Microcalcification of Lumbar Spine Intervertebral Discs and Facet Joints is Associated With Cartilage Degeneration, But Differs in Prevalence and Its Relation to Age

Thelonius Hawellek,¹ Jan Hubert,¹ Sandra Hirschke,² Tim Rolvien,³ Matthias Krause,³ Klaus Püschel,⁴ Wolfgang Rüther,¹ Andreas Niemeier¹

¹Department of Orthopaedics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, ²Department of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, ³Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, ⁴Department of Legal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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ABSTRACT: Cartilage calcification (CC) is associated with degeneration in non-vertebral joints, but little is known about CC and lumbar vertebral joints. The goal of this study was to analyze the prevalence of CC in lumbar facet joints (FJ) and intervertebral discs (IVD) and its relation to cartilage degeneration and age in a non-selected cohort of the general population. The segment L4/5 of 85 consecutive donors (mean age 61.9 years) was analyzed by high-resolution imaging digital-contact radiography (DCR). Quantification was achieved by measuring CC in % of total cartilage area. Histological degeneration of FJs and IVDs was determined by OARSI and Boos scores. Prevalence of CC was 36.5% for FJ (95% CI (0.26, 0.48)) and 100% for IVD (95% CI (0.96, 1.00)). The amount of IVD CC (3.36% SD ± 7.14) was 16.3 times higher (p < 0.001) than that of the FJ (0.23% SD ± 0.53) and independent of each other (p = 0.07). The amount of FJ CC correlated significantly with FJ and IVD degeneration (FJ r = 0.44, p = 0.01, IVD r = 0.49, p = 0.006) while the amount of IVD CC correlated only with IVD degeneration (r = 0.54, p < 0.001). Age correlated with IVD CC (r = 0.35, p < 0.001), but not FJ CC (r = 0.04, p = 0.85). We conclude that IVD fibrocartilage is particularly prone to calcification. A causal relationship between lumbar CC and degeneration is possible, but the clear differences in IVD fibrocartilage CC and FJ synovial joint CC in regard to prevalence and in relation to age point to a differential role of CC in single compartments of the respective motion segment in lumbar spine degeneration.

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Keywords: cartilage calcification; intervertebral disc calcification; lumbar disc degeneration; spondylarthrosis

Lumbar spine degeneration is a widely prevalent, multifactorial disorder that usually involves multiple anatomic structures.¹ Clinically, it is often difficult to differentiate between symptomatic and asymptomatic pathology of the respective structures within a motion segment.² For therapeutic decision making, a clear distinction between symptomatic intervertebral discs (IVD) and facet joints (FJ) is of great importance. There is no stringent correlation between the degree of degeneration and clinical symptoms in either IVDs or FJs.² It currently remains unknown why some severely degenerated discs and joints may remain clinically relatively silent while others with only mild degeneration can be severely symptomatic.

Ectopic cartilage calcification (CC) is believed to be a factor that may play a role in joint degeneration and as a trigger of the local expression of pro-inflammatory stimuli as mediators of pain.³–⁵ Therefore, a more detailed understanding of fibrocartilage calcification of the IVD and hyaline cartilage calcification of the synovial FJ in relation to the degree of degeneration and age may help to provide insight into the commonalities and differences of IVD degeneration and FJ osteoarthritis (OA) in low back pain.

Little is known about IVD CC and there is no literature on FJ CC. It has been described that CC can be detected in degenerative human IVD,⁶,⁷ but a correlation between the amount of CC and indices of IVD degeneration has only been described in animal models such as sheep and Dachshund, but not in humans so far.⁸,⁹ In vitro, bovine annulus fibrosus cells can induce mineralization.¹⁰ The trigger for CC in vivo is unknown and it is still a matter of debate whether calcium phosphate crystals in human cartilage are the cause or the result of degenerative processes.¹¹,¹² It is even unclear whether such a putative causal relationship would be uniform throughout the body or whether it may vary from joint to joint.¹³ One of the difficulties in obtaining reliable data from humans is that CC starts in the nano- to micrometer range and initial stages are impossible to detect by standard X-Ray, CT, or MRI.¹⁴ High resolution digital contact radiography (DCR) in contrast is well suited to detect early crystallizations¹⁵ but has the disadvantage of being applicable only to tissue samples ex vivo. To our knowledge there are three autopsy studies in which lumbar IVDs were analyzed by higher resolution imagining techniques.¹⁶–¹⁸ Based on these three studies, the prevalence of IVD CC in the lumbar spine is estimated to be approximately

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Correspondence to: Thelonius Hawellek and Andreas Niemeier (T: +49 (0)40 7410 53670; F: +49 (0)40 7410 55018; E-mail: thelonius.hawellek@med.uni-goettingen.de (TH); niemeier@uke.uni-hamburg.de (AN))

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30–50% in the general population. Of note, these studies have not analyzed the relationship of IVD CC with FJ CC. In general, there is no literature about the prevalence of CC in FJ, which is remarkable in light of the fact that CC of large peripheral joints is known to be associated with the degeneration grade of these joints. There seems to be a consensus that the lumbar level L4/5 is most often affected by IVD degeneration and FJ OA. The superior articular facet of the FJ shows earlier and more severe damage than the inferior facet and there is no difference in the prevalence of FJ OA between right and left facet joints.

The aim of this study was to analyze the prevalence and the amount of L4/5 IVD CC and FJ CC by DCR in the general population and to analyze the relationship between CC, age and histological tissue degeneration.

MATERIALS AND METHODS

The intact lumbar motion segment L4/5 was obtained of an unselected sample of 85 donors who underwent autopsy at the Department for Legal Medicine, University Medical Center Hamburg-Eppendorf (hereafter referred to as “donors”). Only donors without any signs of lumbar disease other than degeneration were included in this study. None of the donors had evidence of previous lumbar spine surgery. Donors with tumors, infections, or rheumatic diseases in the donors had evidence of previous lumbar spine surgery. Other than degeneration were included in this study. None of the donors had evidence of previous lumbar spine surgery. The study was approved by the local ethics committee (PV 4570) and is in compliance with the Helsinki Declaration.

The mean age was 61.9 years, SD = 19.1 (range 20–93 years); 39 of the donors were female and 46 male. Biometric characteristics of the study population are presented in Table 1.

Sample Preparation

The whole intact lumbar motion segment L4/5 was extracted from the lumbar spine and all soft tissue was removed. The right FJ L4/5 was removed in toto and opened gently. Subsequently the superior articular process L5 was isolated. For FJ analysis a standardized 3 mm bone-cartilage slab was cut in the sagittal plane. Both pedicles of the vertebra L4 and L5 were cut off. Thereafter the intact vertebral bodies of L4 and L5 with the corresponding IVD were cut to generate a standardized 5 mm bone-cartilage-bone slab in the parasagittal plane (Fig. 1).

Digital-Contact Radiography (DCR)

The bone-cartilage slab of the FJ L4/5 and the bone-cartilage-bone slab of the IVD L4/5 was washed with physiological saline solution to remove residual bone debris. Standardized radiographs were taken (25 kV, 3.8 mAs, film focus distance 8 cm) using a high-resolution digital radiography device (Faxitron X-Ray, IL). Quantitative computerized analysis of the area of cartilage calcification both of the hyaline cartilage of the superior articular process L5 and of the fibrocartilage of the IVD L4/5 was performed with standard software (ImageJ 1.46, National Institutes of Health, Bethesda) as published previously. The amount of calcification in percent of the hyaline cartilage and fibrocartilage area was determined by dividing the measured area of calcification by the total area of the respective cartilage slab.

Histology

Histological OA assessment was done of the cartilage of each superior articular process L5, directly adjacent to the slab plane. A specimen of full thickness hyaline cartilage was cut to the subchondral bone plate. Fibrocartilage of the annulus fibrosus of the lateral right posterior part of the IVD L4/5 directly adjacent to the slab plane was retrieved for sample preparation. All specimens were fixed in 4% PFA for 24 h, dehydrated in 80% alcohol and embedded in paraffin. Four micrometer sections of all samples were stained with von Kossa to confirm calcium-phosphate deposition (Fig. 1). Staining with 1% Safranin-O for hyaline cartilage of the FJ L4/5 and HE-staining for fibrocartilage of the IVD L4/5 (Fig. 1) was performed to evaluate the histological degeneration grade according to the OARSI osteoarthritis cartilage histopathology assessment system for FJ (grade 0–6) and according to the Boos-Score for IVD (grade 0–22).

Statistical Analysis

The biometric characteristics of donors are reported as mean values ± standard deviations (Table 1). For descriptive analysis the measured amount of cartilage calcification was used. Logarithmic transformation was done if appropriate. For categorical data Fisher’s test was used. For group comparing, t-test respectively Wilcoxon signed-rank test were used. Linear mixed model was used to analyze the difference between the mean amount of cartilage calcification in the facet joint and intervertebral disc joint. Subject was used as a random effect with a compound symmetry covariance structure and joint was used as fixed effect. In addition, the mixed model assumptions were checked using residual plots. To report the association between continuous variables Pearson’s r or Spearman’s rs rank correlation coefficient was calculated. To avoid spurious correlations, a partial correlation was performed using adjustment for the respective excluded parameters (cartilage calcification, histological degeneration grade and age). All statistical analyses were performed with statistical software R version 3.1.1. p values less than 0.05 were considered statistically significant.

RESULTS

Prevalence of Cartilage Calcification

The prevalence of CC of the hyaline cartilage of the right FJ L4/5 was 36.5% (31/85) (95%CI (0.26, 0.48))
and of the IVD L4/5 100% (85/85) (95%CI (0.96, 1.00)) (Table 2).

Gender
In males, FJ CC was detected in 32.6% (95%CI (0.20, 0.48)) (15/46) and IVD CC in 100% (95%CI (0.92, 1.00)) (46/46). In females, the prevalence of FJ CC was 41.0% (16/39) (95%CI (0.26, 0.58)) and of the IVD 100% (39/39) (95%CI (0.91, 1.00)) (Table 2). There was no significant difference in the detection of CC of the FJ (p = 0.5) or the IVD (p = 1.0) for gender.

Quantitative Analysis of L4/5 Facet Joint and Intervertebral Disc Cartilage Calcification
The amount of CC of the right FJ was 0.23% (SD ± 0.53; range: 0.00–2.89) of total cartilage area and the amount of CC of the IVD was 3.36% (SD ± 7.14; range: 0.01–36.14) of total cartilage area. There was no significant correlation between the amount of FJ CC and IVD CC (rs = 0.33, p = 0.07) (Fig. 2A). The amount of IVD CC was 16.3 times higher (OR = 2.79; p < 0.001) per tissue sample volume compared to FJ CC (Fig. 2B).

Facet Joint and Intervertebral Disc Degeneration
The mean histological degeneration score (OARSI) of the FJ was 3.2 (SD ± 1.2, range: 1–6). The mean histological degeneration grade of the IVD (Boos) was 7.3 (SD ± 3.8, range: 2–20). (Table 3). There was no difference in the histological degeneration grade of the FJ or IVD for gender (p = 0.14, respectively p = 0.78) (Table 3). There was no significant correlation between the histological degeneration grade of the FJ and the IVD (rs = 0.11, p = 0.33).

Correlation Between Cartilage Calcification and Histological Degeneration

Facet Joint L4/5
There was a significant correlation between the amount of FJ CC and FJ histological degeneration grade without (r = 0.45, p = 0.01, 95%CI (0.11 0.69)) and after adjustment for age (r = 0.44, p = 0.01, 95%CI (0.11 0.69)) (Fig. 3A).

Intervertebral Disc L4/5
There was a significant correlation between the amount of IVD CC and IVD histological degeneration without (r = 0.50, p = 0.004 95%CI (0.17 0.72)) and after adjustment for age (r = 0.49, p = 0.006, 95%CI (0.19 0.63)).

There was a significant correlation between the amount of FJ CC and IVD histological degeneration without (r = 0.50, p = 0.004 95%CI (0.17 0.72)) and after adjustment for age (r = 0.49, p = 0.006, 95%CI (0.19 0.63)).

Table 2. Prevalence of Cartilage Calcification of the Intervertebral Disc L4/5 and the Right Facet Joint L4/5 Detected by DCR (n = 85)

| Tissue               | Male     | Female    | Total    |
|----------------------|----------|-----------|----------|
| Intervertebral disc L4/5 | 46/46 (100%) | 39/39 (100%) | 85/85 (100%) |
| Facet joint L4/5     | 15/46 (32.6%) | 16/39 (41.0%) | 31/85 (36.5%) |
any more after adjustment for age ($r = 0.13$, $p = 0.25$, 95%CI (0.01 0.20)).

**Correlation Between Cartilage Calcification and Age**

**Facet Joint L4/5**

There was no significant correlation between the amount of FJ CC and age without ($r_s = 0.17$, $p = 0.38$, 95%CI (0.21, 0.50)) and after adjusting for histological degeneration grade ($r_s = 0.04$, $p = 0.85$, 95%CI (0.20, 0.14)) (Fig. 3B).

**Intervertebral Disc L4/5**

There was a significant correlation between the amount of CC of the intervertebral disc L4/5 and age ($r_s = 0.44$, $p < 0.001$, 95%CI (0.25, 0.60)). After adjusting for histological degeneration grade, there was still a significant correlation between the amount of CC of the intervertebral disc L4/5 and age ($r_s = 0.35$, $p < 0.001$, 95%CI (0.23, 0.43)) (Fig. 3D).

**DISCUSSION**

In this cross-sectional post-mortem study of the general population, we detect an unexpectedly high prevalence of CC in 100% of IVD L4/5 and a comparatively low prevalence of 36.5% in the FJ. Interestingly, the amount of CC in the IVD does not correlate with the amount of FJ CC, but the amount of CC in either IVD fibrocartilage or FJ articular cartilage correlates with the degree of cartilage degeneration. In addition, only IVD CC but not FJ CC correlates with age.

Given the stark difference in prevalence, one may conclude that fibrocartilage is particularly prone to calcification. The reasons for this are not entirely clear at present but a number of observations seem to confirm this hypothesis. Touraine et al. have published a higher prevalence of meniscal fibrocartilage calcification than articular hyaline cartilage calcification in knee joints analyzed by CT.33 Here we show by quantitative analysis that even the amount of CC in IVD was 16.3 times higher compared to FJ. In addition, the prevalence of CC in large synovial joints that also contain fibrocartilage, for example, the shoulder and knee, has been described to be higher than 90%.19,20,22 Another factor may be the size of the joint, since CC prevalence of the MTP1-joint was reported to be significantly lower than in the knee or shoulder and rather comparable to the small FJ.34

Another possible explanation for the unequal calcification potential could be found in the different collagen composition. Mineralized tissues are composed of mainly type-I collagen, the exception being calcified hyaline cartilage, which contains type-II collagen.35 All of the collagenous mineralized tissues contain

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**Table 3. Distribution of the Histological Degeneration Grade of the Intervertebral Disc L4/5 (Boos-Score) Respectively Histological Degeneration Grade of the Right Facet Joint L4/5 (OARSI-Score) ($n = 85$)**

| Boos-Score (IVD) | Number of Donors (%) |
|-----------------|----------------------|
| 0–7             | 54 (63.5)            |
| 8–14            | 26 (30.6)            |
| 15–22           | 5 (5.9)              |

| OARSI-score (FJ) | Number of donors (%) |
|-----------------|----------------------|
| 0               | 0 (0.0)              |
| 1               | 11 (12.9)            |
| 2               | 10 (11.8)            |
| 3               | 31 (36.5)            |
| 4               | 23 (27.1)            |
| 5               | 7 (8.2)              |
| 6               | 3 (3.5)              |

IVD, intervertebral disc; FJ, facet joint.
non-collagenous proteins, whose function in mineralization is still a major research area.\textsuperscript{35} The annulus fibrosus of the IVD contains amounts of type-I and type-II collagen,\textsuperscript{36} which is in clear distinction to the FJ that contains type-II collagen. However, it is important to note that also age-related changes in the expression of the structural matrix molecules such as collagen type-I, III, VI, and X and their spatial distribution may contribute to calcification of both IVD and FJ.\textsuperscript{37} Another possibility for the difference of calcification in FJ and IVD could be the load distribution. In healthy lumbar spines the major load affects the IVD while FJs are bearing less than 25\% of the load transmitted across the lumbar motion segment.\textsuperscript{38,39} All of these potential explanations appear to be possible, but clearly remain speculative at present and will have to be further clarified by future research.

To our knowledge, this is the first report in the literature on the prevalence of FJ CC in relation to age and the histological degree of FJ OA. Since we have found a significant association between the amount of CC and histological degeneration grade independent of age it is conceivable that CC plays a role in the pathogenesis of FJ OA. Based on the observation that CC was detectable even in virtually intact cartilage (OARSI $<3$) in three specimen, one may conclude that histologic cartilage degeneration is

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scatter_plots.png}
\caption{Logarithmic scatter plots of the correlation between the mean amount of cartilage calcification in \% of total cartilage area and the histological degeneration grade (A, C) and age (B, D). Data points are jittered to avoid overplotting. Logarithmic scatter plots are shown with the blue orthogonal regression line and with the corresponding correlation coefficient ($r$). Significant correlations were found between: (A) The mean amount of hyaline cartilage calcification and the histological OA grade (OARSI) of the facet joint (FJ) ($r=0.44$, $p=0.01$, 95\%CI (0.11 0.69)). (C) The mean amount of fibrocartilage calcification and the histological degeneration grade (Boos) of the IVD ($r=0.54$, $p<0.001$, 95\%CI (0.43 0.60)). (D) The mean amount of fibrocartilage calcification of the IVD and age ($r_s=0.35$, $p<0.001$, 95\%CI (0.23, 0.43)). There was no correlation between the mean amount of hyaline cartilage calcification of the facet joint (FJ) and age ($p=0.85$) (B).}
\end{figure}
not a prerequisite for articular cartilage calcification. This is compatible with the results of two animal models of OA (partial medial meniscectomy in guinea pig and STR/OR T mice) in which calcification of the cartilage was visible before cartilage damage became distinguishable.40,41

The biomechanical function of a lumbar motion segment depends on the interaction of the IVD and its two corresponding FJ as a complex.42 Thereby altered motion in each individual structure has a biomechanical influence on the remaining two structures.42,43 In this context, it is interesting to note that FJ CC correlated with IVD degeneration. Calcium phosphate crystals can alter biomechanical properties of a joint44,45 and may do so in the L4/5 FJ, resulting in corresponding IVD dysfunction and ultimately degeneration at the same level. There was no correlation between IVD CC and FJ degeneration, meaning that these two pathological processes are independent of each other. It may be possible that calcified IVD reduces the motion in a lumbar segment and therefore the load on the FJ. For future research it would be interesting to analyze if a highly calcified IVD shows more degeneration in adjacent segments. This is a well-described postoperative phenomenon after lumbar fusion with development of adjacent segment disease.46

The high prevalence of 100% of IVD CC that we report here was unexpectedly high, as Cheng et al.17 and Chanchairujira et al.16 also used DCR in a cadaver study, but reported only 53.8% and 27% prevalence of IVD CC. The reason for this difference is not quite clear, but contributing factors may be differences in sample preparation and processing. While Cheng et al. studied bone-cartilage-bone slabs of each intervertebral disc just as we did, Chanchairujira et al.16 performed DCR images of raw lumbar spines, which carries the inherent likelihood of over-projecting multiple layers of tissue structures so that initial stages of calcification can be easily missed. Given that IVD CC was detectable in discs with only mild degeneration (Fig. 3C) and the significant correlation between the amount of IVD CC with histologic degeneration, a causal relationship between CC and cartilage degeneration is possible, but a yet unproven. Similar relationships between CC and IVD degeneration have been described before in humans,47 sheep9 and Dachshund.9

The FJ capsule and the IVD are innervated and containing nociceptors.38,48 In both structures calcium crystals can work as a trigger of the local expression of pro-inflammatory stimuli that could be a possible cause for low back pain.3–5

Age is an important factor that obviously plays a differential role in IVD CC versus FJ CC. While IVD CC is dependent on age, FJ CC is independent of age. Previously published studies have also reported a relation between age and IVD CC.16–18 In this respect the IVD is clearly different from not only the FJ but also from other synovial articular joints, even those that contain fibrocartilage in addition to the hyaline cartilage.19,21,22 The present study therefore helps to establish a pattern of recognition in that even within the same lumbar motion segment the presence of pure fibrocartilage versus synovial articular cartilage appears to be the main factor associated with the dichotomy of CC being or not being associated with age. Our results confirm the results of already published studies that there is a relationship between age and the degeneration of IVD, respectively FJ.1,2,38

There are limitations to this study. For most donors a comprehensive medical history is lacking. Moreover, there was no detailed information about the medical history, for example low back pain. We analyzed only the right facet joint L4/5. In addition, the standardized slab specimens of the right facet joint and intervertebral disc L4/5 reflect representative planes, but only a small part of the total articulating surface of the joint, which opens some room for error. Furthermore we did not characterize the physicochemical nature of the calcifications detected by DCR. Such analysis require complex diagnostic methods for example, FTIR spectroscopy49 or X-Ray diffractometry50 and was not the purpose of the present study. However, none of these limitations is likely to have a profound impact on the major new findings and conclusions that we draw from the present study.

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AUTHORS’ CONTRIBUTION
TH and JH contributed to the conception and design of the study, acquisition of data, analysis and interpretation of data, as well as drafting and revision of the manuscript. SH was responsible for statistical analysis and interpretation of the data. MK and TR contributed to DCR and histological analysis and interpretation of the data. KP contributed to the acquisition of data. WR contributed to the conception and design of the study. AN contributed to the conception and design of the study, analysis and interpretation of data, as well as drafting and revision of the manuscript. All authors read and approved the final manuscript. All authors included on this paper have made substantial contributions to this work and fulfill therefore the criteria of authorship. Thelonius Hawellek (thelonius.hawellek@med.uni-goettingen.de) and Andreas Niemeier (niemeier@uke.uni-hamburg.de) take responsibility for the integrity of the work as a whole, from inception to finished article.

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