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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

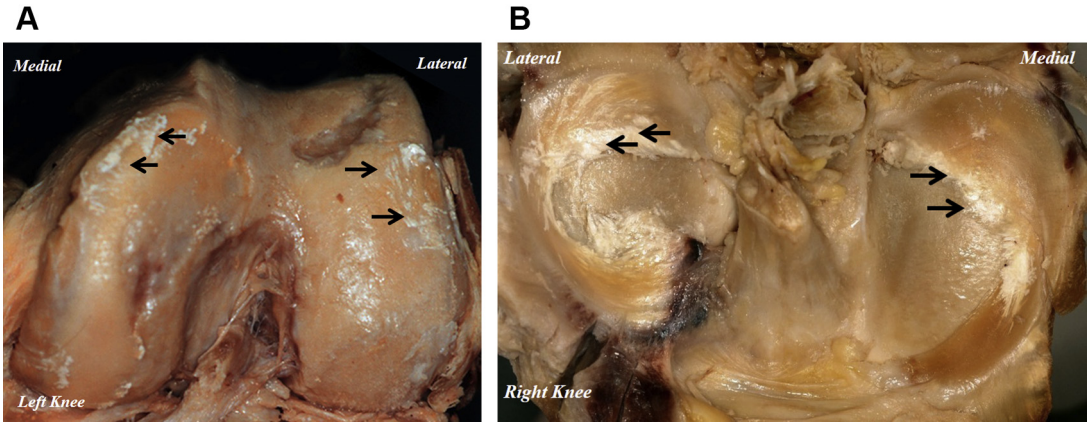

Fig. 1. CPPD deposition in the knee joint. Deposition of CPPD crystals was observed in either the articular surface (hyaline cartilage: A) or meniscus (fibrous cartilages: B).

CPPD deposition in OA-positive knees (Over grade 3) was 17.7% (65 knees out of 367 knees) and the prevalence of CPPD deposition in OA-negative knee was 5.8% (14 knees out of 241 knees). The prevalence of CPPD deposit was significantly higher in OA-positive knees than in OA-negative knees ($P < 0.001$).

In the OA-positive knees, the average age of CPPD deposition and CPPD negative subjects were, 86 ± 8.2, and 79.9 ± 9.6, respectively. The average age of CPPD deposition knees was significantly high ($P < 0.001$).

Discussion

The most important finding of this study was that the prevalence of CPPD deposition disease in the knee of the elderly subjects (13%) was revealed in a relatively large sample size of cadavers. Statistical analysis showed a significant correlation between CPPD occurrence in the knee joint and the subjects' age, gender (female), and depth of cartilage degeneration in the femoro-tibial joint.

The relationship between intra-articular deposition of CPPD crystal and arthritis was first described in 1962 by McCarty *et al.*[20] Following their report, several studies reported on the prevalence of CPPD deposition[2,4–6,8,10,21,22]. The reported prevalence of CPPD deposition disease varies widely 0.46–52.9%[2,4–6,8,10,21,22], and is dependent on the age or study design of the reports. McCarthy *et al.* also reported that CPPD crystal deposition disease was found in almost 50% of asymptomatic subjects older than 80 years of age[4]. These asymptomatic cases might be a cause of the wide variation in the prevalence of CPPD deposition disease reported previously. In order to reveal the accurate prevalence of CPPD deposition disease, including asymptomatic cases, a population based survey using radiography or a cadaveric assessment with a large sample size should be employed. There are few reports concerning the frequency of CPPD deposition identified in cadaveric knees[1]. Recently, Ramonda *et al.* reported a population based survey of chondrocalcinosis using radiographic evaluation[8]. In their study, 169 out of 1629 subjects had chondro-calcinosis. They suggested that female gender and age are both risk factors of chondro-calcinosis. However, radiographic assessment cannot differentiate between CPPD and other types of joint calcification. The European League Against Rheumatism (EULAR) recently suggested that ultrasonography is more sensitive and specific than radiography for the diagnosis of CPPD deposition disease[11]. However, a clinical study using ultrasonography for the diagnosis of CPPD deposition disease in a large sample size has not yet been reported. In this study, the prevalence of CPPD deposition disease in the elderly subjects was evaluated using a relatively large sample size of cadaveric knees. Moreover, the microscopic characteristics of visible crystals in all knee joints were evaluated, and FTIR analysis was done to confirm the presence of CPPD.

Recently, some authors have suggested a correlation between the occurrence of CPPD deposition disease and the progression of OA in the knee joint[6,7]. However, the nature of this correlation has

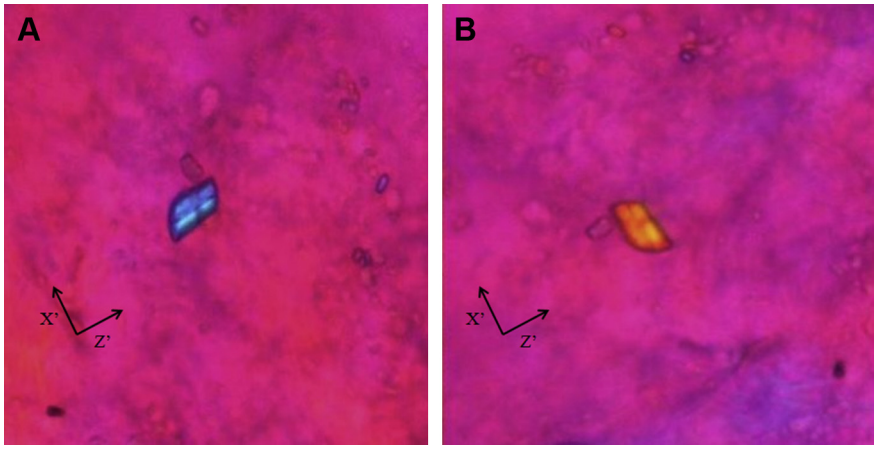

Fig. 2. Compensated polarizing light microscopic findings of CPPD crystal. CPPD crystal appears rhomboid-shaped with triclinic structures and positive birefringent. When the long axis of the crystal was parallel to the Z′ axis (parallel to the axis of the compensator), the color of the crystal was blue (A). When the long axis of the crystal was parallel to the X′ axis (perpendicular to the axis of the compensator), the color of the crystal was yellow.

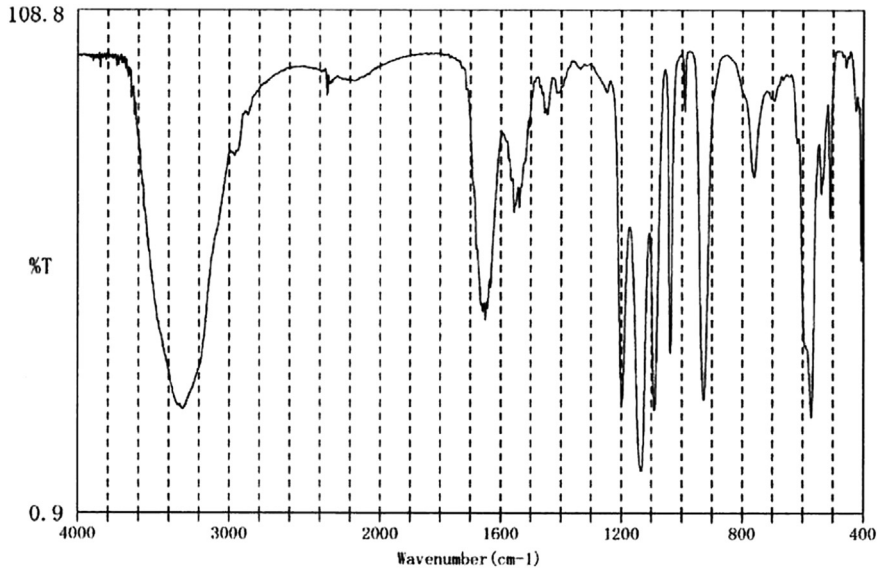

Fig. 3. Characteristic infrared absorption spectra of CPPD crystals. All crystals obtained from the specimens exhibited characteristic infrared absorption spectra under FTIR analysis and were confirmed as CPPD crystals.

not been well investigated. Even the distinction of whether OA drives pathologic cartilage calcification or whether cartilage calcification drives the progression of OA remains unclear[6]. Mitrovic *et al.* reported the prevalence of pathological calcification in knee articular cartilage by macroscopic and radiologic criteria in 130 consecutive autopsies[6]. They concluded that cartilage calcification correlates positively with age and OA severity. Mitsuyama *et al.* reported that the occurrence of CPPD deposition disease on the knee articular surface is correlated with the subjects' age only, not with the severity of knee OA[7].

In this study, statistical analysis showed that the CPPD deposition in the knee joint was significantly correlated with gender (female), age, and the depth of cartilage degeneration in the femoro-tibial joint. These results are useful for the clinical diagnosis of CPPD deposition disease.

The limitations of this study were (1) only knees of elderly subjects were included in this study. (2) only Japanese subjects were included in this study. Knee morphology might be influenced by differences in ethnicity, and therefore, knees of other ethnic groups should be evaluated in future studies. (3) radiological evaluation was not performed in this study. Although FTIR analysis was performed for all knees with crystal deposition and confirmed CPPD presence, radiological evaluation should be included in future studies. (4) no history of gout and other diseases of cadavers have been known in this study. In the method of this study, urate crystal might be regarded as CPPD crystal. This point should be addressed in the future plans.

For clinical relevance, female and elderly subjects, and subjects with severe OA in the femoro-tibial joint are groups with the highest risk of developing CPPD deposition disease in the knee joint.

Conclusion

This study reveals the prevalence of CPPD deposition in the knee of the elderly subjects (13%) using a relatively large sample size of cadaveric knees. Statistical analysis showed that the occurrence of CPPD deposition was correlated with the subjects' age, gender (female), and the severity of cartilage degeneration in the femoro-tibial joint.

Author contributions

Keinosuke Ryu, Yuki Kato, Masato Imada, Shin Aizawa, and Midori Oshida were contributed to the conception and design of this work and collection the data. Takanori Iriuchishima contributed to the analysis of the data, critical revision of the manuscript, and the drafting of the article. Professor Yasuaki Tokuhashi and Professor Junnosuke Ryu finally approved the manuscript.

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Conflict of interest

All authors have no potential conflicts of interest, including financial interests, activities, relationships, and affiliations, to disclose.

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