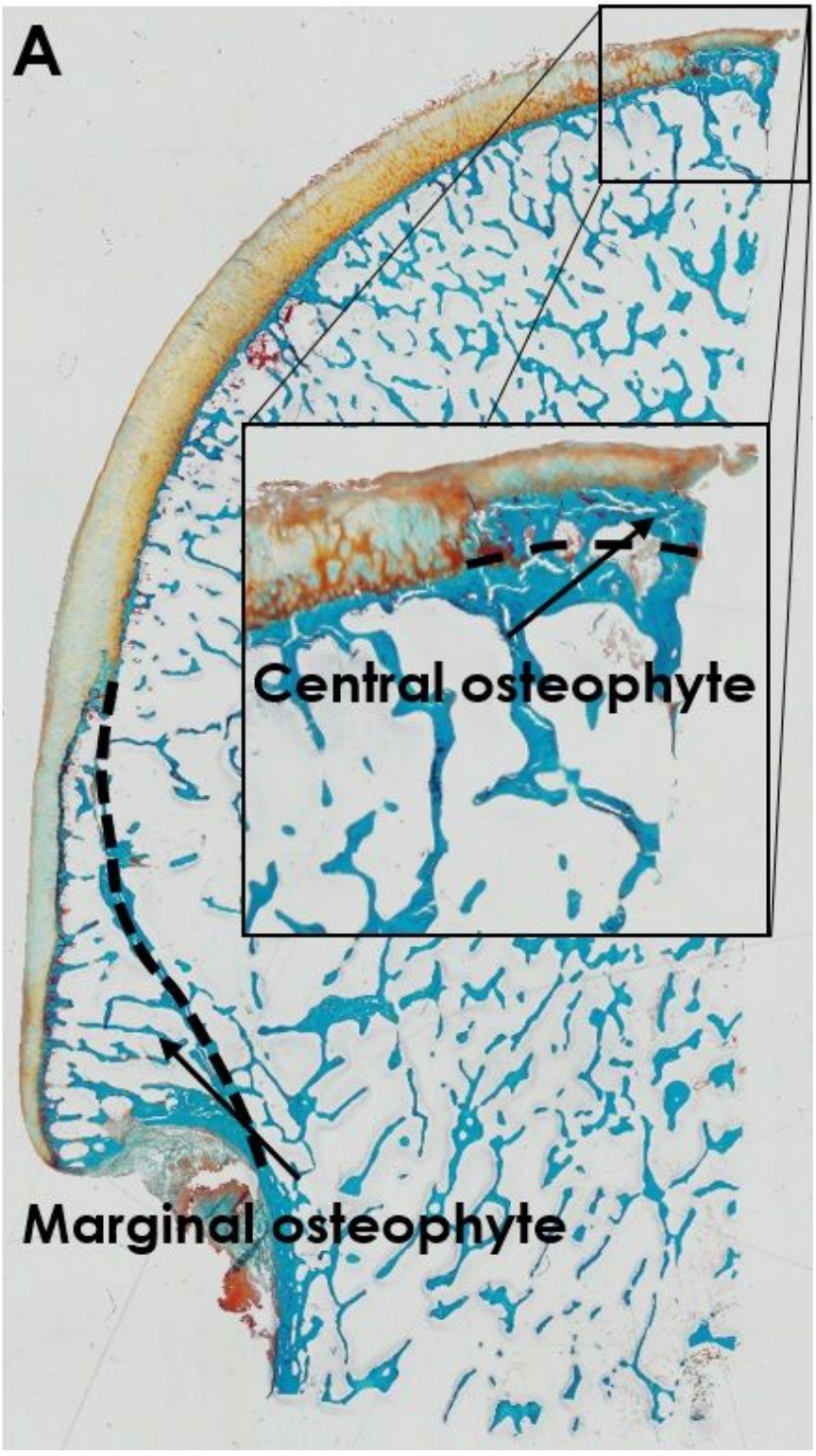

Figure 1

A)The left half of a 7-µm-thick Masson-Goldner trichrome stained tissue section of the femoral head from a patient with osteoarthritis. The dotted line illustrates the normal curvature of the femoral head, without marginal- and central osteophytes.

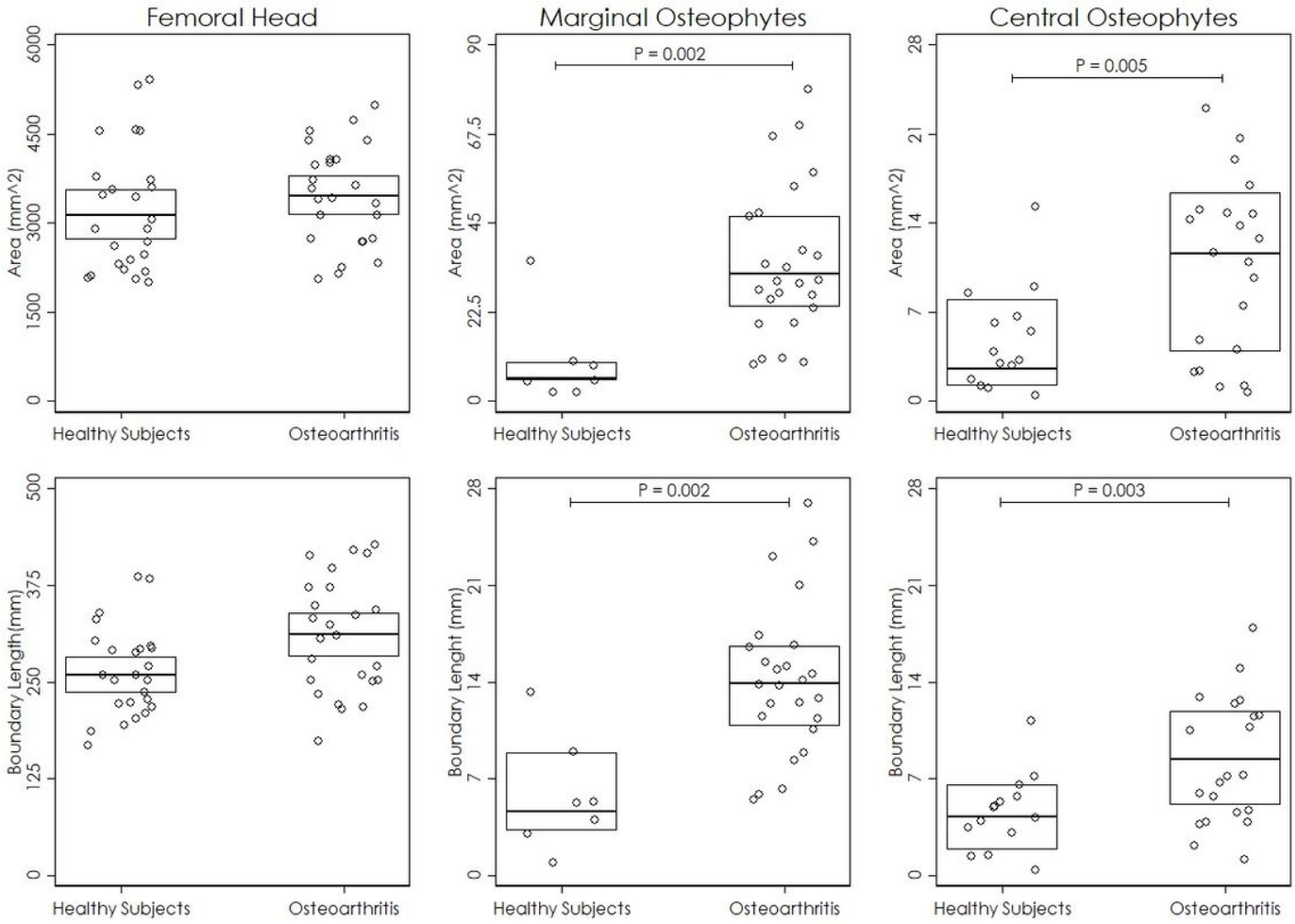

Figure 2

Femoral Head: Each open dot represents the summarised area or boundary length of the femoral heads in an individual. The horizontal line indicates the mean of the healthy subjects and the patients with osteoarthritis, while the boxes represent the 95% confidence interval. Student's t-test was used to test for statistical significance. Marginal- and Central Osteophytes: Each open dot represents the average size of marginal- or central osteophyte sites in an individual. The horizontal line indicates the median of the healthy subjects and the patients with osteoarthritis, while the boxes represent the interquartile range. Mann–Whitney U test was used to test for statistical significance. P-value < 0.05 was considered significant.

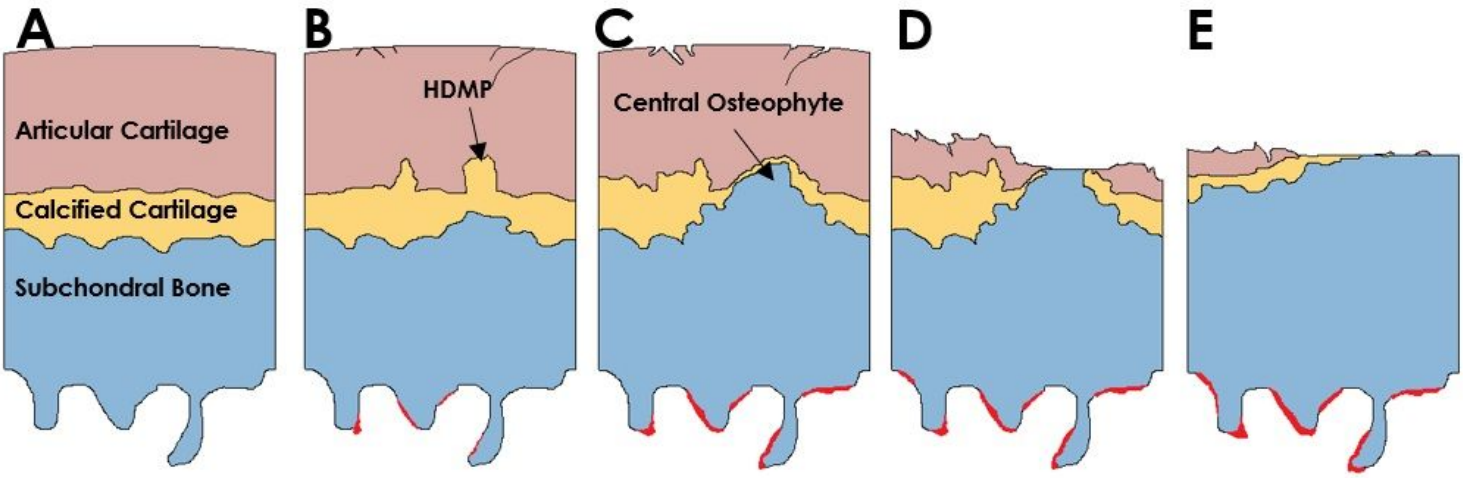

Figure 3

Schematic illustration of the hypothesised growth of central osteophytes into articular cartilage.A) Healthy joint surface with articular cartilage, calcified cartilage and subchondral bone. B) High density mineralised protrusions (HDMP) emerged from cracks in the calcified cartilage and subchondral bone. C) Endochondral ossification of the high density mineralised protrusions resulting in central osteophytes and superficial fibrillation of the articular cartilage. D) The subchondral bone penetrates the articular cartilage. F) Complete loss of the articular cartilage, and subchondral bone sclerosis.