

An Experimental Study for Minimum Level of Decalcification to Detect the Osteolytic Bone Metastasis of Long Bone on Plain Radiography

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Background: In 1951, Ardran reported that metastatic bone lesions could be detectable on plain radiography with 30% to 50% of decalcification. Authors performed experimental study for minimum level of decalcification to detect the osteolytic bone metastasis of long bone with recent technique of radiographs. **Methods:** One pair of fibula and humerus from two cadavers was cut into specimen 1 inch in length. Distal half of specimen was dipped into hydrochloride (HCl) with 15 min interval. All 16 specimens were checked by film-type radiography (FR), computed radiography (CR), digital radiography (DR). To exclude inter-observer's variance, 3 radiologists evaluated images. Calcium amount before and after decalcification was measured and expressed in percentage of decalcification. **Results:** Osteolytic changes were detectable with 11% to 16% of decalcification for fibula and 3% to 8% for humerus on plain radiography with FR, CR, and DR. **Conclusions:** Our study showed that minimum of 3% and maximum of 16% of decalcification is necessary when osteolytic metastatic bone lesions of long bone could be detected on plain radiography.

Key Words: Bone metastasis, Decalcification technique, Osteolysis, Radiography

INTRODUCTION

Skeletal system is the most common metastatic site for malignant tumor, next to lung and liver.[1] In a case where actual post mortem was conducted, the rate of bone metastasis was found to be much higher than the bone metastasis identified using radiology or bone scan.[2] Since the malignant tumor cells metastasized to the skeletal system directly and indirectly irritates the activity of osteoclasts,[3] most of them are osteolytic.[4] Such osteolytic bone metastatic cancer is found as osteolytic lesion in anatomical images including plain radiography, computed tomography (CT), magnetic resonance imaging (MRI), etc.[5] Although functional imaging techniques such as bone scan and positron emission tomography (PET) are more effective in detecting osteolytic bone metastatic cancer and possible to discover it early, the methods of observing the osteolytic lesions of bone metastatic cancer in the anatomical images are still the most valid examination techniques widely used.[5,6] A number of techniques are used now with the

Table 1. Utility of radiographs for bone metastatic cancers

Application of radiological diagnosis
Clarification and differentiation of bone lesions in tumor patients
Detection of bone and bone marrow metastases within the scope of tumor staging and screening
Early detection of possibly threatening complications
Evaluation of stability
Spinal cord compression
Nerve and vessel compression
Differentiation of conspicuous findings of scintigraphy
Control of disease progression and evaluation of therapy success

development of imaging technology, but the plain radiography provides the most important general as well as specific lesion information on the detection of bone metastatic cancer and the degree of the bone destruction (Table 1).[6]

The plain radiography for the skeletal system can be divided into 3 types depending on method of acquiring images.

The first is existing film-type radiography (FR). This can be obtained like a photographic film with black-and-white images using projected radiation with chemical reaction. Researchers named it 'film-type radiography', and marked it as FR.

Second, there is computed radiography (CR) type which composes images by processing digital image obtained from the radiation with CR.

The third type is digital radiography (DR), which obtains the image of radiation cassette that absorbs projected radiation through digital visualization. This method is the most advanced radiography, but it is known that the method has no big difference in resolution from CR.

Likewise, the plain radiography has been much more improved due to the development of digital technology, and the type is rapidly converted from FR to CR or DR. A number of domestic and overseas medical care institutions already use CR or DR-type radiography; FR is still widely used, but it is expected that it will be eventually replaced with CR or DR. Nevertheless, studies on the minimum decalcification rate to detect osteolytic bone metastatic cancer in the plain radiography have shown that the osteolytic bone metastatic cancer in long bone could be discovered in plain radiography, more accurately, FR, only if about 30% to 50% of decalcification should be progressed in the lesion based on the study of Ardran [7] which was conducted in 1951, far before the introduction of CR or DR.

There was no additional studies after that.

Therefore, authors performed an experimental study using cadaver on what level decalcification should be progressed to detect osteolytic bone metastatic cancer in long bone in 3 types of plain radiography including FR, CR and DR. To measure the minimum decalcification to detect osteolytic bone metastatic cancer in long bone in plain radiography, authors conducted simulation of osteolytic bone metastatic lesion of long bone with experimental decalcification using cadaver bones.

METHODS

1. Sample preparations

One pair of fibula and humerus from two cadavers without history of bone metabolic diseases or metastatic cancers was cut into specimen of 3 cm and 2 cm in length, respectively. After dividing into 6 samples of fibula and 10 samples of humerus excluding the articular cartilage, the soft tissue was removed (Fig. 1A, B).

To simulate the environment in vivo in the fibula, all samples were surrounded by thick pork with fat layers. However, in the humerus experiment conducted later, only the humerus itself was taken to the radiography because it was found that the in vivo environment simulation using pork in fibula deteriorated resolution of images due to a number of air gaps occurred between pork surrounding the sample, and there was no big difference from the image only taking the samples.

2. Decalcification

To decalcify only 1/2 of the sample, the non-decalcified site was surrounded by experimental film (Parafilm; Pechiney Plastic Packaging, Chicago, IL, USA), the sample was hung with a string, and only 1/2 of specimen was dipped into hydrochloride (HCl; Duksan Pharmaceutical Industrial Co., Ansan, Korea). The partial decalcification was conducted as a group with 7 specimen of fibula in 1N HCl of weight (g) per 20 mL in the room temperature at 30, 60, 90, 120, 150, and 180 min; the partial decalcification was performed by dividing into 2 groups of 10 specimens of humerus at 15, 30, 45, 60, and 75 min (Fig. 2). After the decalcification was finished, the experimental film was removed, and the specimen was cleansed with the running distilled water to remove HCl in the bone. Then, to remove the residual HCl, specimen was dipped in phosphate

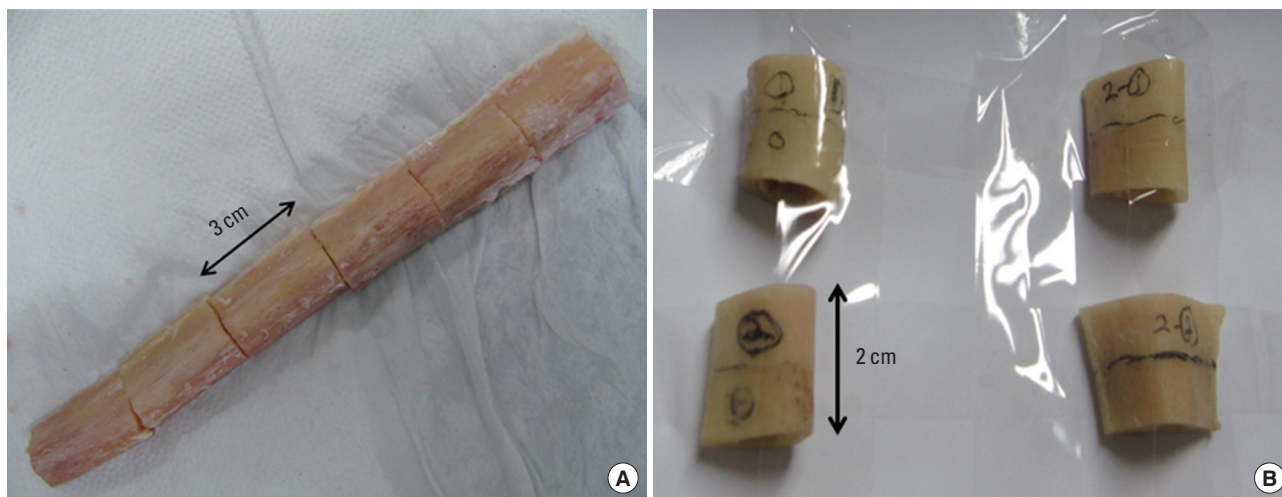


Fig. 1. (A) A cadaveric right fibula shaft was divided into 6 specimens with 3 cm in length. (B) A pair of cadaveric humeral shaft were divided into 10 specimens with 2 cm in length.

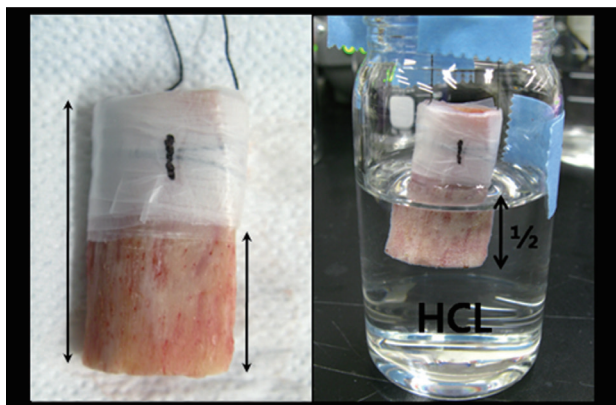


Fig. 2. Distal half of each specimen was immersed into 1 N HCl for decalcification process; 30, 60, 90, 120, 150, 180 min for fibula, 15, 30, 45, 60, 75 min for humerus. Non-decalcified area was covered by Parafilm.

buffered saline (PBS) sufficiently, and cryopreserved at -30°C until conducting the 3 types of plain radiography.

3. Radiological examination

For all specimen after decalcification, fibula was taken to FR image (Dong Kang Medical, Seoul, Korea), and CR image (Siemens, Berlin, Germany), and DR image (Dongyang Medical, Seoul, Korea). Humerus was only taken to CR and DR.

Considering the intra-observers' variance and inter-observer's variance, the plain radiography data of all specimen were mixed with random orders and provided to 3 professors of radiology majoring in the skeletal system from different university hospital, and the minimum decalcification

level that can be identifiable was determined based on the readings. The plain radiographs were stored through the picture archiving and communication system (PACS), and they were sent to the above 3 radiologists for remote reading an online using massive data transmission method.

4. Analysis of calcium amount

The both ends of undecalcified and decalcified parts of the specimen of which radiography was completed were cut using a hacksaw, and bone tissues of size of a grain of rice were collected. Then, the amount of calcium contained in the bone tissues was measured using ICP spectrophotometer (Thermo Scientific, Franklin, MA, USA) after pre-treatment, and then the measured values were converted to investigate the level of decalcification. Finally, they were expressed in % units. After degrading it with a microwave by adding HNO_3 4 mL and H_2O_2 1.5 mL, they were diluted with 2% to 3% HNO_3 and liquidated. After that, the specimen was analyzed.

RESULTS

1. Calcium analysis

The results obtained from the analyzer were calculated into the converted values considering the mass compared to weight. In fibula, the values of decalcified bones from 6 specimens based on undecalcified bones (100%) showed about 5.0% of mean decalcification increase in proportional to addition of decalcification time by 30 min (Fig. 3A).

In 10 specimens of humerus based on undecalcified bones (100%) showed about 3.3% of mean decalcification increase in proportional to addition of decalcification time by 15 min (Fig. 3B).

2. Analysis of plain radiography FR, CR, and DR

The analysis of radiographs was requested to 3 radiologists majoring in the skeletal system in 3 domestic university hospitals regarding FR, CR, and DR types.

In fibula, 30 min (1 professor), 60 min (2 professors), that

is, when the specimens were decalcified 11% and 16%, osteolytic bone metastatic lesion was detected in the CR and DR plain radiography (Fig. 4A, B, Table 2). In humerus, when calculating based on mean increase rate of decalcification, from 3% to 8% of decalcification, which was decalcification of 15 min, the osteolytic lesion different from normal bones was detectable in FR, CR, and DR radiographs (Fig. 5A-C, Table 2). In addition, CR image was slightly clearer than DR image, but there was no difference by decalcification time in readings.

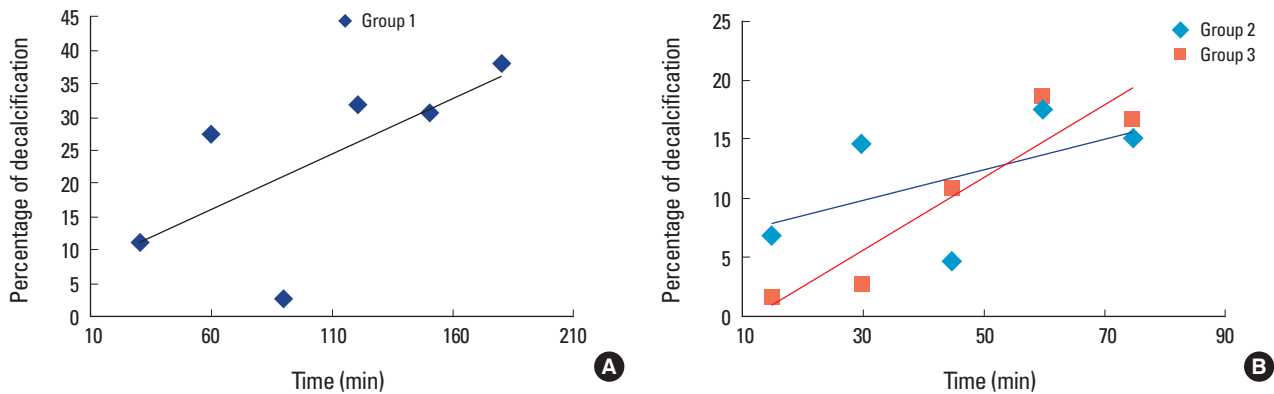


Fig. 3. (A) Rate (%) of decalcification with time (min) for group 1 (fibula). (B) Rate (%) of decalcification with time (min) for group 2, group 3 (humerus).

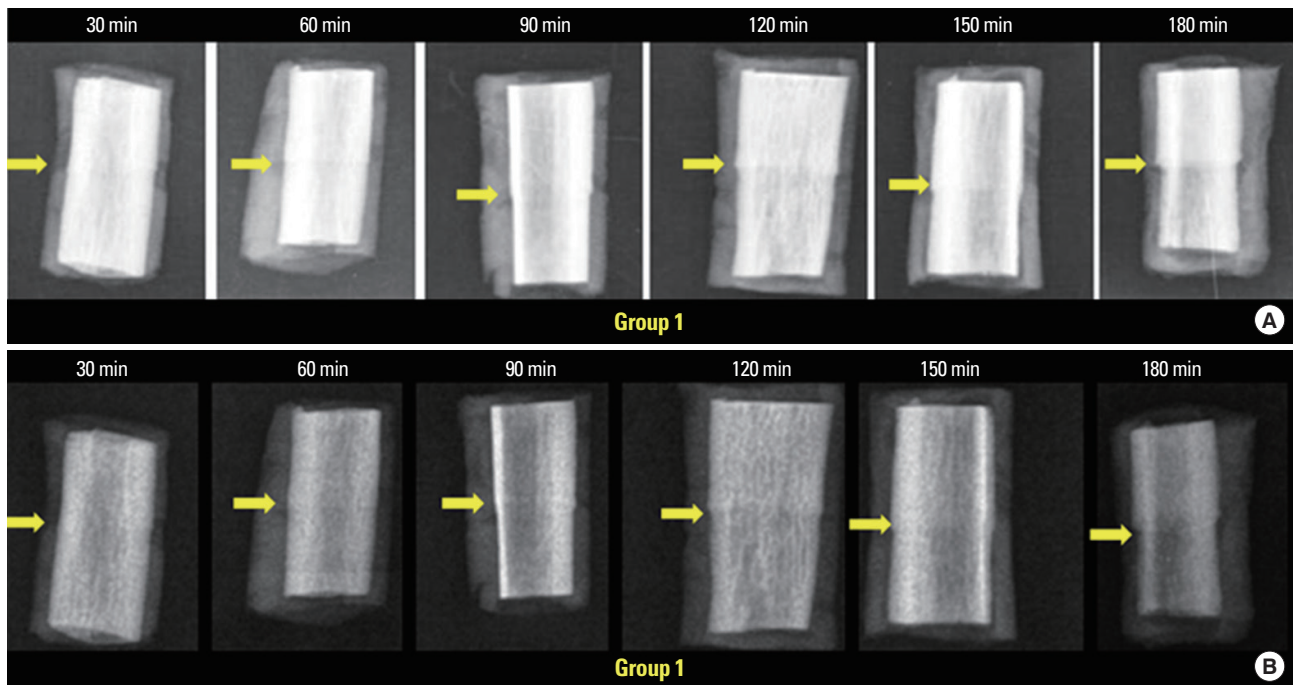


Fig. 4. (A) Serial radiographs of computed radiography showed gradual progression of decalcification with time (min) in fibula. (B) Serial radiographs of digital radiography showed gradual progression of decalcification with time (min) in fibula.

Table 2. Minimal time of immersion to detect the decalcification

Group	Minimal time of immersion to detect the decalcification (min)								
	FR			CR			DR		
	A	B	C	A	B	C	A	B	C
Group1 (fibula)	-	-	-	60	30	60	60	30	60
Group2 (humerus)	15	15	15	15	15	15	15	15	15
Group3 (humerus)	15	15	15	15	15	15	15	15	15

FR, film-type radiography; CR, computed radiography; DR, digital radiography.

DISCUSSION

The skeletal system is the most frequent metastatic site for malignant tumor next to lung and liver.[8] The malignant tumor metastasized to the skeletal system most commonly occurs in the spine, and it is known that metastasis does not frequently occur in the elbow joint of long bone and distal knee joint.[9] Therefore, authors conducted an experiment for cadaver fibula in which the osteolytic bone metastasis is rare but cortical bone is very thick and almost no spongy bone is found in the bone-marrow cavity and humerus in which bone metastasis is common. Then, the fibula and humerus were compared and analyzed.

It is known that the case in which a malignant tumor is metastasized to bones is more frequently detected in the direct post mortem than the regions detected using radiation or bone scan.[2] Excluding some cancers such as prostate cancer, almost all metastatic cancers are osteolytic, because the malignant tumor cells metastasized to bones directly and indirectly stimulates the activity of osteoclast. [4] These osteolytic metastatic cancers can be detected by anatomical imaging diagnostic devices such as plain radiography, CT, and MRI.[5] Functional imaging techniques such as bone scan or PET are more effective. Nevertheless, the methods of observing the osteolytic lesion of metastatic cancers in anatomical images are the definite methods that are still widely used, and they are the most valid examination method to establish therapeutic decision including surgery, etc.

It is often difficult to detect the bone metastatic cancer of spine or pelvis early only with the plain radiography, but the long bone is relatively easy to be detected only with the plain radiography. Especially, in the long bone, the find-

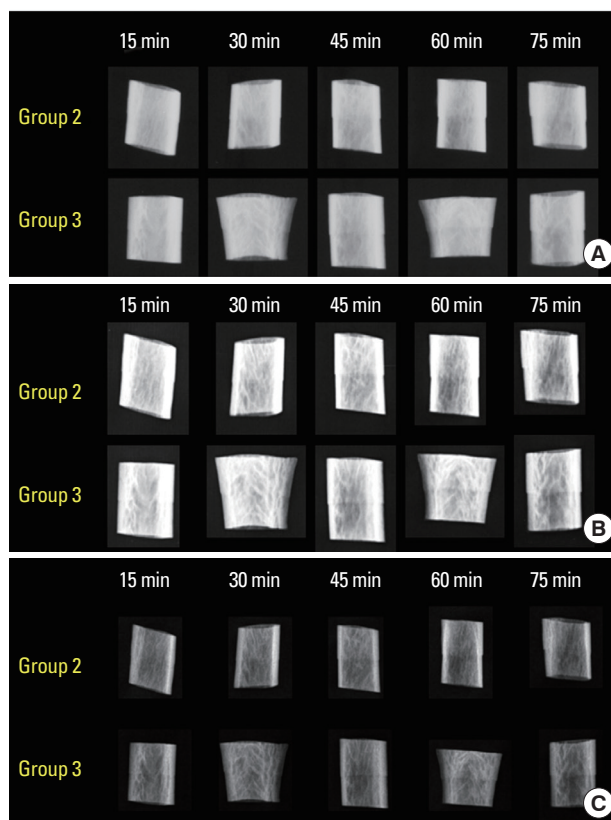


Fig. 5. (A) Serial radiographs of film-type radiography showed gradual progression of decalcification with time (min) in humerus. (B) Serial radiographs of computed radiography showed gradual progression of decalcification with time (min) in humerus. (C) Serial radiographs of digital radiography showed gradual progression of decalcification with time (min) in humerus.

ings of plain radiography for the range of osteoclasia is the most important criterion to determine prevention and treatment of pathological fractures and aggressive intervention surgery, etc. for improvement of quality of life of patients; they play critical role in establishing the therapeutic decision though there are no additional expensive imaging diagnoses such as CT or MRI. Considering the quality of images compared to other imaging techniques, it has a weakness that the sensitivity is relatively low [6] but the plain radiography of long bone is still the most important and fundamental imaging data for osteolytic bone metastatic cancer as well as the essential examination method (Table 1).

CT and MRI can show longitudinal section or cross section and have much better resolution than the plain radiography. However, due to expensiveness and a relatively large radiation exposures of CT, all sites suspected for bone metastatic cancer cannot be examined by CT or MRI. Even-

tually, if metastatic lesion in skeletal system is suspected in functional images such as bone scan or PET, all sites should be examined by the plain radiography to have the primary identification; additional identification through CT or MRI is required only in the undefined case or the case in which influential range of the lesion needs to be examined in more details. This is the established imaging approach for bone metastatic cancers.

In the past, almost all plain radiography types were FR. However, due to the development of digital technology, CR and DR radiographies were developed, and resolution, storage, and period were significantly improved as well. A large number of domestic and overseas medical institutions use CR and DR plain radiographies, and FR is still the method most widely used in the world. In Korea which has the best level of digital technology, most hospitals and clinics already use CR or DR techniques, and FR that is also used in some places is expected to be replaced with CR or DR technology.

Despite the reality, studies on the minimum level of decalcification to detect osteolytic bone metastatic cancer of long bone in radiography showed that the osteolytic bone metastatic cancer can be detected in plain radiography, or more accurately, FR image, only if about 30% to 50% of decalcification is progressed in the lesion, based on the result of Ardran [7]. Even FR images now have much better resolution due to the improvement of generation technology of radiation beam and focusing method, improvement of film-type cassette, etc. New types of plain radiographies were developed including CR and DR, but since Ardran in 1951, there have been no additional studies on the minimum level of decalcification to detect the osteolytic metastatic cancer of long bone in the plain radiography. Therefore, this study was conducted to verify the existing result of 30% to 50% of the minimum level of decalcification to detect the osteolytic bone metastatic cancer site in long bone which has been continuously cited without follow-up studies for last 60 years with the improved radiographic technology.

As a result, when requesting to 3 radiologists majoring in musculoskeletal system in different domestic university hospitals, all of the readings of the 3 professors showed that the specimens of fibula could be distinguished from undecalcified bones in CR and DR when they were decalcified for 30 min (1 radiologist) and 60 min (2 radiologists)

(11% and 16%) in (Fig. 3A, Table 2), and the specimens of humerus could be distinguished from undecalcified bones from when they were decalcified for 15 min (3%-8%) (Fig. 3B, Table 2) in CR and DR images. In addition, DR images seemed slightly clearer than CR images, but there was no difference in readings by decalcification time (Fig. 5A-C).

It is assumed that there were slight increase and decrease in calcium measured values because bone marrow and fat could not be made in the exactly same way in the process of removing soft tissues before decalcification though fibula and humerus specimens were prepared as symmetric sites, and the distribution of spongy bone within the bone-marrow cavity could not be identical. In addition, unlike humerus, 11% and 16% of decalcification was progressed in fibula region, they could be identified in CR and DR images. It is assumed that, when considering the cortical bone of fibula is very big and thick, and that there was almost no spongy bone in the bone-marrow cavity, humerus can be identified in the plain radiography with the level of 3% to 8% of decalcification which was much lower because humerus in which bone metastatic cancer can often occur has bigger diameter and sufficient spongy bone in the bone-marrow cavity.

The existing FR image has disadvantages that it has long waiting time after examination to film development, low reproducibility, and visual communication is impossible, compared to CR and DR techniques. On the other hand, CR and DR have advantages that they can obtain images after the inspection in a few seconds and conduct visual communication, so they can be read in a remote place in real time.[10]

Generally, CR is conducted using imaging plate (IP), a radiate out phosphor, instead of existing imaging film. When the laser beam is injected to the examination information accumulated in the IP, the image information can be obtained. The analogue signals obtained here is converted into digital signals in photomultiplier tube and imaging-processed. After that, they can be printed using a laser printer or stored in an auxiliary storage such as an optical disk or in imaging storage and system. CR needs to go through converting process once, whereas DR has advantages that it does not go through a separate conversion because it is able to recognize the signals themselves in digital method after the radiation is exposed, and it can be transmitted and stored through network.[1,11-13] However, according

to a number of studies, when compared to recent CR, DR is not that superior in work-flow organization and quality of images. Also, the research staff and 3 radiologists majoring in skeletal and muscular system who were requested for observation felt that DR images were slightly clear when distinguishing decalcified and undecalcified parts, but there was no particular difference in the resolution or quality of images for actual readings.

The limitation of this study is that, since it simulated that the osteolytic bone metastatic cancer occurred only in fibula and humerus among long bones, femur was excluded. Also, in fibula, thick pork was surrounded in many folds to present the situation in which long bone is surrounded by soft tissue *in vivo*, but the result was the image was cloudy due to air gap between surrounding pork, and the simulation in which pork is surrounded itself was not variance to distinguish the sites of decalcification. Therefore, the experiment for humerus was conducted with the plain radiography for the long bone itself. However, there remains still a possibility that the radiograph would not be as clear as in this experiment due to thick soft tissue of arms-legs *in vivo*.

In addition, since the phenomenon in which the osteolytic bone metastatic cancer destroys bones is a more complex reaction of uptake or osteolysis of actual bone tissue itself than decalcification, there is a limit when osteolytic bone metastatic cancer simulates the osteoclasia *in vivo* with simple decalcification of the bones. However, because the *in vivo* osteolytic bone metastatic cancer dissolves bones with much more actions than decalcification of bones, it is assumed that they would be detected in radiography at lower level of decalcification than the simulation with only decalcification.

Summing up the above statements, with 1) the possibility that radiograph is not as clear as in this experiment with the thick soft tissues of arms-legs *in vivo*, 2) the phenomenon in which osteolytic bone metastatic cancer destroys bones is a more complex reaction of uptake or osteolysis of actual bone tissue itself than decalcification, the minimum level of decalcification obtained from the artificial decalcification method using HCl used in this experiment can be recognized itself as the effect of an academic index that expresses the upper limit in which the metastatic bone osteolysis of long bone in the plain radiograph can be detected.

CONCLUSION

To measure the minimum level of decalcification to detect the osteolytic bone metastatic cancer site of long bone in the plain radiography of FR, CR, and DR types, an experimental study was conducted using a pair of fibula and humerus from 2 cadavers. As a result, in plain radiographs in types of FR, CR, and DR, the osteolytic bone metastasis was detectable when the minimum of 3% and maximum of 16% of decalcification is progressed. CR and DR were not different in detecting the osteolytic bone metastatic lesions.

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