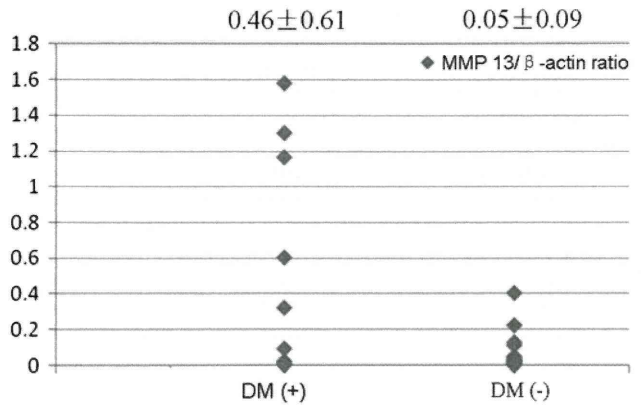

Fig. 3 Expression of MMP13 in the LF from DM (+) LSCS patients and DM (−) LSCS patients

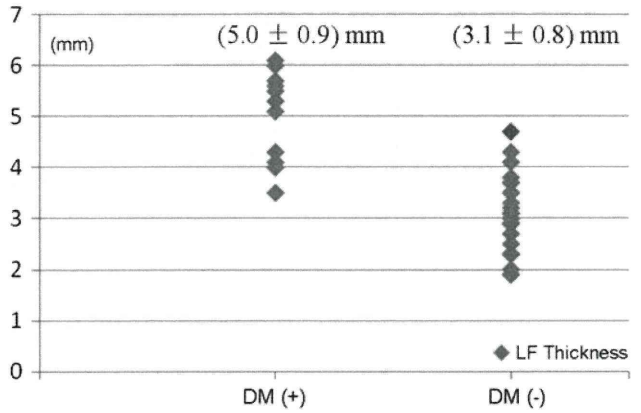

Fig. 4 Thickness of the LF from DM (+) and DM (−) LSCS patients

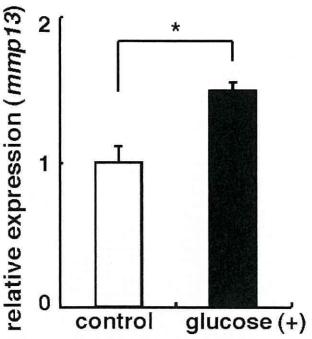

Fig. 5 NIH3T3 cells were cultured in the presence or absence of glucose (3 mg/ml) for 19 h, and *mmp13* expression was analyzed by real-time PCR. Data are shown as mean ± SD of *mmp13/β-actin* in cultured cells with glucose relative to that of without glucose. $*P < 0.001$

Discussion

In this study, we found an increased expression of MMP13 in the LF from DM (+) LSCS patients compared with the LF from DM (−) LSCS patients. In addition, elastin degradation and fibrosis of the LF were more severe in the DM (+) LSCS patients than in the DM (−) patients, and the LF of the DM (+) LSCS patients was significantly thicker. Our results suggest that an increased expression of MMP13, which may be related to DM, can be one of the factors contributing to fibrosis and hypertrophy of the LF, resulting in the progression of stenosis of the lumbar spinal canal.

Previous studies showed that the LF in the lumbar region is rich in elastic fibers, whose principal components are elastin and fibrillin [2]. Fibrillin can be degraded by MMP13, which is an important process in connective tissue remodeling [9]. MMP13 has been described as a trigger for the activation of a positive MMP2 and MMP9 feedback loop in asbestos-induced pulmonary fibrosis in mice [12]. The importance of MMP13 in fibrosis has also been shown in reports on bleomycin-induced pulmonary fibrosis in rats [21] and systemic sclerosis in humans [22]. In vessels and cornea, high plasma glucose increases the expression of MMP13 [14, 15]. In this study, the expression of MMP13 in the LF was higher in DM (+) than in DM (−) LSCS patients, and the expression of MMP13 was correlated with the thickness of the LF. Expression of mmp13 was upregulated by the presence of glucose in mice fibroblastic-like cells. MMP13 degrades both collagen fibers and elastic fibers, and is highly involved in extracellular remodeling [7, 9]. Thus, high plasma glucose levels may increase the expression of MMP13 in the LF and cause fibrosis. However, the exact mechanisms of the upregulation of MMP13 in DM patients should be elucidated by further studies.

In conclusion, we found a higher expression of MMP13 in the LF from DM (+) LSCS patients than in the LF from DM (−) LSCS patients. In addition, the elastin degradation and fibrosis of the LF was more severe in DM (+) patients than in DM (−) patients. These results suggest that the increased expression of MMP13 associated with DM can be one of the factors contributing to LF fibrosis and hypertrophy.

Conflict of interest This study was partly supported by a grant from Ono Pharmaceutical Co., Tokyo, Japan. All authors had no other financial relation regarding the study.

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Mechanism of osteoporosis in adolescent idiopathic scoliosis: experimental scoliosis in pinealectomized chickens

Abstract: To clarify the mechanism of osteoporosis in adolescent idiopathic scoliosis (AIS), we investigated radiological and histological changes in the cervical vertebrae of a chicken thoracic scoliosis model. Forty newly hatched broiler chicks were randomly divided into four equal groups: sham-operated chickens serving as control (CON), pinealectomized chickens (PNX), sham-operated (CON + MLT) and pinealectomized chickens (PNX + MLT) that received intraperitoneal administration of melatonin. Pinealectomy was performed at the age of 3 days, and the chickens were killed at 2 months of age. Postmortem X-rays were examined for the presence of scoliosis, and micro-computed tomography (micro-CT) images were taken to evaluate the microstructure of the cervical vertebrae. Histological specimens of the scanned cervical vertebra were prepared, and a midsagittal section was stained with hematoxylin and eosin and tartrate-resistant acid phosphatase to evaluate the numbers of osteoblasts and osteoclasts, respectively. Scoliosis developed at the thoracic spine in all chickens of the PNX and in two of the PNX + MLT group. Micro-CT data revealed that chickens in the PNX group had a greater degree of generalized osteoporosis compared with the other birds. The number of osteoblasts was significantly decreased in the PNX group, while no significant difference was observed among chickens in the numbers of osteoclasts. Our results suggest that melatonin deficiency reduces osteoblast proliferation and leads to the development of scoliosis and osteoporosis. The restoration of melatonin prevented the development of scoliosis and osteoporosis, indicating that melatonin levels may be crucial to the development of deformity and osteoporosis in AIS.

Hitoshi Kono[1], Masafumi Machida[2], Masashi Saito[3], Yuji Nishiwaki[4], Hiroyuki Kato[1], Naobumi Hosogane[1], Kazuhiro Chiba[1], Takeshi Miyamoto[1], Morio Matsumoto[1] and Yoshiaki Toyama[1]

[1]Department of Orthopaedic Surgery School of Medicine, Keio University, Tokyo, Japan; [2]Clinical Research Center, National Institute of Musculo-Skeletal Disorders, Murayama Medical Center, Tokyo, Japan; [3]Keiyu Orthopedic Hospital, Gunma, Japan; [4]Department of Public Health and Preventive Medicine, School of Medicine, Keio University, Tokyo, Japan

Key words: experimental scoliosis, melatonin, micro-CT, osteoporosis, pinealectomy

Address reprint requests to Masafumi Machida, MD, Clinical Research Center, National Institute of Musculo-Skeletal Disorders, Murayama Medical Center, 37-1, 2-chome, Musashimurayama-shi, Tokyo 208-0011, Japan.
E-mail: machida@murayama.hosp.go.jp

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Introduction

Osteoporosis is one of the most common metabolic bone disorders, and its increasing prevalence in the elderly population has become a major health problem. Osteoporosis rarely affects adolescents [1], but its prevalence is significantly higher in patients with adolescent idiopathic scoliosis (AIS) than the general pediatric and adolescent populations [2]. However, despite the well-known clinical manifestations of AIS, its pathogenesis and relationship to premature osteoporosis remain unanswered.

As Burner et al. [3] first reported an association between osteopenia and AIS using the Singh index, several other investigators have confirmed low bone mineral status and other characteristics of osteoporosis in 27% to 38% of patients with AIS [4–7]. Hung et al. [8] measured the bone mineral density (BMD) of the lumbar spine and femoral neck in 324 patients with AIS and suggested that it might serve to predict the curve progression in AIS. Although the relationship between postmenopausal estrogen deficiency and osteoporosis is well recognized, osteopenia/osteoporosis in young women, particularly in adolescents with hypoestrogenism, has not been sufficiently studied and the cause of low BMD in AIS is unknown.

Pinealectomy in newly hatched chicks results in a significant decrease in serum melatonin levels and consistently produces scoliosis with anatomic characteristics similar to those of human idiopathic scoliosis [9, 10] and have been used to create experimental models of AIS. Furthermore, both autografting of the pineal gland [9] and administration of melatonin [10] prevent the development of scoliosis in this model. Our previous studies have revealed that melatonin deficiency, which contributes to the progression of spine curvature, may determine the prognosis of AIS [11], and that melatonin supplement may prevent the development of progressive scoliosis, especially in mild cases [12]. Recently, several studies have shown that melatonin has the effects on bone metabolism, while its impacts on the pathogenesis of osteopenia and osteoporosis in patients with AIS are unknown [13–16].

To investigate the relationship between osteoporosis and AIS, it is necessary to examine whether the decreased BMD

found in patients with AIS is secondary to the spinal deformity and the resulting imbalance in the mechanical loading of the spine or hips or whether the lower BMD is the primary problem leading to the development of AIS. If changes of bone quality at skeletal sites remote from the scoliotic deformity that are not caused by abnormal mechanical loading, there is a possibility that changes in bone metabolism may be an etiologic factor for osteoporosis. In this study, we studied the microstructural properties of the cervical vertebrae in pinealectomized chickens and demonstrated the effects of melatonin upon the osteogenetic process using morphological, radiological, and histological analyses.

Materials and methods

Birds and treatment

Forty female broiler chickens were kept in individual cages under constant laboratory conditions of 20–22°C room temperature in a 12 hr light/dark cycle (06:00–18:00 light cycle) with cool white fluorescent bulbs yielding approximately 7–54 mphot. They were given water and commercial chicken diet that did not contain melatonin or serotonin. The chickens were randomly divided into four equal groups: sham-operated chickens serving as controls (CON), pinealectomized chickens (PNX), sham-operated (CON + MLT) and pinealectomized chickens (PNX + MLT) that received daily intraperitoneal administration of melatonin (8 mg/kg body weight (BW) in 10% ethanol/saline, s.c.) at 22:00. Sham operation or pinealectomy surgeries were performed under ether anesthesia at 3 days after hatching using techniques described previously [9, 10]. Animal experimentation was approved by the Animal Committee of National Murayama Medical Center.

Tissue collection

Before killing the chickens by pentobarbital injection (40 mg/kg BW) at 2 months of age, blood samples were collected at midnight under a dim red light (640–700 nm, <2 lx) to measure serum melatonin levels. Whole-blood samples were taken by direct puncture of the heart and centrifuged. The serum was stored at −80°C. Serum melatonin concentration was analyzed by radioimmunoassay using a Buhlmann test kit (Buhlmann Lab., AG, Basel, Switzerland). The minimal detection limit was 2.8 pg/mL. Serum melatonin level in the PNX group was 19.7 ± 5.3 pg/mL, while it was restored in the PNX + MLT group (249.7 ± 134.3 pg/mL), in the CON (112.0 ± 39.8 pg/mL) and CON + MLT (185.0 ± 99.1 pg/mL) groups.

Spine evaluation

The spines of all chickens were visually and radiologically examined for the presence or absence of any curvature or bone abnormalities. Posteroanterior radiographs of the vertebral column of each chicken were taken in a standardized supine position. In the evaluation of radiographs of the vertebral column, scoliosis was defined as a lateral curvature $>10°$.

After dissection of the paravertebral muscles and soft tissues from the cervical spine, the fifth cervical vertebra from five chickens per group were scanned with a micro-CT (TOSCANER-32250; Toshiba Medical, Tokyo, Japan) with the spatial resolution set to 25 μm. The fifth cervical vertebra that was not involved in the curve was chosen for analysis to eliminate influence from asymmetrical mechanical forces because of scoliosis. Three-dimensional images were created from two-dimensional radiographs, and microstructural indices were calculated using a 3D image analysis system (TRI/3D-BON; RATOC System Engineering Co., Tokyo, Japan). The region of interest was set to the vertebral cancellous bone between the superior and inferior endplate in the sagittal plane and 150 μm from the inner surface of the vertebral cortices in the axial plane. This cylindrical column with a dimension of 1.6 mm in diameter and 6 mm in height was continuously scanned with a thickness and increment of 25 μm, yielding 515 slices per animal. The gray-scale images were segmented using a medial filter to remove noise and a fixed threshold to exact the mineralized bone phase. After removing the isolated small particles and holes in the marrow space using a cluster-labeling algorithm, the trabecular bone was analyzed for structural indices.

Bone architectural evaluation

The following architectural parameters of the trabeculae were directly evaluated using the three-dimensional model constructed from the micro-CT data: bone volume fraction (bone volume/tissue volume; BV/TV %), trabecular number (Tb.N/mm), trabecular thickness (Tb.Th μm), star volume of the marrow space (V*m.space mm^3) and of the trabeculae (V*Tr mm^3). Of these, BV was calculated using tetrahedrons corresponding to the enclosed volume of triangular surface, while TV was the total tissue volume. BV/TV was then calculated from these values. Tb.Th, Tb.N, and Tb.Sp were determined according to the method described by Hildebrand and Ruegsegger [17]. The star volume is a new stereologic measure that portrays structural changes of the trabecular bone [17, 18]. Each trabecular cube was analyzed for these parameters.

The cervical vertebrae from the five chickens that were scanned using micro-CT were then processed for quantitative histological evaluation. The fifth cervical vertebra was fixed in 10% neutral-buffered formalin, decalcified in 5% hydrochloric acid, and sectioned in the sagittal plane. A midsagittal section from each vertebra was stained with hematoxylin and eosin and tartrate-resistant acid phosphatase to evaluate osteoblasts and osteoclasts, respectively. The number of osteoblasts located at both the upper and lower metaphyses and the number of osteoclasts in the entire vertebral body were manually counted through a microscope by a histologist who was blinded to the study.

Statistical analysis

All data are expressed as arithmetical mean ± standard deviation (S.D.). Statistical analyses were conducted using a

one-way analysis of variance followed by Bonferroni's test. *P*-value < 0.05 was considered to be statistically significant.

Results

All chickens in the CON and CON + MLT groups had straight spines and symmetrical thoracic cages. In contrast, all chickens in the PNX group and two chickens in the PNX + MLT group developed scoliosis at the thoracic spine with rib humps, vertebral rotation, and malformed, asymmetrical thoracic cages (Fig. 1). The deformity was a mild-to-severe lordoscoliosis that was directed to either side with no consistent pattern. The apex of the curves was between the fourth and seventh thoracic vertebrae, with rotation into the convexity, and the maximal intervertebral rotation occurred above and below the sixth thoracic vertebra. In chickens with a severe scoliotic curve, the vertebral bodies were often compressed on the concave side, leading to wedge-shaped deformities, while no such deformity was found in the chickens with moderate scoliotic curves. Limb paralysis and gait disturbance were not observed in any chicken.

Representative images of the 3D trabecular microstructure of cancellous bone from each group are shown in Fig. 2. The cancellous bone from the CON and CON + MLT groups had plate-like trabeculae that were thickened and interconnected (Fig. 2A,B). In comparison, PNX chickens had rod-like trabeculae that were markedly decreased and disconnected with irregular surfaces (Fig. 2C). The 3D measure of trabecular bone architecture in each group is summarized in Table 1. Tb.Th in the PNX was significantly lower than that in the CON + MLT group, and V*Tr in the PNX was significantly lower than that in the CON and CON + MLT groups, while V*m.Sp in the PNX group was significantly higher than that in the other groups. No significant difference in Tb.N or Tb.Sp was found among the groups (Fig. 3A–C).

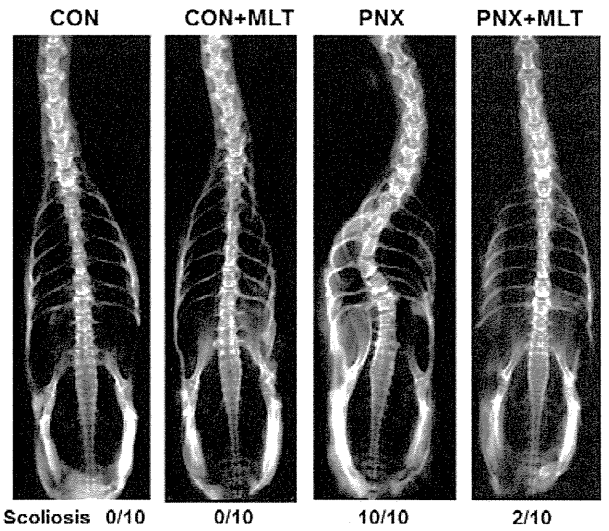

Fig. 1. Posteroanterior radiographs of spine. A straight vertebral column in sham-operated (CON) and sham-operated (CON + MLT) and pinealectomized (PNX + MLT) chickens with melatonin treatment. Scoliosis with a single curve in pinealectomized chickens (PNX). Note the presence of more complicated deformity with vertebral rotation towards the convexity of the curve. The vertebral body at the apex of the curve is compressed (from above downward) on the concave side showing wedge-shaped deformity.

The number of osteoblasts found at the metaphysis decreased significantly ($P < 0.01$) in the PNX group than those in the other groups (Fig. 4). Although osteoclasts were found across the entire vertebra, they were mainly located on the edge of the growth plate, and there was no difference ($P > 0.05$) in the number of osteoclasts among all four groups (Fig. 5).

Discussion

Despite being a widely accepted association, it is not clear why osteopenia/osteoporosis develops prematurely in patients with AIS [4–8]. This is the most common form of scoliosis with a well-established prevalence in women. The condition progresses during prepuberty and puberty, the period when the greatest increase in the bone mass occurs. In fact, recent studies have shown that at least 90% of peak bone mass is accumulated within the second decade of life [19].

Many studies have attempted to uncover the etiology and pathophysiology of AIS [20]. Although hereditary predispositions appear to play some roles, other possibilities include environmental [21], genetic [22, 23], mechanical [24], biochemical [25], neurologic [26], hormonal [27], and muscular factors along with ligament deficiency [28]. More recently, the proposed role of melatonin is based on the formation of scoliosis seen in pinealectomized immature chickens associated with reduced nocturnal serum melatonin levels [11]. To further support this conception, either an autograft of the pineal gland [9] or administration of melatonin [10] prevented the formation of scoliotic deformity in this chicken model. This led us to postulate that the reduced level of serum melatonin was a prerequisite for scoliosis development. In a rat model, induced experimental scoliosis developed only in pinealectomized bipedal, but not in quadrupedal rats [29]. We concluded that melatonin deficiency secondary to pinealectomy alone does not produce scoliosis if the quadrupedal condition is maintained. Similar results were obtained in melatonin 'knock down' C557BL/6J mice without pinealectomy, suggesting the critical influence of a postural mechanism in addition to melatonin deficiency in the development of scoliosis [30].

Burner et al. [3] using plain radiographs and later Cook et al. [4] using Dual Energy X-ray Absorptiometry (DXA) demonstrated that a low bone mass was common in children with AIS. They reported a state of generalized or systemic rather than a localized osteopenia with AIS. Cheng and Guo [5] reported that 68% of patients with AIS had a significantly reduced BMD and suggested that the osteopenia in AIS is related to the primary etiology of the disease rather than secondary to the asymmetrical mechanical forces associated with the back deformity. The follow-up studies indicated that osteopenia in patients with AIS may be a persistent phenomenon [6]. Moreover, Hung et al. [8] reported that poor bone quality or osteopenia may be an important risk factor for curve progression. Prognostic factors related to curve progression during the peripubertal

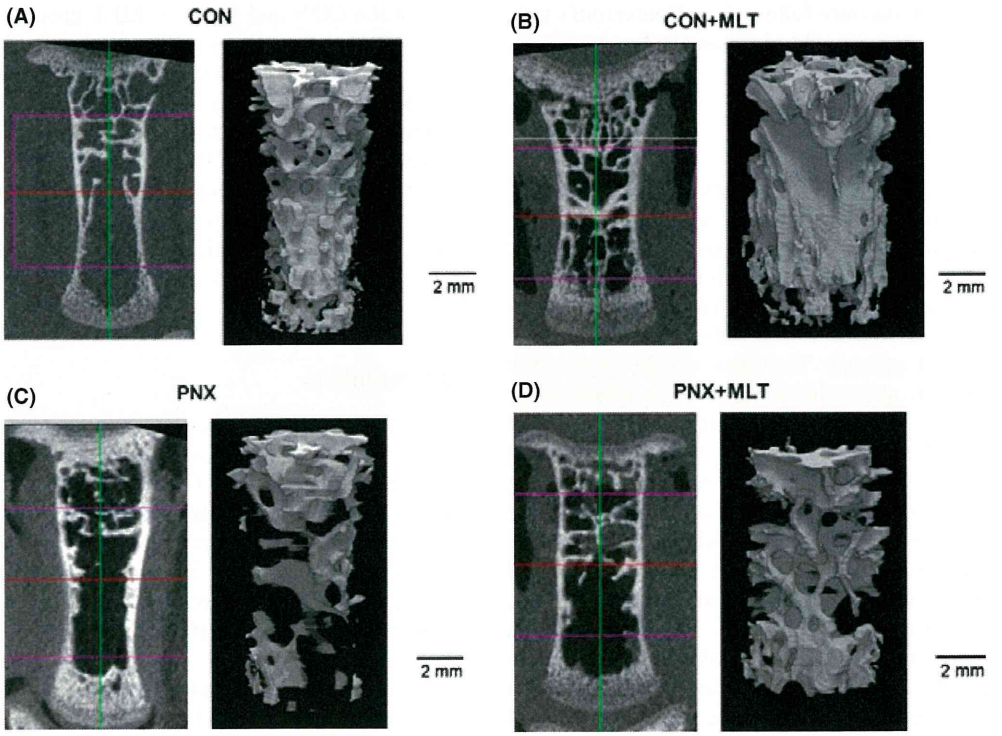

Fig. 2. Micro-CT of the fifth cervical vertebra in each group represents. The cancellous bone from CON and CON + MLT had plate-like trabeculae with thickened and interconnected, while PNX had rod-like trabeculae with disconnected irregular surfaces.

Table 1. 3D parameters of trabecular bone architecture

	CON	CON + MLT	PNX	PNX + MLT
BV/TV (%)	15.2 ± 6.0	23.6 ± 7.2*	10.0 ± 6.0	17.7 ± 8.1
Tb.Th (μm)	208 ± 40.2	232 ± 40.2*	149 ± 63.9	225 ± 45.3
Tb.N (1/mm)	0.65 ± 0.49	0.61 ± 0.12	0.25 ± 0.15	0.44 ± 0.10
Tb.Sp (μm)	643 ± 252	553 ± 105	720 ± 219	636 ± 176
V*m.Sp (mm^3)	1.55 ± 0.95**	1.47 ± 0.42**	3.90 ± 1.80	1.85 ± 0.23**
V*Tr (mm^3)	0.53 ± 0.24**	0.55 ± 0.18**	0.06 ± 0.03	0.33 ± 0.14

All data represent mean ± S.D.
BV/TV, bone volume/tissue volume.
*Significantly different from PNX samples at $P < 0.05$.
**Significantly different from PNX samples at $P < 0.01$.

period reported in previous studies include immaturity seen in a younger age, a premenarcheal status, and a lower Risser grade [31].

In our previous study [11], 12 of 30 patients with AIS had progressive curves and significantly decreased serum melatonin levels, while the remaining 18 patients with stable curves had melatonin levels similar to that of control subjects. From this finding, we proposed that melatonin deficiency may play a role in scoliosis deterioration [11, 12]. In patients with progressive scoliosis, Kindsfater et al. [32] demonstrated a marked increase in the level of platelet calmodulin, a calcium-binding receptor protein. As melatonin, its antagonist, binds to calmodulin with high affinity [33], the inverse change in increased calmodulin and decreased melatonin observed in patients with AIS may be important in the pathogenesis of scoliosis.

Although clinically similar, the etiology of osteopenia/osteoporosis in the aged and young populations is not the same. Postmenopausal osteoporosis occurs following a decline in estrogen levels. Osteopenia/osteoporosis occurs in children, most often secondary to disorders affecting bone metabolism such as renal disease, glucocortical therapy, anticonvulsant therapy, rheumatoid diseases, cystic fibrosis, juvenile diabetes, and endocrine disorders [34]. Because these conditions were excluded from previous studies related to osteopenia/osteoporosis in AIS, and because the menarche status was matched between scoliotic patients and healthy subjects, the pathogenesis of the generalized or systemic osteopenia/osteoporosis in AIS must lie elsewhere.

Recently, several studies demonstrated that melatonin promotes osteoblast differentiation and bone formation [35–38], playing a greater role in the regulation of bone growth than previously considered. Nakade et al. [13] reported that melatonin stimulates type 1 collagen synthesis in human bone cells in vitro, suggesting that it may promote bone formation. Ostrowska et al. [14] analyzed the correlation between the changes in the 24-hr profile of serum melatonin level and the circadian metabolism of type 1 collagen in postmenopausal, obese women and suggested that decreased melatonin levels may be an aggravating factor for postmenopausal loss in the bone mass.

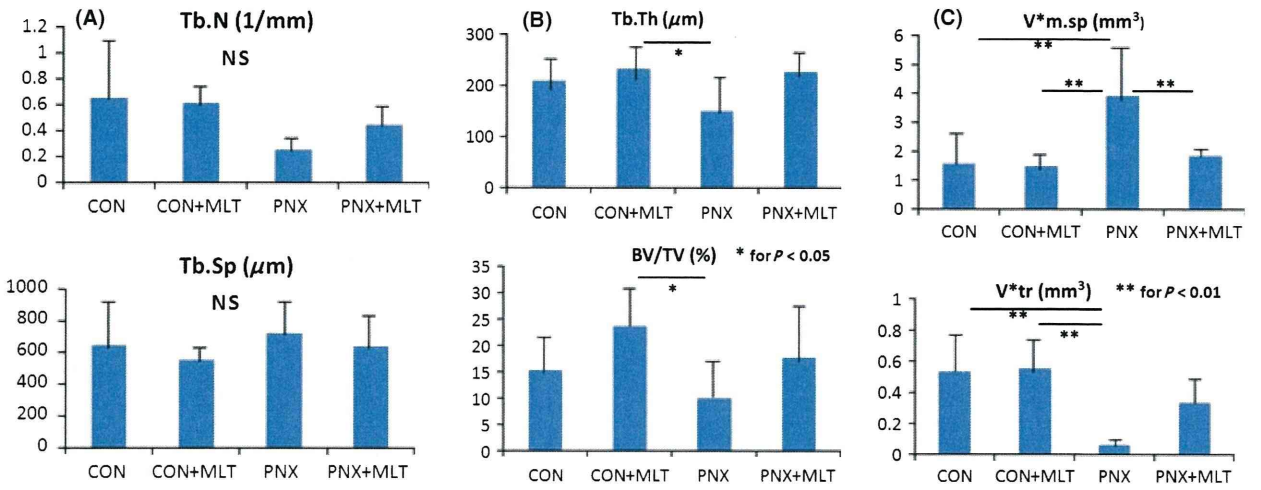

Fig. 3. Tb.N, Tb.Th, Tb.Sp, BV/TV, V*m.Sp, and V*Tr in each group. V*Tr in the PNX was significantly lower than that in the CON and CON + MLT groups. V*m.Sp in the PNX group was significantly higher than that in the other groups. BV/TV, bone volume/tissue volume.

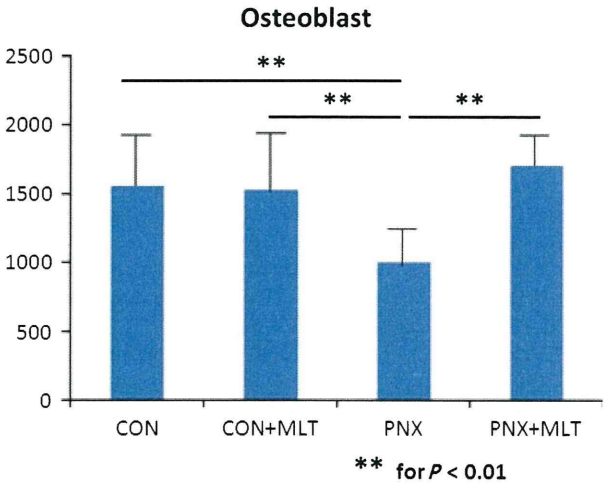

Fig. 4. The number of osteoblasts in each group. The number of osteoblasts was significantly lower in the PNX than that in the other groups.

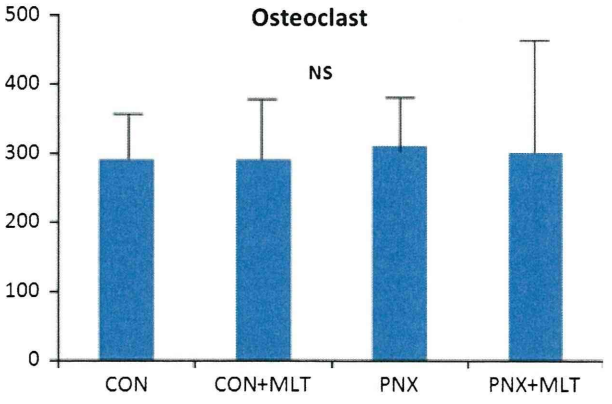

Fig. 5. The number of osteoclasts in each group. There was no statistical difference in the number of osteoclasts among different groups.

Ostrowska et al. [39] also demonstrated that pinealectomy and melatonin administration affects the postovariectomy osteoporotic process in female rats. The in vivo effectiveness of melatonin in preventing bone loss in ovariectomized rats was also reported by Ladizeswky et al. [15]. Turgut et al. [16] investigating the histological and radiological changes in the cervical vertebrae reported all pinealectomized chickens developed scoliotic deformity, showing significantly lower BMD than the nonpinealectomy group. In the cervical vertebrae, the total number of osteocytes, but not osteoblasts, was significantly lower in pinealectomized as compared to nonpinealectomy group, but there was no significant difference in the total number of osteoblasts between the two groups. The observed histological effects of melatonin on osteogenesis were consistent with the results of radiological evaluation and BMD, and they concluded that melatonin may have an osteoinductive effect on bone formation. Their results were different from those of our study, but lead to a similar conclusion. In our study, the number of osteoblasts was significantly decreased in scoliotic chickens after PNX.

However, the mechanism and cause of low BMD in AIS have not been identified. Osteoporosis is a condition of the bone in which the BMD is reduced, bone microarchitecture is disrupted, and the amount and variety of noncollagenous proteins in bone are altered as a result of an imbalance between bone resorption and bone formation. Postmenopausal osteoporosis is reported to correlate with a decline in estrogen levels, and increase in the number of osteoclasts is observed. Although osteoporosis is more common in the elderly than in adolescents, several studies recently reported low BMD in patients with AIS [2–8]. Osteoporosis/osteopenia in adolescents with hypoestrogenism has not been established, and there have been no reports of estrogen function abnormalities in AIS [40]. Previously, we reported that nocturnal melatonin secretion decreased in AIS [11, 12]. Recent studies show that melatonin is a physiological factor that promotes bone formation [13–16, 35–39]. Although the influence of melatonin on skeletal growth and bone formation are evident, research into the

effects of melatonin on human osteoblasts has only started. Satomura et al. [41] reported that melatonin stimulated the proliferation and alkaline phosphate activity of human osteoblasts, promoted the gene expression of type 1 collagen, osteopontin, bone sialoprotein and osteocarcin, and stimulated the in vitro mineral matrix formation in a dose-dependent manner. Sethi et al. [37] and Zhang et al. [38] suggested that melatonin induces osteoblast differentiation from human mesenchymal stem cell and enhances osteogenesis. Intraperitoneal administration of melatonin in mice increased the volume of newly formed cortical bone of the femur. The decrease in BMD and histological and radiological changes observed in melatonin-deficient chickens in this study corroborate the effect of melatonin on osteoblast and bone formation. The fact that intraperitoneal injection prevented osteoporosis and scoliotic deformity in our study further supports the significance of melatonin in the development of osteopenia/osteoporosis and scoliosis in AIS.

In experimental osteoporosis, BMD measured by conventional DXA estimates bone volume through a two-dimensional analysis. In this study, using the three-dimensional evaluation of trabecular microarchitecture in experimental scoliosis, we have evaluated the microstructural changes in bone matrix after pinealectomy. Pinealectomy produced a scoliotic deformity at the thoracic spine that compressed the concave side and stretched the convex side at the apex, causing the deformation of vertebral bodies by mechanical load. Even the cervical vertebrae, not deformed by this mechanics showed a decrease in BMD and fluctuation in BV/TV, Tb.Th, Tb.N, Tb.Sp, V*m.Sp, and V*Tr, as demonstrated by micro-CT. These results indicate that the pinealectomized chickens have generalized or systemic osteoporosis. The morphological characteristics of bone tissue in pinealectomized young chickens were similar to those of postmenopausal osteoporosis in humans. In this study, the number of osteoblasts, but not osteoclasts, in the cervical vertebrae was significantly lower after pinealectomy as compared to sham operation or melatonin treatment. The observed histological effects of melatonin on osteogenesis were consistent with the results of radiological and micro-CT findings.

Based on these results, it seems likely that a melatonin deficiency may be an important contributory factor in the development of scoliotic deformity and osteoporosis. Further study is necessary to determine the level of melatonin necessary to prevent progression of the spinal deformity and osteopenia/osteoporosis in AIS, and such results would confirm the importance of melatonin during adolescence of maturation. It may also lead to the possible prevention of scoliosis and osteopenia/osteoporosis through melatonin replacement therapy.

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ORTHOPAEDIC SURGERY

Evaluation of clinical problems associated with bone metastases from carcinoma from unknown primary sites

Manabu Hoshi · Susumu Taguchi · Keiko Hayakawa · Makoto Ieguchi · Hiroaki Nakamura

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Abstract
Introduction This study focused on the evaluation of data concerning the clinical features of patients who were initially diagnosed with bone metastases of carcinoma from unknown primary sites that could not be detected, even using state of-the-art diagnostic modalities.
Method The oncologic outcome of these patients is discussed.
Patients The clinical records of seven patients who had presented with bone metastases of carcinoma from unknown primary sites were retrospectively reviewed. Clinical features, treatment and outcome were analyzed. Extraskeletal metastases were located in the lymph nodes, liver, skeletal muscle, kidney, adrenal gland, and pleura. Six cases were observed in the pelvis, three in the femur, three in the skull, two in the rib, two in the cervical spine, two in the thoracic spine, two in the lumbar spine, one in the humerus, one in the radius, one in the clavicle, one in the scapula and one in the sternum. Four patients received systemic chemotherapy including platinum.
Results At the last follow-up time of average 272 days, six patients were dead of disease and one patient was alive with disease. Although considerable progress has been made in the development of diagnostic modalities, including more recently FDG-PET, the primary tumor site cannot always be identified. Multiple bone and visceral organ metastases are often present in patients whose primary tumor was not detected.
Conclusion In the present study, it was found that systemic chemotherapy can appreciably increase the survival time of the patients with carcinoma metastases from unknown primary sites.

Keywords Unknown primary site · Bone metastasis · Treatment · Chemotherapy

M. Hoshi (✉) · S. Taguchi · K. Hayakawa · M. Ieguchi · H. Nakamura
Department of Orthopedic Surgery,
Osaka City University Graduate School of Medicine,
1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
e-mail: hoshi@med.osaka-cu.ac.jp

Introduction

The prognosis of patients with various types of carcinoma has been gradually improved due to early diagnosis using modern imaging techniques and improved treatment protocols. Because of higher control rates of the primary tumor in recent decades, the prolonged survival of patients has significantly increased the risk of developing distant metastases. Carcinoma metastasis from unknown primary sites is frequently detected in the lymph nodes due to swelling [1] and bone is also a frequent target site. 10–15% of patients present with symptoms of occult cancer [2].

Identification of the primary site is critical in enabling a rapid start to treatment. Unfortunately, even with the recent progress in the development of variable diagnostic techniques, the primary tumor site cannot be determined always. Failure to locate the primary tumor using multimodal diagnostic approaches leads to increased treatment delays and costs. As a consequence, patients cannot undergo cancer-specific treatment and their overall prognosis is generally poor.

In the present study, the clinical features and oncological outcomes of a group of patients treated for carcinoma metastases in the bone were examined. Only patients whose primary site of carcinoma could not be detected using the latest diagnostic modalities were admitted into the study.

Patients in whom the primary location of the carcinoma could be identified were excluded.

Materials and methods

From March 1994 to 2009, seven patients with carcinoma metastases in the bone were treated and followed up. All patients were evaluated with physical examinations, various radiological evaluations and pathological investigation using biopsy. The primary site of the carcinoma in these patients could not be identified. There were three male and four female patients, with a mean age at diagnosis of 61.9 years (range 49–80 years), were followed up for a median period of 272 days (range 16–469 days). No patient was lost during follow-up. The clinical information reviewed in this study included performance status, laboratory date of tumor marker [alkaline phosphatase (ALP), lactate dehydrogenase (LDH), carcinoembryonic antigen (CEA)], anatomical site of bone metastases or extraskeletal metastases, histology and treatment modalities. Oncologic outcome at the last follow-up was also examined.

Results

The characteristics of patients with bone metastases from carcinoma originating from unknown primary sites are summarized in Table 1. At their first visit to our orthopedic department, the performance status was zero in two patients, one in two patients, two in one patient and three in two patients. Laboratory data showed elevated levels of ALP in five out of seven patients, LDH in four out of seven patients and CEA in four out of seven patients. Radiological imaging of patients, including FDG-PET, revealed that extraskeletal metastases were located in the lymph nodes in five cases (Fig. 1a), liver in two cases, skeletal muscle of the buttock in two cases (Fig. 1b, c), lower leg in two cases, kidney in one case, adrenal gland in one case and pleura in one case.

Table 1 Characteristics of patients with bone metastases from carcinoma of primary unknown site

		Number	%
Age	61.9 (mean)		
PS	0	2	28.6
	1	2	28.6
	2	1	14.3
	3	2	28.6
	4	0	
Laboratory			
ALP	Elevated	5	71.4
LDH	Elevated	4	57.1
CEA	Elevated	4	57.1
Extraskeletal metastases			
Lymph node		5	71.4
Liver		2	28.6
Kidney		1	14.3
Skeletal muscle		2	28.6
Adrenal gland		1	14.3
Pleura		1	14.3

The incidence of bone metastases at different sites and the oncologic outcome of patients are summarized in Table 2. There were six cases of bone metastases in the pelvis (Fig. 1b, c), three in the femur, three in the skull, two in the ribs, two in the cervical spine, two in the thoracic spine, two in the lumbar spine, two in the humerus, one in the radius, one in the clavicle, one in the scapula and one in the sternum.

For histopathological diagnosis, six patients were biopsied. One patient underwent endoprosthetic replacement (Fig. 2a, b). Biopsy specimens and surgical specimens were identified as adenocarcinoma in three patients (Fig. 3a),

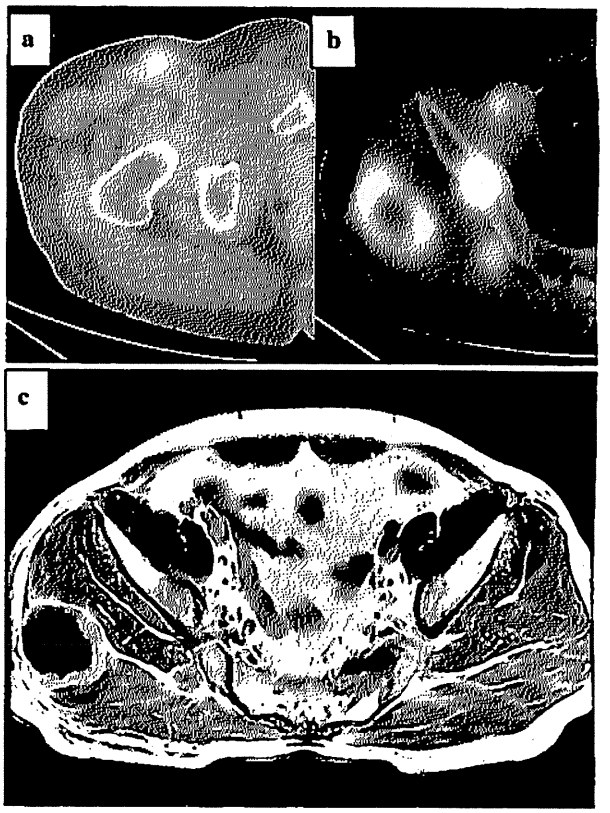

Fig. 1 Case 1. a PET/CT shows the lymph node swelling in the right inguinale. b PET/CT demonstrates the abnormal uptakes in intrapelvic lymph node, ilium, and buttocks. c Axial T2 weighted MRI shows the bone metastases of the right ilium and soft tissue metastasis in buttock

Table 2 Bone metastases and oncologic outcome in carcinoma of unknown primary sites

Case	Age	Sex	Histology	Bone metastasis	Treatment	Follow-up (days)	Prognosis
1	54	M	Adenocarcinoma	Pelvis	CT (CBDCA + PTX, TS1), RT (pelvis)	131	AWD
2	75	F	Adenocarcinoma	Skull, rib, pelvis, thoracic	RT (pelvis, thoracic)	407	DOD
3	55	F	Adenocarcinoma	Femur, pelvis, clavicle	CT (TS1), surgery (femur)	469	DOD
4	49	M	Poorly differentiated carcinoma	Femur	RT (femur)	33	DOD
5	64	F	Signet ring cell carcinoma	Skull, cervix, lumbar, thoracic, rib, pelvis	CT (CBDCA + PTX)	420	DOD
6	80	F	Poorly differentiated carcinoma	Carpal, lumbar, pelvis	RT (pelvis)	16	DOD
7	56	M	Signet ring cell carcinoma	Scapula, sternum, pelvis, femur, skull, cervix, lumbar, humerus	CT (CBDCA + PTX, TS1 + GEM)	429	DOD

RT Radiation therapy, *CT* Chemotherapy, *CBDCA* carboplatin, *PTX* paclitaxel, *GEM* gemcitabine hydrochloride, *DOD* Die of disease, *AWD* Alive with disease

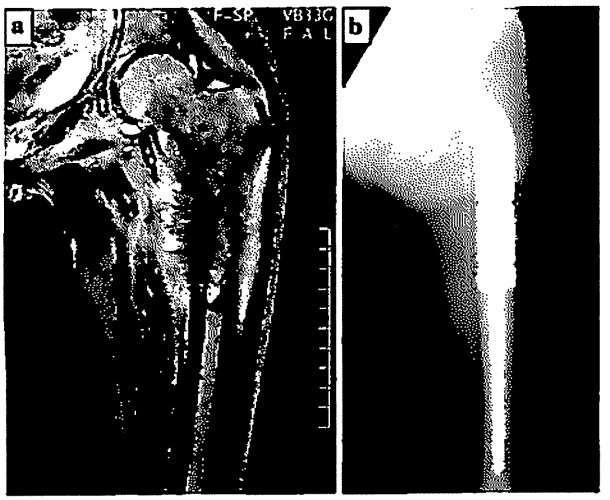

Fig. 2 Case 3. **a** Coronal T2 view of MRI shows the bone metastases of proximal femur. **b** Plain film shows the endoprosthetic replacement after tumor resection

signet ring cell carcinoma in two patients (Fig. 3b) and poorly undifferentiated carcinoma in two patients (Fig. 3c).

After diagnosis of metastatic cancer, three patients had major health problems. Two patients were in frail health, due to age (cases 2 and 6). One patient (case 4) had severe chronic renal failure. Four patients (cases 1, 3, 5 and 7) received systemic chemotherapy, and their regimen consisted of three sessions of carboplatin (CBDCA). The median survival time was 429 days for patients receiving chemotherapy, and 33 days for patients that did not undergo chemotherapy.

Three patients received local radiation therapy. One patient (case 3) showed prominent sclerotic changes after radiation therapy, indicating efficacy in treating the cancer (Fig. 4). In another patient (case 6), health suddenly deteriorated during radiation treatment and death followed after 10 days. At an average follow-up time of 272 days, six patients were DOD and only one patient was AWD. The median survival time of all patients was 420 days.

Discussion

Carcinoma metastases originating from an unknown primary site have been defined by Pavlidis et al. [3] as cancer occurring in patients who present with histologically confirmed metastatic disease, in whom a detailed medical history, complete physical examination, histopathological review of biopsy material with use of immunohistochemistry, chest radiography, CT of abdomen and pelvis failed to identify the primary site. The incidence of carcinoma metastases from unknown primary sites is approximately 3% of all malignant neoplasms. The common sites of occurrence of these metastases are the lymph nodes, liver, lung and bone [1].

Patients with bone metastases are often referred to an orthopedic oncologist by general physicians for investigation of the location of the primary tumor site. There is a need to identify the primary site as quickly as possible, in order to finalize the diagnosis and begin treatment. The incidence of carcinoma metastases in bone originating from unknown primary sites in the gastro-intestinal tract is relatively low. However, lung, prostate and breast cancer metastases occur more frequently in bone. As a consequence, endoscopy of the digestive tract is not the first choice for the primary investigation [4, 5]. This is due to the fact that it is neither time- nor cost-effective to do so and can delay treatment.

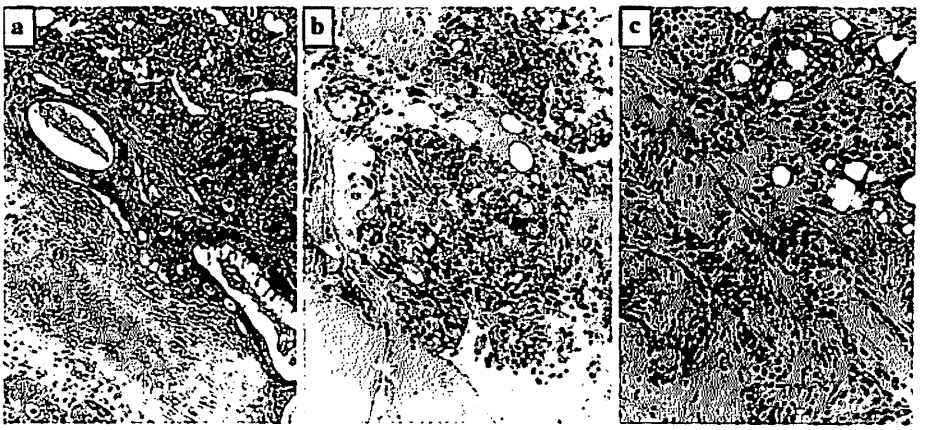

Fig. 3 a Histopathology confirms adenocarcinoma (Case 3), b signet ring cell carcinoma (Case 5), and c poorly differentiated carcinoma (Case 6) (magnification ×200)

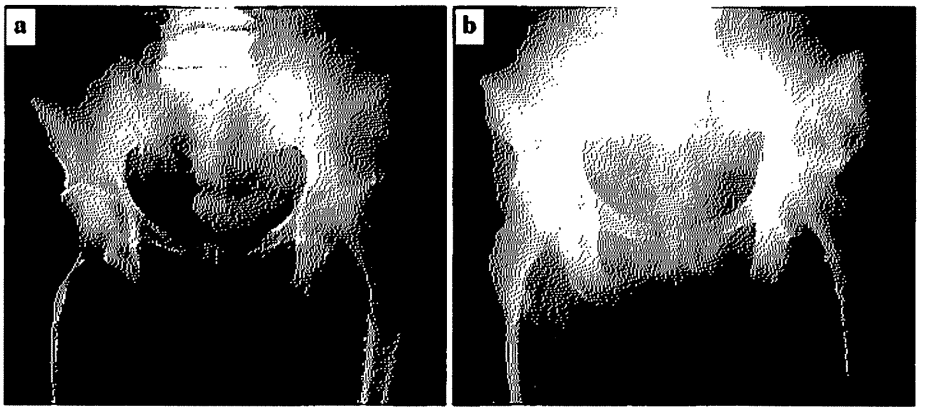

Fig. 4 Case 2. a Plain film shows the osteolytic lesion of bone metastases in the left ilium. b Plain film shows the prominent sclerotic change after local radiation therapy

If radiological imaging fails to confirm the location of the primary carcinoma site, biopsy is essential to rule out the possibility of the metastases being primary malignant bone tumors and to predict the cancer origin. In the present study, histopathological analysis revealed that the carcinoma types were adenocarcinoma in three cases, signet ring cell carcinoma in two cases and poorly differentiated carcinoma in two cases. Pavlidis et al. [3] reported that the histopathological types of carcinomas from primary unknown sites were mainly adenocarcinoma, followed by squamous cell carcinoma and poorly differentiated carcinoma. Prognosis of the patient differs depending on the type of carcinoma. Non-adenocarcinomas such as squamous cell carcinoma and neuroendocrine carcinoma have more favorable prognostic factors [1].

To investigate the location of the primary site of a carcinoma, FDG-PET imaging is being increasingly used. However, the efficacy of this approach is disputable. FDG-PET cannot be used to detect the presence of bone metastases, but can contribute in detecting extraskeletal metastases. Kole et al. [6] demonstrated that 27% of primary sites could be identified using FDG-PET after unsuccessful results using conventional diagnostic tools. In a review by Sève et al. [7] the detection rate of previously unknown primary tumor sites was found to be 41% using FDG-PET. We have been successful in using this modality for detecting primary carcinoma tumors at previously unknown sites.

At the first examination, multiple metastases, including bone and extraskeletal metastases, were already present in most cases. Systemic chemotherapy is the first choice of management for carcinoma metastases from unknown primary sites. Although systemic cancer chemotherapy should essentially be carried out according to conventional therapy protocols, an optimal standard chemotherapy regimen for carcinoma metastases from unknown primary sites has yet to be established. Multidrug regimens are generally administered as a first choice option. Previous reports [8–14] concerning representative systemic chemotherapy for carcinoma metastases from unknown primary sites, involving more than 50 patients, are summarized in Table 3.

Over the last two decades, the median survival time of patients with carcinoma metastases has been extended by approximately 7 months, due to the introduction of systemic chemotherapy using platinum. The administration of chemotherapy depends upon the general condition of the patient, but factors related to the therapeutic efficacy of chemotherapy may outweigh this consideration. More recently,

Table 3 Previous report on chemotherapy for carcinoma of unknown primary site

Authors	Year	No. of patients	Regimen	Response rate (%)	Survival median (months)	Reference
Bécouarn et al.	1989	85	5Fu/DOX/CDDP/HMM	21	7	[8]
Briasoulis et al.	1998	62	CBDCA/EPI/VP16	37.1	10	[9]
Greco et al.	2000	71	CBDCA/PTX/VP16	48	11	[10]
Briasoulis et al.	2000	77	CBDCA/PTX	38.7	13	[11]
Greco et al.	2002	120	CBDCA/PTX/GEM	25	9	[12]
Piga et al.	2004	102	CBDCA/DOX/VP16	26.5	9	[13]
Pittman et al.	2006	51	CBDCA/GEM	30.5	7.8	[14]

the administration of variable drug combinations, such as gemcitabine (GEM) and paclitaxel (TAX) in combination with platinum, has delivered improved response rates of 25–48%, and the median survival time has been increased to 9–11 months.

It appears that the patients diagnosed with carcinoma metastases from unknown primary sites have relatively limited life expectancy. The median survival time of patients involved in the present study was only 420 days. Hess et al. [1] reported that in their analysis of 1,000 patients, the median survival time was 11 months. Poor prognostic factors were age >61.5 years, the presence of liver metastases and tumor histologies other than neuroendocrine carcinoma and adrenal metastases [1]. In addition, the effect of bone metastases on survival depends on whether patients also have liver metastases. These factors may be important in the orthopedic oncologists' decision about whether or not to perform palliative surgery.

The limitation of the present study is that the number of patients was small and the power of the study is so limited, and the present study included the patients who were treated in a long span of 15 years, from March 1994 to 2009. In recent decades, early diagnosis using modern imaging techniques, such as whole body MRI and PET/CT, contribute to detect origin of primary sites in some cases [15]. And the extensive investigation with histopathological technology, immunohistochemistry [16, 17], electron microscopy [18] also gave some improvement for detection of primary site. Especially the therapeutic modality is also improving. Therefore, we may not evaluate the accurate up to date clinical features and result of the patients initially diagnosed with bone metastases from carcinoma with primary sites.

More recently, molecular gene profiling of carcinoma metastases biopsy samples has contributed to the detection of the primary site, and patients with metastases identified as originating from the colon have had a better response to their therapy [19]. It is now believed that in the future, such genetic analysis of biopsy samples will enable the delivery of cancer-specific treatments without the need for other costly and time-consuming diagnostic techniques.

Conflict of interest statement The authors declare that they have no conflict of interest.

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Prognostic factors for reduction of activities of daily living following osteoporotic vertebral fractures

Tomiya Matsumoto, MD; Masatoshi Hoshino, MD, PhD; Tadao Tsujio, MD; Hidetomi Terai, MD, PhD; Takashi Namikawa, MD, PhD; Akira Matsumura, MD, PhD; Minori Kato, MD, PhD; Hiromitsu Toyoda, MD, PhD; Akinobu Suzuki, MD, PhD; Kazushi Takayama, MD, PhD; Kunio Takaoka, MD, PhD; and Hiroaki Nakamura, MD, PhD

From the Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan

Corresponding author: Tomiya Matsumoto, MD

Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine

1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Tel: +81-6-6645-3851; Fax: +81-6-6646-6260

E-mail: tomiya@med.osaka-cu.ac.jp

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Structured Abstract

Study Design. Prospective cohort study

Objective. To elucidate the prognostic factors indicating reduced activities of daily living (ADL) at the time of the 6-month follow-up after osteoporotic vertebral fracture (OVF).

Summary of Background Data. OVF has severe effects on ADL and quality of life (QOL) in elderly patients and leads to long-term deteriorations in physical condition. Many patients recover ADL with acceleration of bony union and spinal stability, but some suffer from impaired ADL even months after fracture. Identifying factors predicting reduced ADL following OVF may prove valuable.

Methods. Subjects in this prospective study comprised 310 OVF patients from 25 institutes. All patients were treated conservatively without surgery. Pain, ADL, QOL and other factors were evaluated on enrollment and at 6 months. ADL were evaluated using the criteria of the Japanese long-term care insurance system to evaluate the degree of independence. We defined reduced ADL as a reduction of at least single grade at 6 months after fracture and investigated factors predicting reduced ADL following OVF using uni- and multivariate regression analysis.

Results. ADL were reduced at 6 months after OVF in 66 of 310 patients (21.3%). In univariate analysis, age >75 years (P=0.044), female sex (P=0.041), □2 previous spine fractures (P=0.009), presence of middle column injury (P=0.021), and lack of regular exercise before fracture (P=0.001) were significantly associated with reduced ADL. In multivariate analysis, presence of middle column injury (odds ratio (OR), 2.26; P=0.022) and lack of regular exercise before fracture (OR, 2.49; P=0.030) were significantly associated with reduced ADL.

Conclusion. These results identified presence of middle column injury of the vertebral body and lack of regular exercise before fracture as prognostic factors for reduced ADL. With clarification and validation, these risk factors may provide crucial tools for determining subsequent OVF

treatments. Patients showing these prognostic factors should be observed carefully and treated with more intensive treatment options.

Key words: osteoporosis; vertebral fracture; activities of daily living; prognostic factor

Mini Abstract

Prognostic factors inducing reduced ADL following osteoporotic vertebral fracture were investigated. The percentage of patients with reduced ADL was 21.3%. Prognostic factors were the presence of middle column injury and lack of regular exercise before fracture.

Key points

- We investigated prognostic factors for reduced ADL following OVF in a prospective multicenter study.
- Reduced ADL after OVF were seen in 21.3% of patients after 6 months of conventional conservative treatment.
- Factors significantly associated with reduced ADL were the presence of middle column injury of the vertebral body and lack of regular exercise before fracture.

INTRODUCTION

Fracture associated with osteoporosis has become a major problem with the increasing population of elderly individuals[1]. Vertebral body fracture is the most frequent type of osteoporotic fracture[2]. The lifetime risk of osteoporotic vertebral fracture (OVF) for a 50-year-old woman is estimated as 32%, compared with a 15.6% lifetime risk of hip fracture[3].

OVF has a severe impact on activities of daily living (ADL) and quality of life (QOL) in elderly patients and can represent the beginning of a long-lasting deterioration in health[4-8]. Symptomatically, severe back pain caused by such fractures gradually subsides with progression of bony union and spinal stability[9,10]. However, severe and intractable pain with[9] or without associated neurological symptoms occasionally continues in a limited number of cases[11], and ADL in these patients are markedly impaired[6,12]. Identification of prognostic factors for reduced ADL following OVF in a prospective study may be particularly valuable. To date, almost all studies of the relationships of compression fracture to pain, ADL and QOL have been retrospective[13-17]. Several possible factors may affect ADL in patients with OVF. However, factors inducing impairment of ADL remain unclear. The purpose of this study was to elucidate prognostic factors inducing reduced ADL by 6-month follow-up after OVF.

MATERIALS AND METHODS

Patient population

Twenty-five institutes in the Osaka area of Japan participated in this prospective cohort study. Patients >65 years old and with a fresh OVF were enrolled in this study after providing written informed consent. Upon an initial visit to the respective institute, fresh vertebral fracture was diagnosed based on acute back pain, a deformed vertebral body on radiography and abnormal intensity within the vertebral bodies on magnetic resonance imaging (MRI). The study design was approved by the ethics committees for clinical research at each institute.

A total of 485 patients were enrolled. Of these, 15 patients died, 11 were excluded because of other diseases and 39 patients were lost to follow-up. As a result, 420 patients completed the 6-month follow-up (follow-up rate, 86.6%). Among these 420 patients, 310 patients (44 men,