CASE REPORT

Accumulation and washout of 99mTc-sestamibi in osteoarthritic subchondral bone may indicate increased osteoclastic activity accompanying microfractures : a case study

Ryo Tanaka and Masato Ebine

Department of Medical Risk and Crisis Management, Faculty of Risk and Crisis Management, Chiba Institute of Science, 15-8 Shiomi-cho, Choshi, Chiba 288-0025, Japan

Abstract : We show for the first time that imaging of osteoarthritic subchondral bone with 99mTc-sestamibi (MIBI) may be a valuable diagnostic tool to assess the severity of subchondral sclerosis. This is a report on a patient who had osteoarthritis of both knees and where MIBI was accumulated in the lesion sites. Due to the accumulation of MIBI in the bone, we believe that excessive stress had been applied to the bone and microfractures had activated bone remodeling. Typically, MIBI accumulation is not observed in the bone, but MIBI is believed to exhibit high accumulation where there is an increased presence of osteoblasts and osteoclasts. Osteoclasts absorb calcium, consequently causing a depolarization of the mitochondrial membrane potential, which may decrease the ability to retain MIBI and enhance washout. Pathological examination of the tissues of this case confirmed an abundance of osteoclasts. This suggests that using MIBI may be useful for determining the increased presence of osteoblasts and osteoclasts. J. Med. Invest. 63 : 127-130, February, 2016

Keywords **:** *osteoarthritis of the knee, osteoblasts, osteoclasts, bone marrow edema, ^{99m}Tc-sestamibi*

INTRODUCTION

99mTc-sestamibi (MIBI) was originally developed as an agent for use in scintigraphy of myocardial blood flow. MIBI has also been previously used to visualize bone damage because MIBI spreads through the flow of blood and accumulates in cells, in a process that is dependent on the mitochondrial membrane potential (1, 2). About 90% or more of MIBI binds to the mitochondria (3), where it is retained for a relatively long time ; it has been confirmed that there is no evident redistribution phenomenon (4).

In addition to its use in determining myocardial blood flow, MIBI has also been reported to accumulate in locations such as benign tumors, areas of inflammation, granulomas, and scars (5-9). These reports, however, mainly address tissues including mammary glands and tumors ; accumulation in bone has not been reported.

It is difficult to visualize local microfractures that accompany osteoarthritis of the knee with radiographs, but the use of magnetic resonance imaging (MRI) or bone scintigraphy enables early diagnosis. The bone scintigram reflects bone metabolism, but it is thought that MIBI reflects increased vascular flow to the fracture region. However, MIBI has not been used previously to visualize bone damage.

We hereby report, along with a discussion of some of the literature, on a case where early- and late-stage MIBI images were helpful in ascertaining the clinical condition of osteoarthritis of the knee.

Received for publication July 15, 2015 ; accepted August 28, 2015.

Address correspondence and reprint requests to Ryo Tanaka Department of Medical Risk and Crisis Management, Faculty of Risk and Crisis Management, Chiba Institute of Science 15-8 Shiomi-cho, Choshi, Chiba 288-0025, Japan and Fax : +81-479-30-4741.

CASE REPORT

A 71-year-old man had been experiencing pain in both knees for 1 year. One month previously, the pain intensified, and the pain in his left knee during walking became so bad as to make walking difficult, so he visited our hospital.

Plain radiography and computed tomography (CT ; Fig. 1) findings showed narrowing of the medial joint space and the appearance of osteophytes. The patient was subsequently diagnosed with osteoarthritis of both knees and underwent left knee joint replacement surgery.

Scintigraphy of the lower leg was performed in a preoperative nuclear medicine examination. We explained the exposure level of MIBI to the patient, obtained written informed consent with a full understanding of the study based on ethical rules, and carried out the study. In MIBI scintigraphy imaging, intravenous injection of 740 Mq was followed by capturing of planar early-stage images (after 1 hour) and delayed-stage images (after 4 hours) ; in the early-stage images, single-photon emission CT imaging was also performed.

The early MIBI images showed a high accumulation from the medial side of the distal left femur to a medial site in the proximal tibia. The measured radioactive isotope count for a region of interest (ROI) set to the high-accumulation portion of the patient's left knee was 17.8 counts in the early images and 6.6 counts in the delayed images, representing a washout rate of 62.7%. The measured ROI count for an ROI set to the left quadriceps muscle, however, was 11.9 counts in the early images and 8.6 counts in the delayed images, representing a washout rate of 28.0% (Fig. 2).

Postoperative pathological findings showed that the medial and lateral portions of the left tibia and femur all had fuzzing of the articular cartilage, loss of articular cartilage, and eburnation ; this was accompanied by thickening of the subchondral trabecular bone and cyst formation. In tartrate-resistant acid phosphatase staining, the formation of cysts under the cartilage was noticeable on the medial side of the femur and tibia, and the trabecular bone

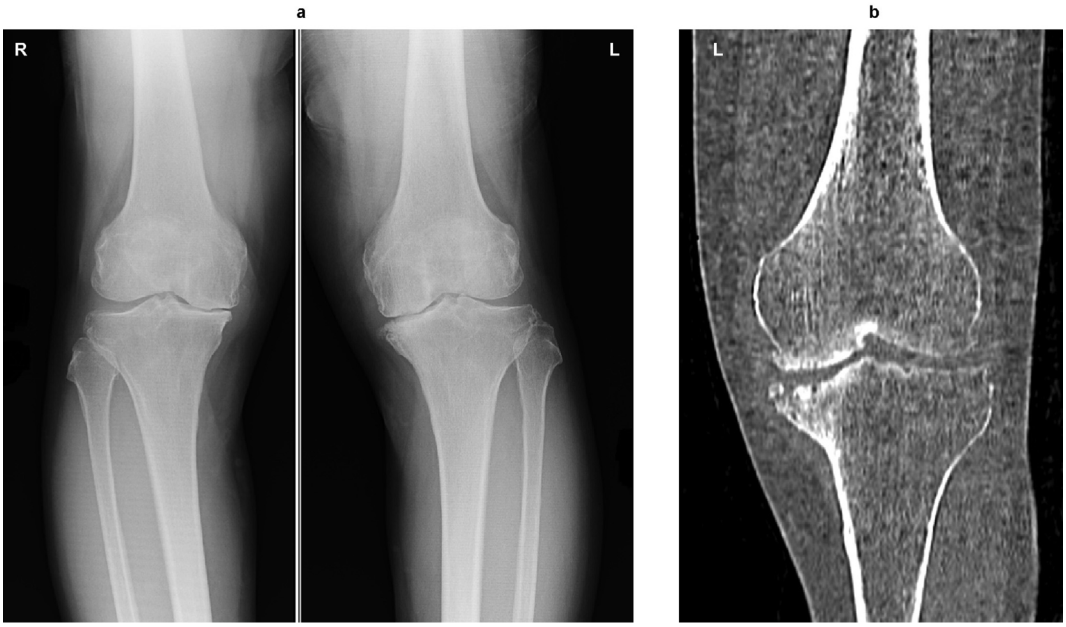

Figure 1
a : Bilateral knee radiographs ; b : left knee computed tomography image

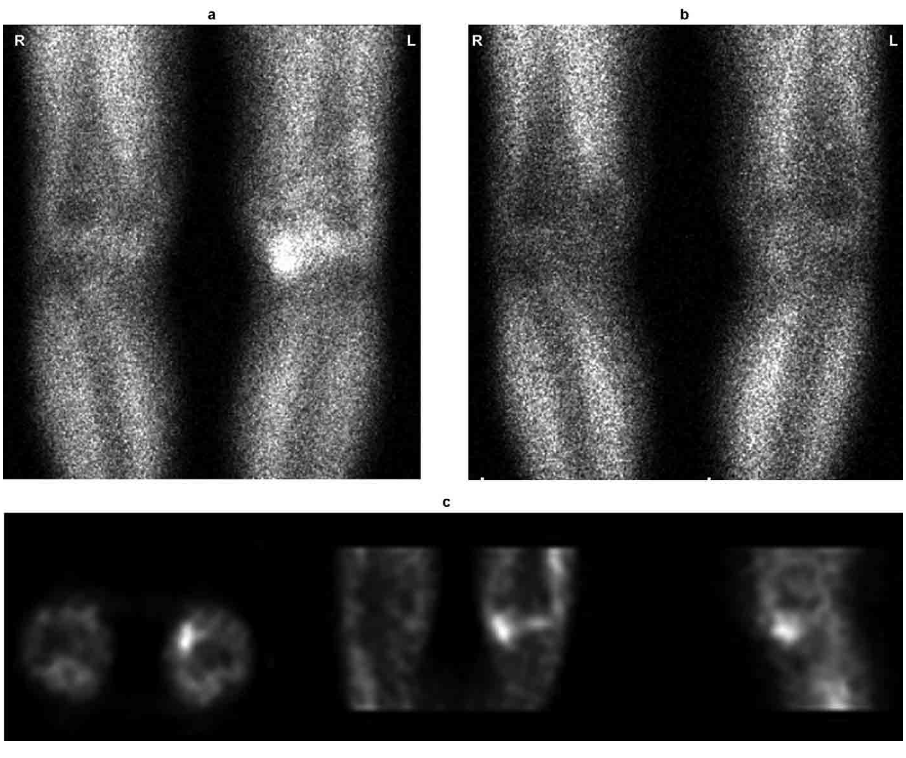

Figure 2
a : Early-stage image at 1 hour after intravenous 99mTc-sestamibi (MIBI) ; b : delayed-stage image at 4 hours after intravenous MIBI ; c : MIBI single-photon emission computed tomography image (early-stage image)

surrounding the cysts exhibited increased activity of osteoblasts and osteoclasts (Fig. 3).

DISCUSSION

Osteoarthritis of the knee occurs when long-term wear of the knee cartilage and meniscus causes osteophytes to form, which intensifies deformation of the joint and leads to changes in the

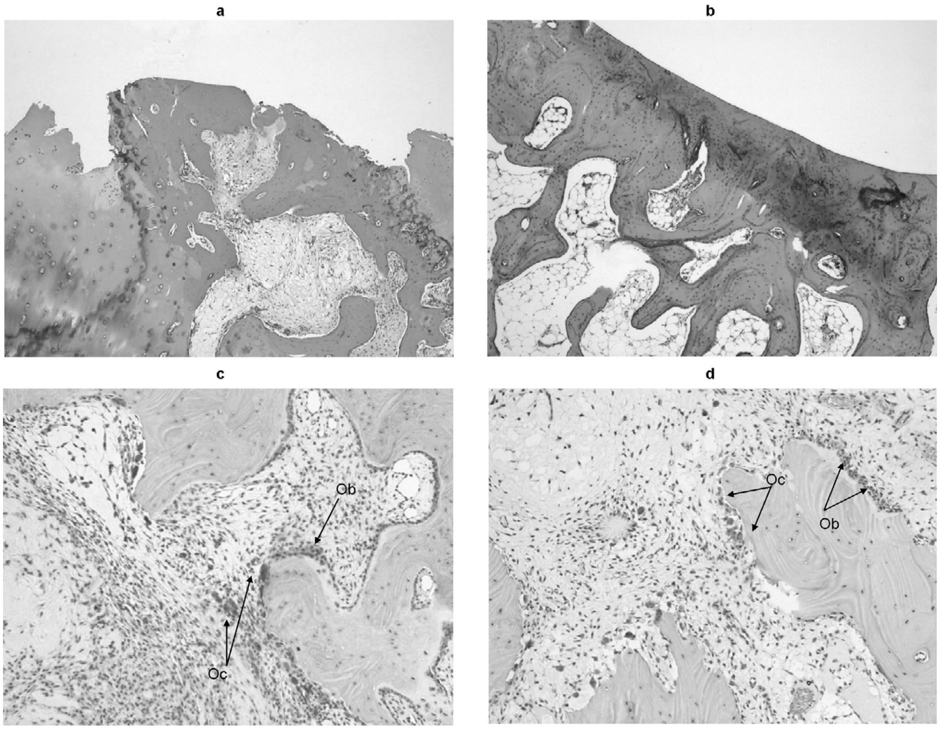

Figure 3
Pathological findings. a : Lateral tibia : cartilage loss ; b : lateral tibia : eburnation ; c : medial tibia tartrate-resistant acid phosphatase (TRAP) staining : increased osteoblasts and osteoclasts ; d : medial femur TRAP staining : increased osteoblasts and osteoclasts

subchondral bone tissue. Radin *et al.* (10) stated that structural changes in the subchondral bone tissue produced thickening of the trabecular bone due to microfractures, leading to osteoarthritis of the knee.

The early stages of osteoarthritis of the knee often show wear of the articular cartilage and meniscal tearing ; bone marrow edema of the subchondral bone has been reported as being correlated with pain, the extent of cartilage damage, and prognosis (11). Arnoldi *et al.* (12) have also reported that venous stasis and elevated venous pressure in the subchondral bone tissue are closely related to pain at rest. In the present case, as well, the patient had a repeated history of locally applied direct external force, which is believed to have generated microfractures that could not be distinguished in radiographs or CT images. In terms of symptoms, the patient experienced pain on the medial side of the knee joint, and it is reasonable to believe that bone marrow edema occurred, owing to reactive changes associated with synovitis. MRI is superior to CT in depiction of the bone narrow edema, but it is difficult to identify the region of the microfracture. However, MIBI accumulates in the microfracture region and may be more useful in early diagnosis than MRI.

Christensen *et al.* (13) have reported that with osteoarthritis of the knee, a high accumulation of 99mTc-MDP is detected in bone scintigraphy at sites where new bone formation is underway, especially the subchondral tissue and osteophytes.

Typically, intracellular accumulation of MIBI is dependent on mitochondrial function, but there are no reports regarding where it accumulates in bone cells. The present case exhibited high accumulation in early MIBI images at the medial part of the left knee joint, where bone deformation was observed in CT findings. Postoperative bone pathology findings showed osteoblast and osteoclast proliferation on the medial sides of the femur and tibia. A bone contusion associated with the progression of osteoarthritis is believed to have caused the proliferation of osteoblasts and osteoclasts associated with bone remodeling. As they differentiate into osteoblasts, osteoprogenitor cells have increased mitochondrial biosynthesis and activity, and mitochondria have been shown to be abundant in osteoclasts (14). Therefore, MIBI is understood to have accumulated in the mitochondria of the increased osteoblasts and osteoclasts. In addition, MIBI accumulates depending on mitochondrial membrane potential. Therefore, osteoclasts with a high energy demand have a greater mitochondrial membrane potential ; thus, they would accumulate MIBI more abundantly. In contrast, other bone cells have poor mitochondrial quantity and accumulation of MIBI with a gamma camera cannot be imaged.

According to the report from Piwnica *et al.* (2), increased MIBI accumulation was observed in an enhancement test of uptake kinetics using lipophilic anions, and a mitochondrial uncoupling agent produced a decrease in accumulation of the myocardial mitochondrial fraction. Crane *et al.* (15) reported in a study of the MIBI myocardial retention mechanism that the result is a mitochondrial calcium "overload" and loss of mitochondrial metabolic function. In light of these results, it is believed that MIBI accumulation is also dependent on metabolic status, that transport by cationic channels is not involved, but that mitochondrial membrane potential plays an important role (16, 17). An abnormality in the mitochondrial membrane potential is implicated as participating in the enhanced washout between early- and delayed-stage images, due to the decreased MIBI-retaining ability.

Osteoblasts survive for 3 months in vivo, whereas osteoclasts are short-lived cells that experience apoptosis and die in about two weeks (18). In one proposed idea, calcium produced when osteoclasts engage in bone resorption is wrapped in a vesicle and transported from the apical side to the basolateral side (19). The osteoclastic system of calcium ion inflow to the mitochondria is known to involve co-transport dependent on the mitochondrial respiratory chain ; this pathway operates via sodium-calcium exchange across the inner mitochondrial membrane or through a pathway into the mitochondria via MF2, which forms a bridge between the endoplasmic reticulum and the mitochondria. Moreover, the mitochondria have reportedly been observed to exert a buffer effect (20). Therefore, it is believed that osteoclasts have mitochondrial uptake of calcium, which causes a depolarization of the mitochondrial membrane potential, and that this affects the MIBI-retaining ability. In other words, the results suggest the presence of osteoclasts at sites where MIBI washout is enhanced in osteoarthritis of the knee.

This report represents the first case of MIBI accumulation in osteoarthritis of the knee and suggests that using MIBI may be useful to detect the presence of osteoblasts and osteoclasts. Furthermore, this method may detect osteoarthritic lesions at stages earlier than can be detected by joint space narrowing. We also feel that the extent of MIBI accumulation would be an indicator for ascertaining the severity of microfractures.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

1. Piwnica-Worms D, Kronauge JF, Chiu ML : Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence. Circulation 82 : 1826-38, 1990
2. Piwnica-Worms D, Kronauge JF, Chiu ML : Enhancement by tetraphenylborate of technetium-99m-MIBI uptake kinetics and accumulation in cultured chick myocardial cells. J Nucl Med 32 : 1992-1999, 1991
3. Carvalho PA, Chiu ML, Kronauge JF, Kawamura M, Jones AG, Holman BL, Piwnica-Worms D : Subcellular distribution and analysis of technetium-99m-mibi in isolated perfused rat hearts. J Nucl Med 32 : 1516-1521, 1992
4. Okada RD, Glover D, Gaffney T, Williams S : Myocardial kinetics of technetium-99m-hexakis-2-methoxy-2-methylpropyl-isonitrile. Circulation 77 : 491-8, 1988
5. Mekhmandarov S, Sandbank J, Cohen M, Lelcuk S, Lubin E : Technetium-99m-MIBI scintimammography in palpable and nonpalpable breast lesions. J Nucl Med 39 : 86-91, 1998
6. Khalkhali I, Cutrone J, Mena I, Diggles L, Venegas R, Vargas H, Jackson B, Klein S : Technetium-99m-sestamibi scintimammography of breast lesions : clinical and pathological follow-up. J Nucl Med 36 : 1784-1789, 1995
7. Lu G, Shih WJ, Huang HY, Long MQ, Sun Q, Liu YH, Chou C : 99Tcm-MIBI mammoscintigraphy of breast masses : early and delayed imaging. Nucl Med Commun 16 : 150-156, 1995
8. Ohta H, Ichii H, Arimoto A, Ukikusa M, Awane H : Tc-99m sestamibi uptake in tuberculosis of the breast. Clin Nucl Med 23 : 106-108, 1998
9. Ohta H, Komibuchi T, Nishio T, Kitai T, Yamamoto S, Ukikusa M, Awane H, Irie K, Shintaku M : Technetium-99m-sestamibi scintimammography of benign and malignant phyllodes tumors. Ann Nucl Med 11 : 37-39, 1997
10. Radin EL, Parker HG, Pugh JW, Steinberg RS, Paul IL, Rose RM : Response of joints to impact loading. 3. Relationship between trabecular microfractures and cartilage degeneration. J Biomech 6 : 51-57, 1973
11. Wen DY, Propeck T, Kane SM, Godbee MT, Rall KL : MRI description of knee medial collateral ligament abnormalities in the absence of trauma : edema related to osteoarthritis and medial meniscal tears. Magn Reson Imaging 25 : 209-214, 2007
12. Arnoldi CC, Lemperg K, Linderholm H : Intraosseous hypertension and pain in the knee. J Bone Joint Surg Br 57 : 360-363, 1975
13. Christensen SB, Arnold CC : Distribution of 99mTc-phosphate compounds in osteoarthritic femoral heads. J Bone Joint Surg Am 62 : 90-96, 1980
14. Miyazaki T, Neff L, Tanaka S, Horne WC, Baron R : Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. J Cell Biol 160 : 709-718, 2003
15. Crane P, Laliberte R, Heminway S, Thoolen M, Orlandi C : Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. Eur J Nucl Med 20 : 20-25, 1993
16. Platts E, North T, Pickett R, Kelly J : Mechanism of uptake of technetium-tetrofosmin. I : Uptake into isolated adult rat ventricular myocytes and subcellular localization. J Nucl Cardiol 2 : 317-326, 1995
17. Younes A, Songadele J, Maublant J, Platts E, Pickett R, Veyre A : Mechanism of uptake technetium-tetrofosmin. II : Uptake into isolated adult rat heart mitochondria. J Nucl Cardiol 2 : 327-333, 1995
18. Suzuki Y : Role of osteoclasts in bone destruction of rheumatoid arthritis. Jpn J Clin Immun 24 : 87-97, 2001
19. Salo J, Lehenkari P, Mulari M, Metsikkö K, Väänänen HK : Removal of osteoclast bone resorption products by transcytosis. Science 276 : 270-273, 1997
20. Kawahara I, Koide M, Tadokoro O, Udagawa N, Nakamura H, Takahashi N, Ozawa H : The relationship between calcium accumulation in osteoclast mitochondrial granules and bone resorption. Bone 45 : 980-986, 2009