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The prevalence of and factors related to calcium pyrophosphate dihydrate crystal deposition in the knee joint

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SUMMARY

Objectives: The purpose of this study was to reveal the accurate prevalence and related factors to the presence of calcium pyrophosphate dihydrate (CPPD) crystal deposition in cadaveric knee joints.
Design: Controlled laboratory study.
Methods: Six hundred and eight knees from 304 cadavers (332 male knees and 276 female knees, formalin fixed, Japanese anatomical specimens) were included in this study. The average age of the cadavers was 78.3 ± 10.7 years. Knees were macroscopically evaluated for the existence of CPPD, and the depth of cartilage degeneration of the femoro-tibial joint following the Outerbridge's classification. CPPD crystal was confirmed under Fourier transform infrared spectroscopy (FTIR) analysis using light microscopy. Statistical analysis was performed to reveal the correlation between the occurrence of CPPD deposition in the knee joint and gender, age, and the depth of cartilage degeneration of the femoro-tibial joint.
Results: The prevalence of grossly visible CPPD crystal was 13% (79 knees). In all of these knees, CPPD crystal was confirmed under FTIR analysis. Statistical analysis showed significant correlation between the occurrence of CPPD deposition and gender ($P < 0.001$), and depth of cartilage degeneration in the femoro-tibial joint ($P < 0.001$). In the cartilage degeneration positive knees (Over grade 3 in Outerbridge's classification), average age of CPPD deposition knee was significantly higher than CPPD negative knees.
Conclusions: In this study, the prevalence of CPPD deposition disease was evaluated in a relatively large sample size of cadaveric knees. The prevalence of CPPD deposition disease was 13%, and was significantly correlated with the subject's age, gender, and severity of cartilage degeneration in the femoro-tibial joint.

Introduction

Crystal-induced arthritis is the acute or subacute arthritis caused by various chemical mediators to deposited crystals in and around joints[1–4]. There are a variety of crystals that may induce arthritis; the one of the frequent crystal in the knee joint is calcium pyrophosphate dihydrate (CPPD)[1,5,6]. CPPD deposition around the knee can be observed in all articular and peri-articular tissues such as cartilage, synovium and ligaments, however, it is most commonly found in hyaline articular cartilage and meniscal fibro cartilage[2,3]. For the elderly population, CPPD crystal deposition disease is a common disorder[1,6–9]. However, its actual prevalence has not been well investigated. Because there are many asymptomatic cases of CPPD, revealing the actual prevalence of CPPD deposition disease in a clinical setting is difficult[8,10,11]. Some studies have tried to reveal the accurate prevalence of CPPD deposition

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disease in the knee using radiograph assessments[3,10] or joint fluid examinations[6,12], but these methods are relatively insensitive and can detect only sizable CPPD deposits. The most accurate way in which to identify CPPD crystal deposits is through examination of tissues, an opportunity that is rarely available except in end stage patients undergoing surgery.

It has also been reported that CPPD deposition is frequently observed with severe osteoarthritic (OA) changes and cartilage degeneration[13–15]. However, the exact correlation between CPPD deposition and the severity of cartilage degeneration in the knee joint has yet to be revealed.

The purposes of this study were to reveal the accurate prevalence of CPPD deposition disease in the knee joint using a relatively large number of cadaveric knees, and to investigate factors correlated to its occurrence.

Materials and methods

This study has been approved by the ethics committee of Nihon University School of medicine (IRB number: 20-14). Six hundred and eight knees from 304 cadavers (Male: 332 knees from 166 cadavers, Female: 276 knees from 138 cadavers) were included in this study. All cadaveric knees were evaluated bilaterally. Cadavers were fixed in 10% of formalin (Wako Co., Ltd., Osaka, Japan) within 48 h after the death. Formalin was injected through the femoral artery using catheter. The dissection of the knee was performed within 12 months after the formalin fixation. The ethnicity of all cadavers was Japanese, and information on the age, sex and cause of death of the donor was available. All cadavers were donated mainly for the medical education. The average age of the cadavers was 78.3 ± 10.7 years, ranging from 52 to 103 years.

Dissection

Muscles inserted at the knee joint (biceps femoris, semimembranosus, sartorius, gracilis, popliteus, and gastrocnemius) were removed. Then, the vastus medialis, vastus lateralis, and vastus intermedius were cut approximately 5 cm above the patella and were reflected distally with the rectus femoris tendon. After the joint capsule was incised horizontally at its insertion to the anterior portion of the femur, the capsule and retinaculum patellae were incised longitudinally on both sides of the patella. Then the anterior cruciate ligament was resected, and the knee joint was opened and observed. If necessary, the medial collateral ligament or popliteus tendon were also removed.

After this procedure, the existence of crystal deposit and the area of deposition was evaluated and the cartilage degeneration was assessed by macroscopically visible findings.

Fibrous cartilage (medial and lateral meniscus) and hyaline cartilage (femoral and tibial condyle, and patello-femoral joint articular surface) were evaluated to detect deposition of crystals and degenerative changes.

Evaluation of cartilage degeneration

The depth of cartilage degeneration was determined using Outerbridge's classification[16]. Grading was as follows:

Grade 1: normal cartilage or softening and swelling of the cartilage.
Grade 2: partial-thickness defect which did not reach the subchondral bone and was less than 1.3 cm in diameter.
Grade 3: partial-thickness defect which did not reach the subchondral bone and was more than 1.3 cm in diameter.
Grade 4: exposed subchondral bone and visible reactive tissue formation.

When there were multiple lesions of different Outerbridge's classification grades, the sizes of the lesions were added up. Lesions with degenerative changes more severe than Outerbridge's classification grade 3 were regarded as OA lesions.

Compensated polarized light microscopy of CPPD crystals

Free bodies of crystals or scraped crystals from the cartilage surface were mounted on slides. A drop of 0.9% saline was added, and the crystals were examined with compensated polarizing light microscopy[17]. When crystals showed weak positive birefringent, and appeared as rhomboids, rectangles, or rods, with triclinic structures, they were identified as CPPD crystals[17]. Confirmation of the presence of CPPD crystals was performed by two observers.

Fourier transform infrared spectroscopy (FTIR) of CPPD crystals

Crystals were placed on Smart-Tech reflective slides and dehydrated by incubation at 37°C. FTIR spectra were documented using a VIR-9500 Portable FT-IR Spectrometer (JASCO Co. Ltd. Tokyo, Japan). The infrared absorption spectra obtained from the crystals in the specimens were compared with standard spectra of CPPD crystals[18,19]. In all cases, the identities of the crystals under FTIR analysis confirmed the light microscopy findings.

Statistical analysis

Data are presented as the mean ± standard deviation. Data were analyzed using SPSS software (SPSS for Windows version 19). Pearson's χ square test was performed to reveal the correlation between CPPD deposition and gender, and CPPD deposition and depth of cartilage degeneration (Degeneration over grade 3 was regarded as osteoarthritis positive). In the osteoarthritis positive subjects (Degeneration over grade 3), Student's t test was performed to reveal the correlation of age and CPPD deposition. A P-value of 0.05 or less was considered to be statistically significant.

Results

Prevalence of CPPD deposition

Deposition of CPPD crystals was observed in either the articular surface (hyaline cartilage) or meniscus (fibrous cartilages) in a total of 79 knees (13.0%) in 46 cadavers (33 bilateral, 13 unilateral) (Fig. 1. A: CPPD on articular surface. B: CPPD on meniscus). In all 79 knees, the characteristic morphological structure of the CPPD crystals was confirmed by compensated polarized light microscopy (Fig. 2). In addition, spectra of the crystals were obtained through FTIR analysis and compared with characteristic spectra of CPPD crystals, confirming the presence of CPPD deposition in all knees (Fig. 3). Analyzing in the prevalence of CPPD deposit by gender, deposit occurred in 21 male knees (6.3%) in 13 cadavers (8 bilateral, 5 unilateral), and 58 female knees (21.0%) in 33 cadavers (25 bilateral, 8 unilateral). The frequency of CPPD deposition was significantly higher in females than in males ($P < 0.001$).

Evaluation of cartilage degeneration in the femoro-tibial joint

Cartilage degeneration of the femoro-tibial joint was observed as follows. Grade 1: 176 knees (29%), Grade 2: 65 knees (11%), Grade 3: 148 knees (24%), Grade 4: 219 knees (36%). The prevalence of