Vertebral Histomorphometry in a Child with Glucocorticoid-Induced Osteoporosis

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Vertebral fractures are an under-recognized problem in children with glucocorticoid-induced osteoporosis (GIO). They cause severe back pain and spinal column deformity with a decrease of quality of life. For evaluating the bone mass, bone mineral density measurements have been widely carried out using dual energy X-ray absorptiometry. However, bone histomorphometric analyses of GIO in children are scarce. Bone histomorphometric analyses of vertebral bodies have not been reported. Our aim is to report the first bone histomorphometric data for vertebrae from an autopsied child with GIO. A 15-year-old girl with systemic lupus erythematosus was started on a daily oral dose of 10 mg of prednisolone at 6 years of age. She presented with back pain from 12 years of age. Magnetic resonance imaging at 14 years of age showed a compression fracture of the first lumbar (L1) vertebral body. At 15 years of age, she died of heart failure owing to pulmonary hypertension. Collapsed (L1) and non-collapsed (seventh thoracic vertebrae; T7) vertebral bodies were autopsied for bone histomorphometry and compared. T7 showed severe osteoporosis (bone volume, 4.99%; trabecular thickness, 59 μm; trabecular separation, 1,134 μm). Compared with T7, L1 showed increased bone volume (33.9%) and trabecular thickness (77 μm), and decreased trabecular separation (156 μm) owing to the impact of the vertebral fracture. The bone formation and bone resorption parameters were comparable between the two vertebrae. These histological findings suggest that severe osteoporosis developed after long-term glucocorticoid administration, and that the remodeling activities were similar in the fractured and non-fractured vertebrae.

Keywords: bisphosphonate; bone histomorphometry; glucocorticoid-induced osteoporosis; growth; osteoblast

Glucocorticoid-induced osteoporosis (GIO) is the most common cause of secondary osteoporosis in adults, and is the result of profound effects of glucocorticoids on bone cells (Canalis et al. 2004). Glucocorticoids inhibit osteoblastogenesis and promote osteoblast apoptosis, thereby leading to significant reductions in bone formation (Weinstein et al. 1998). The growing skeleton may be especially vulnerable to the adverse effects of glucocorticoids on bone formation, which could possibly compromise trabecular and cortical bone accretion.

Compression fractures of the vertebral body are an under-recognized problem in children with GIO (Varonos et al. 1987; Valta et al. 2007; Huber et al. 2010). They cause severe back pain and spinal column deformity with a decrease of quality of life. For evaluating the bone mass, bone mineral density measurements have been widely carried out using dual energy X-ray absorptiometry. However, bone histomorphometric analyses of GIO in children are scarce. A histomorphometric evaluation of GIO in children with chronic illnesses has been reported for specimens obtained by iliac bone biopsy (Ward et al. 2004). To the best of our knowledge, however, bone histomorphometric analyses of vertebral bodies in children with GIO have not been reported in the literature. Here, we report the first bone histomorphometric data for vertebrae from an autopsied child with GIO.

Patient and Methods

A 15-year-old girl with systemic lupus erythematosus (SLE) developed a fever at 6 years of age, and was started on a daily oral dose of 10 mg of prednisolone. The dosage of glucocorticoid was doubled stepwise to control the SLE. For the purpose of preventing GIO, she was administered 0.25 μg/day of 1,25-dihydroxyvitamin D from 8 years of age, and 2.5 mg/day of risedronate (a bisphosphonate)
from 11 years of age. However, she presented with back pain from 12 years of age.

On physical examination at 12 years of age, the height was 129.2 cm, the weight was 47.5 kg and the body mass index was 28.4. There were no abnormal findings in routine blood tests, and liver and renal function tests. X-ray examinations indicated a delayed bone age, because a secondary ossification center for the iliac crest did not appear on pelvic X-rays. From 13 years of age, she was complicated with pulmonary hypertension. Menarche did not occur until 15 years of age.

Magnetic resonance imaging (MRI) of the thoracic and lumbar spine at 12 years of age showed no abnormalities. The bone mineral density (BMD) of the lumbar spine was 0.505 g/cm² (normative data for Japanese girls at 12 years of age: 0.824 g/cm²) (Nishiyama and Okada 2001). MRI of the spine at 13 years of age revealed fatty marrow changes of the vertebrae, and her BMD had decreased to 0.488 g/cm² (Nishiyama and Okada 2001). MRI at 14 years of age showed a compression fracture of the first lumbar (L1) vertebral body with mild vertebral collapse. Six months later, the fourth and fifth lumbar vertebral bodies also collapsed and thoracolumbar kyphosis increased.

At 14 years of age, a peripheral blood stem cell auto-transplantation was performed, with little or no improvement in the SLE. Six months later, the patient died of heart failure owing to pulmonary hypertension.

**Bone sample preparation**

The seventh thoracic (T7; non-collapsed) and L1 (collapsed) vertebral bodies were autopsied for bone histomorphometry. The specimens were fixed with 10% ethanol and decalcified with K-CX (FALMA Co. Ltd., Tokyo, Japan). Sections of 5-µm thickness were obtained and subjected to Cole’s hematoxylin-eosin staining. This study was approved by the Ethical Committee of Nakadori General Hospital.

**Bone histomorphometry**

Bone histomorphometry was performed using a semiautomatic graphic system (Histometry RT Camera; System Supply Co., Nagano, Japan). Measurements were obtained in regions at 390 µm from the lowest point of the growth plates in both the cranial and caudal directions as well as at 390 µm from the endocortical surfaces at a magnification of 100 ×.

The following histomorphometric parameters were measured: percent bone volume (BV/TV, %), defined as the percentage of trabecular bone volume (BV) to tissue volume (TV); trabecular thickness (Tb.Th, µm); trabecular separation (Tb.Sp, µm); percent osteoid surface (OS/BS, %), defined as the percentage of osteoid surface (OS) to bone surface (BS); and percent eroded surface (ES/BS, %), defined as the percentage of eroded surface (ES) to BS. In addition to the measurements on conventional bone histomorphometry, we measured the percentage of fat tissue to TV (Fat/TV, %) at the same region by bone histomorphometry.

To evaluate the trabecular connectivity, we evaluated the autopsied vertebrae using a node-strut analysis. We defined the parameters of the node-strut analysis according to the methods described by Mellish et al. (1991). The trabecular structure was subdivided into nodes and termini, and the node number (N.Nd/TV, per µm²) and terminus number (N.Tm/TV, per µm²) were expressed per square micrometer of tissue volume. A node (Nd) was defined as the junction point between two or more trabeculae, while a terminus (Tm) was defined as the end of a trabecula that was unconnected to any other trabecular element within the space of the section. The following struts were defined by drawing lines between the nodes and termini: node-to-node (NdNd); terminus-to-terminus (TmTm); and node-to-terminus (NdTm). The strut length was expressed as the percentage of the total strut length (TSL), and NdNd/TSL (%), TmTm/TSL (%) and NdTm/TSL (%) were measured.

Using these methods, high node parameters indicated high trabecular connectivity, while high terminus parameters indicated low trabecular connectivity.

**Results**

**Histological findings**

The non-collapsed T7 vertebra showed a substantial decrease in cancellous bone and the bone marrow was extensively filled with adipose tissue (Fig. 1). The trabecular bones were very thin and the trabecular separations were wide. Most of the trabecular bones of T7 showed rod-like shapes with less trabecular connectivity. Compared with the non-collapsed T7 vertebra, the collapsed L1 vertebra showed less fatty marrow, and increased trabecular bone and connectivity with many osteoblasts and newly formed bone (Fig. 2). These histological findings of L1 were consistent with the healing process after the fracture.

![Fig. 1. Histological findings of the non-collapsed seventh thoracic vertebral body.](image-url)

The histological sections of the non-collapsed T7 vertebral body show the fatty marrow and the substantial decreases in cancellous bone and trabecular connectivity. Hematoxylin-eosin staining. Original magnifications: a, × 2; b, × 20.
Bone Histomorphometric Data of Autopsied Vertebrae

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Bone and fat histomorphometry

The bone and fat histomorphometric findings are shown in Table 1. T7 showed severe osteoporosis with lower BV/TV and Tb.Th and wider Tb.Sp compared with L1. L1 showed increased BV/TV and Tb.Th and decreased Tb.Sp owing to the impact of the vertebral fracture. OS/BS and ES/BS in the two vertebrae were comparable. Thus, the remodeling activity in the fractured vertebra after healing may have been similar to that in the non-fractured vertebra during glucocorticoid administration. The Fat/TV of T7 (31.3%) was larger than that of L1 (4.3%), indicating fatty marrow changes of the vertebral body in GIO.

Node-strut analysis

The results of the node-strut analysis are shown in Table 2. Owing to the few trabecular connections in the non-fractured T7, the node-related parameters including N.Nd/TV, NdNd/TSL and NdTm/TSL of T7 were lower than those of L1. Conversely, the N.Tm/TV of L1 was higher than that of T7. The TmTm/TSL of L1 was smaller than that of T7.

Discussion

Secondary osteoporosis is increasingly recognized as a complication of chronic diseases in children, particularly when glucocorticoids are necessary for treatment. Iliac bone histomorphometry of secondary osteoporosis in children has been reported (Ward et al. 2004). However, to the best of our knowledge, bone histomorphometric evaluations of autopsied vertebral bodies in children with GIO have not been reported in the literature. This is the first report regarding bone histomorphometric and node-strut analyses.

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**Table 1. Data from the bone histomorphometric analysis.**

<table>
<thead>
<tr>
<th></th>
<th>BV/TV (%)</th>
<th>Tb.Th. (μm)</th>
<th>Tb.Sp. (μm)</th>
<th>OS/BS (%)</th>
<th>ES/BS (%)</th>
<th>Fat/TV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>4.99</td>
<td>59</td>
<td>1,134</td>
<td>19.85</td>
<td>20.80</td>
<td>31.3</td>
</tr>
<tr>
<td>L1</td>
<td>33.9</td>
<td>77</td>
<td>156</td>
<td>15.57</td>
<td>16.56</td>
<td>4.3</td>
</tr>
</tbody>
</table>

BV/TV, bone volume/tissue volume; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; OS/BS, osteoid surface/bone surface; ES/BS, eroded surface/BS; Fat/TV, fat tissue/TV.

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**Table 2. Data from the node-strut analysis.**

<table>
<thead>
<tr>
<th></th>
<th>N. Nd/TV (N/μm²)</th>
<th>N.Tm/TV (N/μm²)</th>
<th>NdNd/TSL (%)</th>
<th>TmTm/TSL (%)</th>
<th>NdTm/TSL (N/μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7</td>
<td>0.0000000</td>
<td>0.000004</td>
<td>0.87</td>
<td>92.9</td>
<td>6.2</td>
</tr>
<tr>
<td>L1</td>
<td>0.000011</td>
<td>0.000016</td>
<td>47.0</td>
<td>11.1</td>
<td>41.9</td>
</tr>
</tbody>
</table>

N.Nd/TV, node-number/tissue volume; N.Tm/TV, terminus-number/TV; NdNd/TSL, node-to-node/total strut length; TmTm/TSL, terminus-to-terminus/TSL; NdTm/TSL, node-to-terminus/TSL.

Owing to the few trabecular connections in the non-fractured T7 vertebra, the node-related parameters N.Nd/TV, NdNd/TSL and NdTm/TSL are lower for T7 than for L1. Conversely, the N.Tm/TV of L1 is higher than that of T7. The TmTm/TSL of L1 is smaller than that of T7.

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**Fig. 2.** Histological findings of the collapsed first lumbar vertebral body.

The histological sections of the collapsed L1 vertebral body show the healing process after the fracture with many osteoblasts, newly formed bone and the increased trabecular bone and connectivity. Hematoxylin-eosin staining. Original magnifications: a, × 2; b, × 20.
of vertebral bodies in a child with GIO.

It has been noted that the BV/TV of a vertebral body is correlated with that of the iliac bone (Meunier et al. 1973; Mellish et al. 1991). The BV/TV of T7 in the present patient (4.99%) was severely low compared with that of the iliac bone in healthy females at 15-19 years of age (24.5%) (Meunier et al. 1973). In addition, the percentage of fat tissue was increased compared with the bone volume, indicating fatty marrow changes caused by the glucocorticoid treatment.

Chappard et al. (1996) performed both histomorphometric and node-strut analyses on biopsied iliac bone from asthmatic patients under glucocorticoid treatment, and concluded that trabecular perforations developed with Tb.Th below 70 μm when the BV/TV was less than 11%. In the present case, the BV/TV and Tb.Th were 4.99% and 59 μm at T7, respectively. The results of the node-strut analysis also showed severely decreased trabecular connectivity in the present patient. These findings strongly suggest that our patient had seriously advanced osteoporosis with trabecular disconnectivity caused by glucocorticoid administration as a child.

With regard to bone turnover under glucocorticoid treatment in children, it has been reported that long-term glucocorticoid therapy causes a reduction in bone turnover (Canalis and Delany 2002; Ward et al. 2004). In the present study, the bone formation parameter (OS/BS) was 19.85% and 15.57% in the non-fractured T7 and fractured L1 vertebrae, respectively. The bone resorption parameter (ES/BS) was 20.8% and 16.56% in the non-fractured T7 and fractured L1 vertebrae, respectively. Since there are no previous bone histomorphometric studies of autopsied vertebral bodies in children with GIO, we have difficulty in evaluating these values. However, the OS/BS in the present patient was slightly lower than the normative data for the iliac bone of children at 14-16 years of age (25.7%) (Glorieux et al. 2000). The ES/BS was comparable to that for the iliac bone of children at 14-16 years of age (18.0%) (Glorieux et al. 2000). Thus, it is suggested that the systemic and continuous glucocorticoid administration to this child for 6 years since puberty may have caused slight inhibition of bone formation, resulting in a negative bone balance and osteoporosis. The present patient was given a daily oral dose of 2.5 mg of a bisphosphonate (risedronate) for 36 months. Bisphosphonates have been widely used in the prophylaxis and treatment of GIO in adults, and their efficacy and safety in children with GIO are gradually being reported (Bianchi et al. 2000; Rudge et al. 2005). Brumsen et al. (1997) studied the long-term effects of bisphosphonates in the growing skeleton. They reported that there was no excessive suppression of bone remodeling, and bone biopsies of the iliac crest showed bone with a normal lamellar structure without mineralization defects. To the best of our knowledge, no studies on bone repair or fracture healing in children with bisphosphonate therapy before closure of the growth plates have been reported. The histological features of L1 in our patient showed marked osteoblasts around the trabecular bone and new bone formation following fracture healing. These histological findings suggest that the process of bone fracture healing existed under administration of bisphosphonates, and was not severely disturbed.

In conclusion, we used histomorphometric and node-strut analyses to evaluate autopsied vertebral bones from a 15-year-old girl with GIO after treatment with a bisphosphonate for 6 years. The bone volume was severely reduced with thinning and disconnectivity of the trabeculae caused by glucocorticoid therapy, even though the treatment was a bisphosphonate. The bone turnover was suppressed and fat tissue was increased in the non-fractured vertebral body examined. Trabecular bone repair after vertebral fracture was observed by bone histomorphometry in the condition of GIO treated with a bisphosphonate.

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Conflict of Interest

The authors declare that they have no conflict of interest in this study.

References


