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Posterior lumbar interbody fusion with total facetectomy for low-dysplastic isthmic spondylolisthesis: effects of slip reduction on surgical outcomes

Clinical article

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Object. The management of isthmic spondylolisthesis remains controversial, especially with respect to reduction. There have been no reports regarding appropriate slip reduction. The purpose of this study was to investigate the following issues: 1) surgical outcomes of posterior lumbar interbody fusion (PLIF) with total facetectomy for low-dysplastic isthmic spondylolisthesis, including postoperative complications; 2) effects of slip reduction on surgical outcomes; and 3) appropriate slip reduction.

Methods. A total of 106 patients who underwent PLIF with total facetectomy for low-dysplastic isthmic spondylolisthesis and who were followed for at least 2 years were reviewed. The average follow-up period was 8 years. Surgical outcomes, including the scores assessed using the Japanese Orthopaedic Association scoring system, the recovery rate, and postoperative complications were investigated. As for radiographic evaluations, pre- and postoperative slip and disc height, instrumentation failure, and fusion status were also examined.

Results. The pre- and postoperative average Japanese Orthopaedic Association scores were 14 (range 3–25) and 25 (range 11–29) points, respectively. The average recovery rate was 73% (range 0%–100%). The average pre- and postoperative slip was 24% and 10%, respectively. A significant correlation between postoperative slip and clinical outcomes was found; clinical outcomes were better in proportion to slip reduction. Although no statistical difference was detected in clinical outcomes between postoperative slip of less than 10% and from 10% to 20%, patients with postoperative slip of more than 20% showed significantly worse clinical outcomes. Postoperative complications included neurological deficits in 7 patients (transient motor loss in 6 and permanent motor loss in 1), instrumentation failures in 7, adjacent-segment degeneration in 5, and nonunion in 4. Instrumentation failures occurred significantly more often in patients with more slip reduction, although slip reduction did not affect the other postoperative complications. All patients with instrumentation failure showed postoperative slip reduction within 10%.

Conclusions. The use of PLIF with total facetectomy for low-dysplastic isthmic spondylolisthesis appears to produce satisfactory clinical outcomes, with an average of 73% recovery rate and few postoperative complications. Although clinical outcomes were better in proportion to slip reduction, excessive reduction caused instrumentation failure, and patients with less reduction demonstrated worse clinical outcomes. Appropriate reduction resulted in a postoperative slip ranging from 10% to 20%.
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Key Words • **posterior lumbar interbody fusion** • **total facetectomy** • **isthmic spondylolisthesis** • **reduction** • **surgical outcome**

Radicular pain of the lower extremity is one of the important symptoms of isthmic spondylolisthesis. The causes of this symptom are compression and/or irritation of nerve roots at the foraminal level around the isthmic site. The compressive factors are proliferative fibrocartilagenous tissue at the isthmic site, a bulged or herniated intervertebral disc at the slipped segment, foraminal stenosis associated with large slip, and a combination of these factors. Due to the mechanically unstable nature of isthmic spondylolisthesis, a fusion procedure is standard surgical management for this pathological condition.

Abbreviations used in this paper: ASD = adjacent-segment degeneration; JOA = Japanese Orthopaedic Association; MMT = manual muscle test; PLIF = posterior lumbar interbody fusion.

We perform posterior lumbar interbody fusion (PLIF) with total facetectomy to treat isthmic spondylolisthesis. The PLIF procedure provides sufficient decompression of the nerve root and stabilization of the affected segment. Use of PLIF with pedicle screw fixation has produced satisfactory clinical results,[18,21–23] but the necessity for slip reduction and restoration of disc height is still a controversial subject. Slip reduction has an advantage for fusion because the contact area of the interbody arthrodesis site is larger with reduction.[2,3,19,31] On the other hand, the reduction maneuver sometimes causes neurological deficits or instrumentation failure.[8,17,20,29,31] There have been few reports of the degree of slip reduction,[10,15,32] and furthermore there have been no reports about appropriate slip reduction.

The purpose of this study was to investigate the following issues: 1) surgical outcomes of PLIF with total facetectomy for low-dysplastic isthmic spondylolisthesis, including postoperative complications; 2) effects of slip reduction on surgical outcomes; and 3) appropriate slip reduction. To the best of our knowledge, this is the first report to describe the surgical outcomes of PLIF and appropriate reduction for isthmic spondylolisthesis with uniform instrumentation and a uniform fusion technique, involving more than 100 patients with an average follow-up of 8 years.

Methods

This is a retrospective study. All patients gave informed consent and the study was approved by the institutional review board.

Patient Population

In the present study, low-dysplastic isthmic spondylolisthesis was characterized by a normal vertebral shape, a normal lumbosacral profile, and a balanced pelvis without retroversion.[7] Of 109 consecutive patients who underwent PLIF with total facetectomy for low-dysplastic isthmic spondylolisthesis between 1996 and 2010, 106 patients who were followed for at least 2 years were included in this study. Patients with high-dysplastic spondylolisthesis, which is characterized by a wedge L-5 and a domed and vertical sacrum, were excluded.[14] Patients who had undergone previous lumbosacral fusion surgery were also excluded in this series. The follow-up rate was 97%. There were 36 women and 70 men. The mean age at surgery was 57 years (range 22–79 years), and the average follow-up period was 8 years (range 2–17 years). The levels of operation were L4–5 in 30 patients and L5–S1 in 76 patients.

Surgical Indication and Procedure

All patients considered for surgery had severe, disabling radicular pain with or without low-back pain that was unresponsive to conservative treatment such as medication, physical therapy, and root and/or epidural block. All PLIF procedures were performed using the same technique, which has been described elsewhere.[23] The procedure involves complete resection of the spondylolytic floating lamina including bilateral inferior articular processes; bilateral resection of the superior articular processes; decompression of the nerve root by excising proliferative fibrocartilagenous tissue at the isthmic site and performing subtotal discectomy; insertion of the interbody fusion cages and trimmed autologous bone to the intervertebral space; and pedicle screw fixation with the Steffee Variable Spine Plating System (DePuy Spine). We performed bilateral total facetectomies to have wide exposure of the neural elements and the disc space.

Interbody arthrodesis was performed by placing 2 cages that were sandwiched between a minimum of 2 autologous bone blocks, and chips that were trimmed from the excised lamina and facets. The posterior iliac crest was not harvested, and posterolateral arthrodesis was not performed at any level. Cages with a width of 9 mm, a height of 9 mm, and a length of 25 mm were generally used in patients with preoperative slip of less than or equal to 25%, and cages with a width of 9 mm, a height of 7 mm, and a length of 22 mm were used in patients with preoperative slip of greater than 25%. The first size of cage was used in 81 patients, the second size of cage was used in 21 patients, and autologous bone alone was used in 4 patients.

Basically, an interbody arthrodesis procedure was performed bilaterally, but 3 patients underwent unilateral arthrodesis because it was difficult to expose the unilateral interbody space due to the conjoint nerve root. Fluoroscopic guidance or computer navigation was not used during pedicle screw insertion. Pedicle screw fixation was applied at the fused segment, but instrumentation was extended to the cranial adjacent level in 1 patient because of difficulty in screw insertion due to a hypoplastic pedicle. As for technical features of plates and pedicle screw systems, slip reduction depended on plate bending and plate connection with pedicle screws. We did not attempt complete slip reduction, but attempted to achieve it for the length of the grafted cages.

Clinical Assessment

The complete medical records of all patients were available for review. These records were reviewed to determine demographic data, clinical results, and postoperative complications. Clinical outcomes were assessed using the scoring system proposed by the Japanese Orthopaedic Association (JOA).[22] Briefly, the JOA score consists of subjective symptoms (low-back pain, leg pain, and gait; 3 points each); clinical symptoms (straight leg raising test, sensory abnormality, and motor disturbance; 2 points each); restriction of activities of daily living (14 points); and urinary bladder function (6 points). A normal JOA score is 29 points (Table 1). The recovery rate of the clinical outcomes, which indicates the degree of normalization after surgery, was evaluated using the Hirabayashi method, as follows[22]: Recovery rate (%) = (postoperative score – preoperative score) × 100/(full score – preoperative score).

Clinical assessments were performed for all patients before surgery and at 1, 6, 12, 18, and 24 months after surgery, and then annually. Patients were divided into 4 groups according to the recovery rate (excellent, recovery rate greater than or equal to 75%; good, 50%–74%; fair,

TABLE 1: Surgical outcomes in 106 patients who underwent PLIF with total facetectomy

Outcome	Value (range)	
	Preop	Postop
clinical		
JOA score	14 (3–25)	25 (11–29)
% recovery rate		73 (0–100)
radiological		
% slip	24 (5–55)	10 (−8 to 33)
% slip reduction		14 (0–36)
disc height in mm	5 (0–9)	10 (6–15)
disc height restoration in mm		5 (1–12)

25%–49%; poor, less than 25%), and the number in each group was analyzed.

In the current study, postoperative complications were defined as spine-specific complications such as neurological deficits, instrumentation failure, adjacent-segment degeneration (ASD), and fusion failure. Adjacent-segment degeneration was defined as a condition in which additional surgery was required to treat neurological deterioration caused by the adjacent segment. Complications that were not specific for spine surgery or did not affect recovery (for example, urinary tract infection and anemia) were excluded.

Radiological Assessment

Plain radiographs were obtained in all patients at 1, 6, 12, 18, and 24 months after surgery, and annually thereafter. Slip and disc height were measured on lateral radiographs. As the index of slip, the percentage of slip was used. The maximum value of slip on dynamic lateral radiographs was used as the degree of preoperative slip. Slip reduction was evaluated by comparing the value on the lateral radiograph immediately after operation with the preoperative value. As the index of disc height, the distance between the upper and lower vertebral endplates perpendicularly measured from a point equidistant on the bisector line drawn connecting the middle points of the anterior and posterior disc heights on a neutral lateral radiograph was measured.[16] The change in disc height was evaluated by comparing the immediate postoperative value with the preoperative value. Measurements for slip and disc height were calculated by 3 surgeons (R.Y., T.H., and T.M.) who were blinded to the clinical results.

Fusion status was also examined. Solid fusion was defined as a condition in which bony continuity between graft bone and the vertebra was detected, without loosening of the pedicle screws or motion at the fused segment in flexion and extension lateral radiographs.[21,22] If solid fusion was not detected 1 year after surgery, plain and reconstruction CT scanning was performed to confirm bony continuity between graft bone and the vertebra. Nonunion was defined as a condition in which bony continuity between graft bone and vertebra was not detected on plain radiographs or reconstructed CT scans, along with loosening of pedicle screws or apparent motion at the fused segment on flexion and extension lateral radiographs for more than 2 years.

Effects of Slip and Disc Height Reduction on Clinical Outcomes

The effects of pre- and postoperative slip and pre- and postoperative disc height on surgical outcomes were investigated.

Statistical Analysis

Findings from these measurements were analyzed statistically by using simple regression analysis, the Student t-test, chi-square analysis, the Fisher exact probability test, and Spearman rank correlation coefficients. A p value less than 0.05 was the minimum level of statistical significance.

Results

Clinical Outcomes

The clinical outcomes are shown in Table 1. Overall, pre- and postoperative average JOA scores were 14 (range 3–25) and 25 (range 11–29) points, respectively. The average recovery rate was 73% (range 0%–100%). Surgical outcome was excellent in 64 patients (60%), good in 24 (23%), fair in 11 (10%), and poor in 7 (7%).

Radiological Outcomes

The average pre- and postoperative slip values were 24% (5%–55%) and 10% (−8% to 33%), respectively. The average slip reduction was 14% (0%–36%). The average pre- and postoperative disc heights were 5 mm (0–9 mm) and 10 mm (6–15 mm), respectively. The average disc height restoration was 5 mm (1–12 mm) (Table 1).

Postoperative Complications

Postoperative neurological deficits were observed in 7 patients (7%) (Table 2). Motor loss was seen immediately after surgery in 4 patients and a few days after surgery in 3 patients. Of the 4 patients who showed motor loss immediately after surgery, slight motor loss with a manual muscle test (MMT) grade of 4 was observed in 3 patients and they recovered fully, whereas severe motor loss (MMT Grade 1) was seen in 1 patient and was permanent. This patient with permanent motor loss showed a postoperative

TABLE 2: Postoperative complications in 106 patients who underwent PLIF with total facetectomy

Complication	No. (%)
neurological deficits	7 (7)
transient motor loss	6 (6)
permanent motor loss	1 (1)
instrumentation failure	7 (7)
ASD	5 (5)
nonunion	4 (4)

slip value of 33%. Of 3 patients who showed motor loss a few days after surgery, slight motor loss (MMT Grade 4) was observed in 2 patients and they recovered fully, whereas severe motor loss (MMT Grade 2) was seen in 1 patient, who underwent additional surgery for postoperative hematoma and recovered fully thereafter.

Instrumentation failures were observed in 7 patients (7%) (Table 2); pedicle screw loosening was observed in 4 patients, and pedicle screw breakage was seen in 3. All pedicle screw loosening was observed at the cranial side. These patients did not report back pain or radicular pain, but nonunion was detected in 1 asymptomatic patient. In the other 3 patients, bony union was observed at the final follow-up. All pedicle screw breakages were observed at the caudal side. Of the 3 patients with pedicle screw breakage, 2 had no complaints, and bony union was observed at the final follow-up. The remaining patient, who had suffered a traffic accident, complained of severe low-back pain and showed bilateral pedicle screw breakage and nonunion at 3 years after surgery. This patient underwent revision surgery including implant removal, curettage of the bone graft area with addition of iliac bone graft, and extension of the fusion area.

Adjacent-segment degeneration was observed in 5 patients (5%: 3 men and 2 women) (Table 2). The mean age at primary surgery was 49 years (range 34–71 years). Progression of ASD was observed at the cranial segment in all cases. The conditions encountered at the secondary operations were degenerative spondylolisthesis in 1 patient and disc herniation in 4 patients. The average period between the primary and secondary operations was 5.4 years (range 2–9 years).

Nonunion was observed in 4 patients (4%) (Table 2). Of the 4 patients with nonunion, 2 had no complaints, but 2 complained of severe low-back pain that was unresponsive to conservative treatment and was treated with revision surgery. These patients underwent replacement of pedicle screws and addition of iliac bone graft at the revision surgery. Interbody cages and bone graft that had been inserted at the primary surgery were not replaced. One of these patients was mentioned in the "instrumentation failure" section and another showed a postoperative slip value of 33%.

Effects of Slip and Disc Height Reduction on Clinical Outcomes

Significant correlations were detected between final JOA scores and postoperative slip ($p = 0.001$) and slip reduction ($p = 0.010$), whereas preoperative slip did not affect clinical outcomes ($p = 0.734$) (Fig. 1). Significant correlations were also detected between recovery rate and postoperative slip ($p = 0.013$) and slip reduction ($p = 0.016$) (data not shown). Clinical outcomes were better in proportion to slip reduction. Although no statistical difference was detected in clinical outcomes between postoperative slip of less than 10% and from 10% to less than 20%, patients with postoperative slip of more than 20% showed significantly worse clinical outcomes (Table 3). Significant differences in postoperative slip ($p = 0.030$) and slip reduction ($p = 0.026$) were detected between the excellent and poor groups. Postoperative slip values in the excellent and poor groups were 8% and 18%, respectively, and slip reductions in the excellent and poor groups were 14% and 4%, respectively (Fig. 2). On the other hand, no correlation was detected between pre- and postoperative disc height and clinical outcomes.

As for postoperative complications, pre- and postoperative slip values and disc height did not affect the occurrence of neurological deficits and ASD. Nonunion appeared to occur more often in patients with less slip reduction, although no significant difference was detected (Fig. 3). The average postoperative slip values with and without nonunion were 20% (7%–33%) and 10% (–8% to 33%), respectively. Instrumentation failure was significantly more common in patients with more reduction than in those without instrumentation failure ($p = 0.009$). The average postoperative slip values with and without instrumentation failure were 4% (0%–10%) and 10% (–8% to

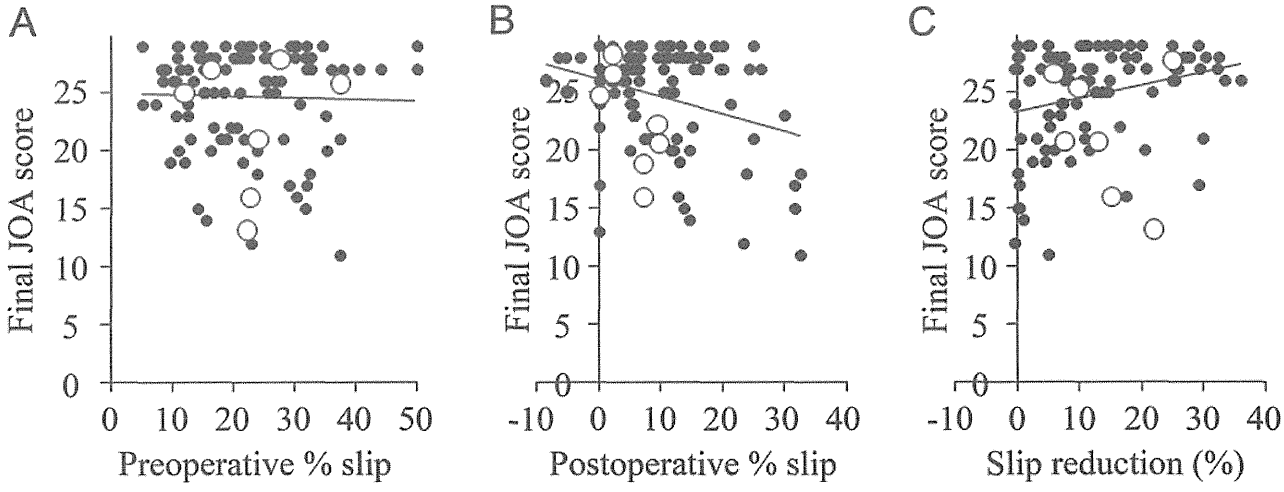

Fig. 1. Scatterplots showing that significant correlations were detected between final JOA scores and postoperative slip ($p = 0.001$) and slip reduction ($p = 0.010$). Clinical outcomes were better in proportion to slip reduction. All patients with instrumentation failure showed postoperative slip values within 10%. *Empty circles* refer to the patients with instrumentation failure.

TABLE 3: Postoperative slip and clinical outcomes in 106 patients who underwent PLIF with total facetectomy

Postop Slip	No. of Patients	JOA Score		Recovery Rate
		Preop	Postop	
<10%	61	14.4	25.4	75.3
10%–20%	33	14.6	25.1	72.9
>20%	12	14.8	20.1*	37.3*

* $p < 0.05$.

33%), respectively. All patients with instrumentation failure showed postoperative slip values within 10% (Fig. 1).

Discussion

The management of isthmic spondylolisthesis remains controversial, especially with respect to slip reduction. Our concept for slip reduction was to achieve it for the length of the grafted cage between the upper and lower vertebrae by using a plate connection system, not to perform intentional reduction. Although slip reduction can provide better function of the lumbosacral junction, at least in theory, several authors have recommended in situ fusion surgery rather than reduction surgery, considering postoperative complications such as neurological deficits.[1,4–6,11,13,17,20,26,27,29] On the other hand, previous reports have detailed that pseudarthrosis rates were decreased by reduction.[2,3,12,19,28,30,31,33] Therefore, surgery for isthmic spondylolisthesis requires management of both postoperative neurological deficits and the union rate.

A postoperative neurological deficit is generally considered to be a serious complication. Current instrumentation systems have provided tools to correct slippage. However, these operations involve an increased risk of neurological complications from the screws, and the possibility of distracting neurological elements during the corrective procedure. Ogilvie reviewed the incidence of neurological complications with isthmic spondylolisthesis and found that it has increased with progress in instrumentation surgery, although the incidence of complications with degenerative spondylolisthesis has not increased.[20] In laboratory studies the strain on the L-5 nerve root is nonlinear during reduction, with a small amount occurring in the first 50% of reduction and the remaining 50% of reduction resulting in 71% of the strain.[25] Therefore, our concept for reduction, with an average 14% slip reduction appears reasonable. Additionally, total facetectomy can provide more space for PLIF maneuvering and can facilitate retraction of nerve roots.

In previous reports, the incidence of neurological deficits associated with PLIF ranged from 2% to 8%, and the incidence of permanent neurological deficits ranged from 1.7% to 6.5%.[8,17,20,21,29] The current incidence of neurological complications is substantially lower than that of previous reports. Despite such advances, however, 7 patients (7%) in the present series showed neurological deficits after the primary surgery, and permanent motor loss was observed in 1 patient (1%). No correlation was detected between reduction and the occurrence of postoperative neurological deficits. Thus, it appears that the low rate of permanent motor loss in the current series, compared with previous reports, was due to surgical innovations such as appropriate reduction and total facetectomy.

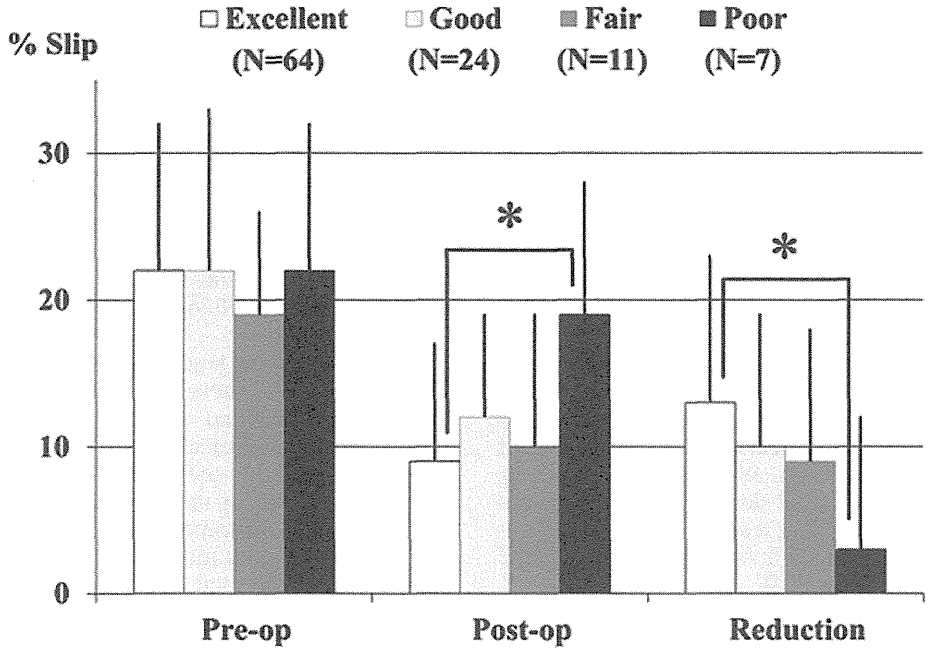

Fig. 2. Bar graph showing that clinical outcomes were better in proportion to slip reduction. Significant differences in postoperative slip ($p = 0.030$) and slip reduction ($p = 0.026$) were detected between the excellent and poor groups. Postoperative slip values in excellent and poor groups were 8% and 18%, respectively, and slip reduction values in the excellent and poor groups were 14% and 4%, respectively. *$p < 0.05$.

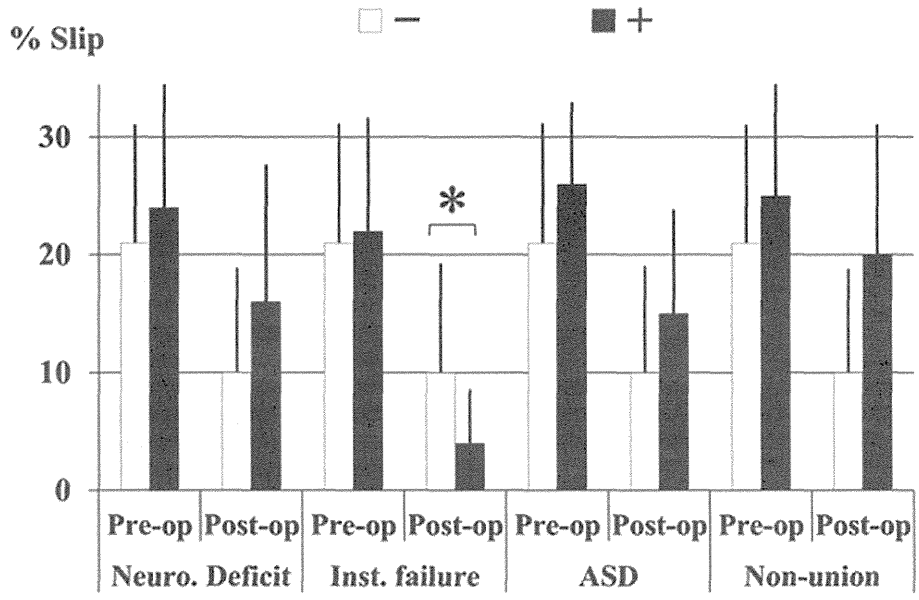

FIG. 3. Bar graph showing postoperative slip with and without complications. Minus (–, *white bars*) designates patients without postoperative complications and plus (+, *black bars*) denotes patients with postoperative complications. *$p < 0.05$. Inst. = instrumentation; neuro. = neurological.

In the present series, nonunion was observed in 4 cases and the union rate was 96%. The causes of these nonunions were not indicated by the patient history or laboratory data. In previous reports, the fusion rate ranged from 65% to 100%.[2,3,8,19,21,22,31] Several reports have noted that the union rate for isthmic spondylolisthesis was worse in nonreduction surgery than in reduction surgery.[12,20] In the current series, nonunion was found more often in patients with less reduction, although no significant difference was detected. From the point of view of interbody arthrodesis, a larger contact area of the bone graft site by reduction appeared to be desirable. Furthermore, total facetectomy enabled a low postoperative neurological deficit rate combined with a high union rate.

Instrumentation failures were observed in 7 patients, including 4 with pedicle screw loosening and 3 with pedicle screw breakage. In previous reports, the instrumentation failure rate ranged from 2% to 12%.[20,21,31] Instrumentation failures were significantly more common in patients with more reduction, although the clinical outcomes were not affected. All patients with instrumentation failure showed postoperative slip values within 10%. Excessive reduction might cause mechanical stress in the instrument or vertebral bone. Interestingly, all pedicle screw loosening was observed at the cranial side, and all breakage occurred at the caudal side. The reasons for this were unclear, but these phenomena might be plate system–specific instrumentation failures for PLIF.

Adjacent-segment degeneration after PLIF is one of the most important sequelae affecting long-term outcome. Although the development of ASD can occur as a part of the normal aging and degenerative process, this phenomenon appears to be at least partly influenced by the alteration of stresses that occurs as a consequence of lumbar fusion. Poussa and colleagues reported that slip reduction accelerated ASD compared with in situ fusion.[26,27] Furthermore, Kaito et al. reported that excessive disc height lifting was a risk factor for ASD after PLIF.[9] In previous reports, the revision rate for ASD ranged from 1.4% to 16.8%.[9,21,22,24] In the present series, 5 patients (5%) had ASD, and the rate was almost equal to that in previous reports. No correlation was seen between the reduction in slip and disc height and the occurrence of ASD in this study. These results suggested that our reduction procedure did not affect the occurrence of ASD.

One of limitations of this study was that spinopelvic parameters such as sacral slope, pelvic tilt, and sagittal vertical axis could not be investigated. However, global sagittal imbalance is rarely observed in low-dysplastic spondylolisthesis. In the present series, no patient presented with either low-back pain alone or global sagittal imbalance as a chief complaint, unlike patients with adult spinal deformity.

In the present study, slip reduction appeared to affect the clinical outcomes, which were better in proportion to slip reduction. Although no statistical difference was detected in clinical outcomes between postoperative slip less than 10% and from 10% to 20%, patients with postoperative slip of more than 20% showed significantly worse clinical outcomes. Twelve patients (11%) retained a postoperative slip more than 20% in spite of our PLIF techniques with total facetectomy and plate systems. These patients showed significantly worse clinical outcomes due to severe postoperative complications such as permanent motor loss and nonunion that required revision surgery. In the present study, it remained unclear whether further forcible reduction should be performed for these rigid cases. On the other hand, excessive reduction caused

instrumentation failure. All patients with instrumentation failure showed postoperative slip values within 10%. Although our initial goal for reduction was neither a degree of slip nor an attempt at complete reduction, the present results suggest that appropriate reduction resulted in a postoperative slip ranging from 10% to 20%.

Conclusions

The use of PLIF with total facetectomy for low-dysplastic isthmic spondylolisthesis appears to produce satisfactory clinical outcomes, with an average of 73% recovery rate and few postoperative complications. Although clinical outcomes were better in proportion to slip reduction, excessive reduction caused instrumentation failure and less reduction demonstrated worse clinical outcomes. Appropriate reduction resulted in a postoperative slip ranging from 10% to 20%.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Okuda. Acquisition of data: Okuda. Analysis and interpretation of data: Okuda. Drafting the article: Okuda. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Okuda. Statistical analysis: Okuda. Administrative/technical/material support: Okuda. Study supervision: all authors.

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Effect of Intermittent Administration of Teriparatide (Parathyroid Hormone 1-34) on Bone Morphogenetic Protein-Induced Bone Formation in a Rat Model of Spinal Fusion

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Background: Although clinical bone morphogenetic protein (BMP) therapy is effective at enhancing bone formation in patients managed with spinal arthrodesis, the required doses are very high. Teriparatide (parathyroid hormone 1-34) is approved by the U.S. Food and Drug Administration to treat osteoporosis and is a potent anabolic agent. In this study, intermittent administration of parathyroid hormone 1-34 combined with transplantation of BMP was performed to elucidate the effect of parathyroid hormone 1-34 on the fusion rate and quality of newly formed bone in a rat model.

Methods: A total of forty-eight male Sprague-Dawley rats underwent posterolateral lumbar spinal arthrodesis with one of three different treatments with recombinant human (rh) BMP-2: (1) 0 μg (control), (2) 2 μg (low dose), or (3) 50 μg (high dose). Each of the rhBMP-2 treatments was studied in combination with intermittent injections of either parathyroid hormone 1-34 (180 μg/kg/wk) or saline solution starting two weeks before the operation and continuing until six weeks after the operation. Osseous fusion was assessed with use of radiographs and a manual palpation test. Microstructural indices of the newly formed bone were evaluated with use of micro-computed tomography. The serum markers of bone metabolism were also quantified.

Results: The fusion rate in the group treated with 2 μg of rhBMP-2 significantly increased (from 57% to 100%) with the administration of parathyroid hormone 1-34 ($p < 0.05$). The fusion rates in the other groups did not change significantly with the administration of parathyroid hormone 1-34. The bone volume density of the newly formed bone significantly increased in both the 2-μg and 50-μg rhBMP-2 treatment groups with the administration of parathyroid hormone 1-34 ($p < 0.01$). Micro-computed tomography scans of the newly formed bone clearly demonstrated an abundance of trabecular bone formation in the group treated with parathyroid hormone 1-34. In addition, serum levels of osteocalcin were significantly increased in the parathyroid hormone 1-34 treatment group.

Conclusions: Intermittent administration of parathyroid hormone 1-34 significantly increased fusion rates in the group treated with low-dose rhBMP-2, and it improved the quality of the newly formed bone in both the high and low-dose groups in a rat model of rhBMP-2-induced spinal fusion.

Clinical Relevance: Our results suggest that the combined administration of rhBMP-2 and parathyroid hormone 1-34 may lead to efficient bone regeneration.

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Autogenous bone-grafting is the current gold standard for achieving spinal fusion. However, its use is limited by the amount of bone available and by the associated donor-site morbidity[1,2]. Moreover, the rate of pseudarthrosis has been reported to range between 5% and 43%[3,4]. These problems have prompted surgeons to identify alternative means for stimulating bone formation.

One possibility is the use of bone morphogenetic proteins (BMPs), which are a group of growth factors belonging to the transforming growth factor superfamily that are known to elicit new bone formation[5-7]. However, the uncontrolled release of high concentrations of BMPs can cause inflammation, soft-tissue edema, and unintended bone formation[8,9]. Thus, the U.S. Food and Drug Administration has approved the use of BMPs in the spine for anterior lumbar spinal arthrodesis only; uses for posterior spinal arthrodesis have been "off label." Therefore, an efficient method for reducing the required dose of BMPs by enhancing the bioactivity is required.

Teriparatide (recombinant human parathyroid hormone [PTH] 1-34) is the only anabolic agent that has been approved by the U.S. Food and Drug Administration for the treatment of osteoporosis[10,11]. Intermittent administration of PTH 1-34 results in osteoblastic proliferation and differentiation, thereby leading to an increase in bone mass[12-14]. Recently, upregulation and modulation of BMP signaling by PTH have been reported[15,16]. These mechanisms indicate the possibility of synergistic bone regeneration by the co-administration of rhBMP-2 and PTH 1-34.

In the present study, intermittent administration of PTH 1-34 combined with BMP transplantation was performed to elucidate the effect of PTH 1-34 on the fusion rate and quality of the newly formed bone in a rat model of spinal fusion.

Materials and Methods

Experimental Design

A total of four dozen eight-week-old male Sprague-Dawley rats (weight, 270 to 290 g) were used. The animals were allocated to one of six different treatment groups by assigning one of three surgical treatments and one of two injection treatments to each rat. Each treatment group consisted of eight animals (see Appendix). The three surgical treatments consisted of (1) implantation of a collagen-only carrier (Groups A and B), (2) implantation of a collagen carrier loaded with 2 μg of rhBMP-2 (Groups C and D), and (3) implantation of a collagen carrier loaded with 50 μg of rhBMP-2 (Groups E and F). The animals in each treatment group were further divided into two subgroups, including rats that also received either (1) injections of PTH 1-34 (Groups B, D, and F) or (2) injections of saline solution (Groups A, C, and E) (see Appendix). rhBMP-2 doses for the low and high-dose groups were based on a preliminary spinal fusion study (data not shown). We chose 2 μg (fusion rate, 50%; concentration, 20 μg/mL) as the low dose because this amount of rhBMP-2 is better suited for elucidating the effect of PTH 1-34 on the fusion rate, and we chose 50 μg (fusion rate, 100%; concentration, 500 μg/mL) as the high dose because this amount of rhBMP-2 reflects the clinical use of high-dose (1500-μg/mL) rhBMP-2.

Injections of PTH 1-34

Rats in the control group were given subcutaneous injections of 0.9% saline solution, whereas rats in the PTH groups were given subcutaneous injections of PTH 1-34 (60 μg/kg) three times a week (total, 180 μg/kg/wk). Injections were initiated two weeks prior to surgery and were continued for six weeks after surgery, at which time the animals were killed.

TABLE I Results of Radiographic Analysis of Spinal Fusion

rhBMP-2 Dosage (μg)	Injected Material* (%)	
	Saline Solution	PTH 1-34
0	0 of 8 (0)	0 of 8 (0)
2	4 of 7 (57)	8 of 8 (100)†
50	7 of 8 (88)	8 of 8 (100)

*The values are given as the number of spines that were found to have successfully fused as assessed with radiographs, with the percentage in parentheses. †At this dosage, the rate of fusion was significantly higher in the group that received PTH 1-34 ($p < 0.05$).

Posterior Spinal Fusion

Preparation of a Collagen Carrier Vehicle

A commercially available absorbable collagen sponge (CollaCote; Integra LifeSciences, Plainsboro, New Jersey) was cut into 5 × 10-mm fragments. Thereafter, the appropriate concentration of rhBMP-2 (0, 2, or 50 μg) was dissolved in phosphate buffered saline solution and applied to the carrier just before implantation.

Surgical Technique of L4-L5 Posterolateral Spinal Arthrodesis

All of the animal procedures were conducted in accordance with the guidelines of the Regulations on Animal Experimentation at Osaka University. The rats were anesthetized with a combination of 0.15 mg/kg of medetomidine (Domitor; Nippon Zenyaku Kogyo, Fukushima, Japan), 2 mg/kg of midazolam (Dormicum; Astellas Pharma, Tokyo, Japan), and 2.5 mg/kg of butorphanol (Vetorphale; Meiji Seika, Tokyo, Japan). As per the usual method[16,17], a posterior midline skin incision was made, followed by two separate paramedian incisions in the lumbar fascia 3 mm from the midline, through which the transverse processes were exposed. The L4 and L5 transverse processes were decorticated with use of a high-speed burr. Subsequently, a collagen sponge containing 0, 2, or 50 μg of rhBMP-2 was implanted on each side. The rats were housed in separate cages and allowed to eat and drink ad libitum while their condition was monitored daily.

Euthanasia and Analyses

Just prior to euthanization of the animals, blood samples were collected and stored at −80°C until the serum markers of bone metabolism were analyzed. The rats were killed with an overdose of anesthetics at six weeks after surgery. The spinal segments and femora were harvested and fixed with 10% formalin.

Assessment of Fusion

Radiographic Assessment

Fusion between L4 and L5 was evaluated with radiographs made with use of an MX-20 Specimen Radiography System (Faxitron X-Ray, Lincolnshire, Illinois) under consistent conditions (35 kV, 300 μA, 300 seconds). Fusion was considered to have occurred when there was clear evidence of new bone formation and osseous bridging with cortical continuity between the L4 and L5 transverse processes.

Manual Assessment

The explanted lumbar spines were manually tested for intersegmental motion. Any motion detected on either side between the facets or between the transverse processes was considered to be a failure of fusion.

In both assessments, the spines were scored as either fused or not fused independently by three examiners. The L4-L5 segments were considered to be fused only when all three observers agreed.

TABLE II Results of Spinal Fusion as Assessed with Manual Palpation

	Injected Material* (%)	
rhBMP-2 Dosage (μg)	Saline Solution	PTH 1-34
0	0 of 8 (0)	0 of 8 (0)
2	2 of 7 (29)	8 of 8 (100)†
50	8 of 8 (100)	8 of 8 (100)

*The values are given as the number of spines that were found to have successfully fused as assessed with manual palpation, with the percentage in parentheses. †At this dosage, the rate of fusion was significantly higher in the group that received PTH 1-34 ($p < 0.05$).

Micro-Computed Tomography Analysis

Following manual evaluation, the spines were scanned with use of high-resolution micro-computed tomography (micro-CT) (R_mCT; Rigaku Mechatronics, Tokyo, Japan); each sample was scanned twice. The micro-CT data were collected at 90 kV and 200 μA. Visualization and data reconstruction were performed with use of TRI/3D-BON software (RATOC System Engineering, Tokyo, Japan).

Analysis of the Microstructural Indices of the Newly Formed Fusion Mass

The quality of the newly formed fusion mass between the transverse processes where bone did not originally exist was analyzed as described previously[18] (see Appendix). Scanning of the newly formed bone was initiated from the lower end plate level of the L4 vertebral body and continued cranially in 2.0-mm increments (fifty slices) at a resolution of 40 μm per voxel. Bone volume density, trabecular thickness, trabecular number, trabecular separation, thickness of cortical bone, and cortical bone ratio were estimated. The bone volume density corresponds to the ratio of bone volume to fusion-mass volume.

Microstructural Analysis of the Fused Spinal Segments

The tissue volume and bone volume of the total fusion mass were measured (from the bottom to 15 mm cranially from the bottom of the L5 transverse process, for a total of 254 slices) at a resolution of 59 μm per voxel.

Analysis of the Systemic Effects of PTH 1-34

To evaluate the systemic effects of PTH 1-34, the bone volume density of the distal femoral epiphysis and L6 vertebral body was analyzed at a resolution of 40 μm per voxel. Scanning of the distal part of the femur was initiated at 1.5 mm proximal to the growth plate and continued at 3.0-mm increments (for a total of seventy-five slices). Scanning of the L6 vertebral body was initiated at 1.0 mm cranial to the lower growth plate and continued at 3.2-mm increments (for a total of eighty slices).

Analysis of Serum Markers of Bone Metabolism

Serum markers of bone metabolism were analyzed with use of an enzyme-linked immunosorbent assay specific for osteocalcin (Osteocalcin High Sensitive EIA kit [rat]; Takara Bio, Shiga, Japan), type-I collagen cross-linked C-telopeptides (RatLaps ELISA; Immunodiagnostic Systems, Fountain Hills, Arizona), and tartrate-resistant acid phosphatase-5b (RatTRAP Assay; Immunodiagnostic Systems), according to the manufacturer's instructions. Serum from all animals ($n = 47$) was measured once for each marker, with comparisons performed between the groups treated with PTH 1-34 (Groups B, D, and F) and those treated with saline solution (Groups A, C, and E).

Histologic Analysis

The dissected and formalin-fixed spines were demineralized with 50% formic acid and 10% sodium citrate, dehydrated in a graded ethanol series, and

TABLE III Microstructural Indices of Newly Formed Bone

		Injected Material*	
Parameter	rhBMP-2 Dosage (μg)	Saline Solution	PTH 1-34
Tissue volume (mm^3)	2	5.5 ± 5.4	17.1 ± 13.3†
	50	34.8 ± 22.2	29.1 ± 12.0
Bone volume (mm^3)	2	0.7 ± 0.9	3.8 ± 3.2†
	50	2.3 ± 2.7	6.3 ± 2.8†
Bone volume density (%)	2	12.1 ± 5.2	22.1 ± 5.5†
	50	7.1 ± 4.6	22.2 ± 5.5†
Trabecular thickness (μm)	2	142.6 ± 15.1	166.8 ± 31.1‡
	50	153.0 ± 35.9	181.8 ± 17.2†
Trabecular number (mm^{-1})	2	0.8 ± 0.8	0.7 ± 0.2
	50	0.3 ± 0.2	0.6 ± 0.2†
Trabecular separation (μm)	2	305.5 ± 97.2	268.9 ± 56.4
	50	756.9 ± 428.7	298.7 ± 64.9†
Cortical bone ratio (%)	2	41.5 ± 7.6	39.4 ± 11.0
	50	28.8 ± 17.1	30.1 ± 4.8‡
Cortical bone thickness (μm)	2	313.2 ± 77.4	391.3 ± 90.8†
	50	272.4 ± 109.5	365.1 ± 53.0†

*Values are given as the mean and the standard deviation. †At this dosage, the value was significantly higher in the group that received PTH 1-34 ($p < 0.01$). ‡At this dosage, the value was significantly higher in the group that received PTH 1-34 ($p < 0.05$).

embedded in paraffin wax. Serial coronal sections (thickness, 5 μm) of the involved segments were cut and stained with hematoxylin and eosin.

Statistical Analysis

The PASW Statistics computer software program (version 18.0, SPSS, Chicago, Illinois) was used for all of the analyses. The Mann-Whitney U test was used for the comparison of microstructural indices and serum markers of bone metabolism. The chi-square test was used for the comparison of fusion assessments. In all analyses, the level of significance was set at $p < 0.05$.

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Results

Spinal Fusion

Radiographic Analysis

One rat in Group C died the day after surgery; thus, a total of forty-seven rats were used for the final analysis. Fusion at L4-L5 in the groups treated with 2 μg of rhBMP-2 significantly increased, from four of seven (57%) to eight of eight (100%), with the administration of PTH 1-34 ($p < 0.05$). Fusion in the groups treated with 0 or 50 μg of rhBMP-2 did not change significantly with the administration of PTH 1-34 (in both groups treated with 0 μg of rhBMP-2, fusion occurred in zero of eight [0%] spines regardless of whether PTH 1-34 was used; in the groups treated with 50 μg of rhBMP-2, fusion increased from seven of eight [88%] to eight of eight [100%] with the use of PTH 1-34) (Table I).

Manual Assessment

All the spines in Groups E and F, which were treated with 50 μg of rhBMP-2, were assessed with manual palpation and were considered to have fused (eight of eight in each group, 100%). None of the spines in Groups A or B, which were treated with 0 μg of rhBMP-2, were considered to have fused (zero of eight in each group, 0%). Fusion in the spines that were treated with

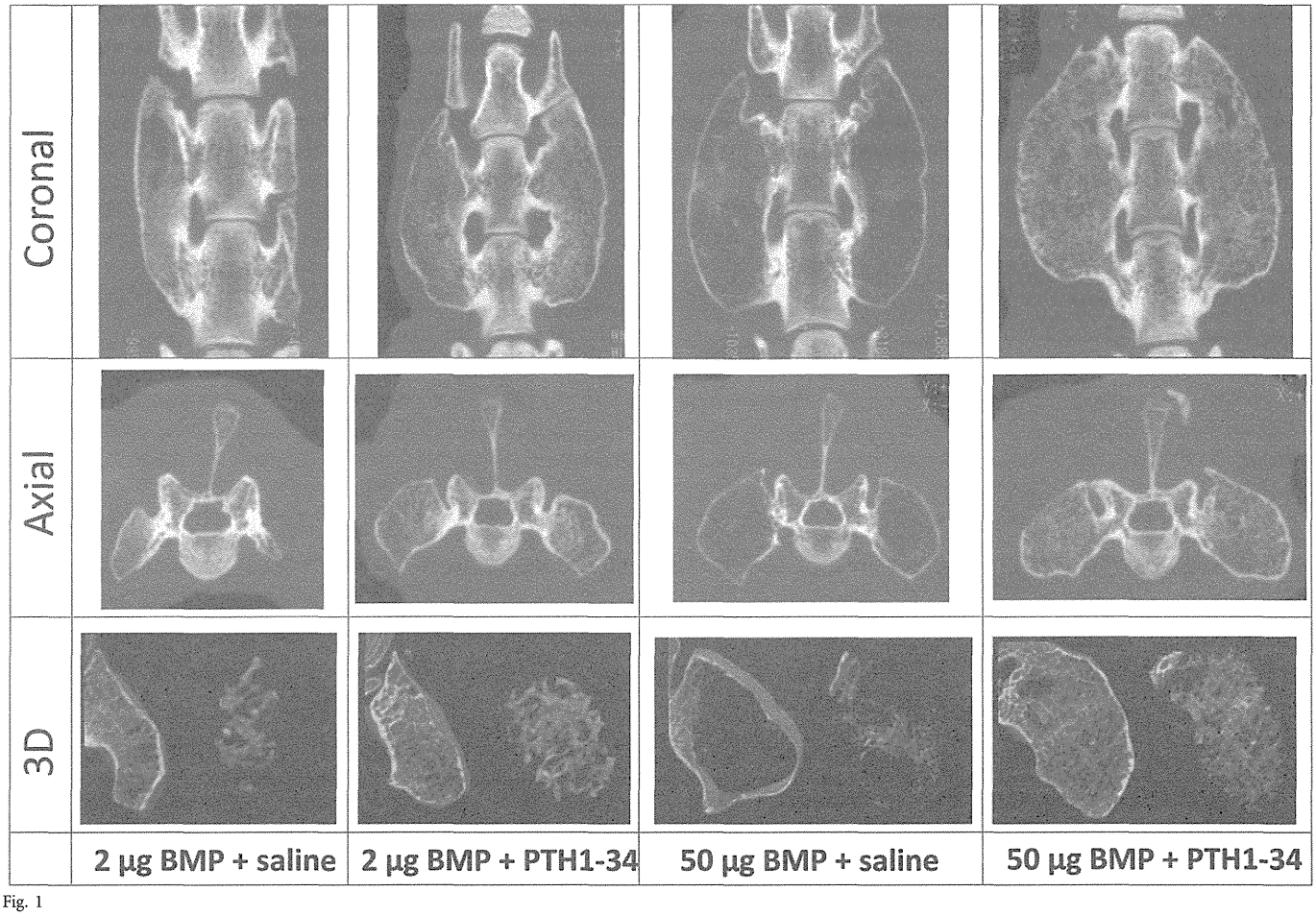

Fig. 1

In the groups treated with 2 μg of recombinant human bone morphogenetic protein (BMP)-2, the administration of teriparatide (parathyroid hormone [PTH] 1-34) improved osseous bridging between the transverse processes, the volume of the fusion mass, and the trabecular bone volume inside the fusion mass. In the group treated with 50 μg of rhBMP-2 and saline solution, the induced fusion mass resembled an eggshell (a thin outer layer of cortical bone with scarce trabecular bone inside). PTH 1-34 administration with 50-μg rhBMP-2 treatment resulted in the formation of a fusion mass that was completely filled with thick trabecular bone. 3D = three-dimensional.

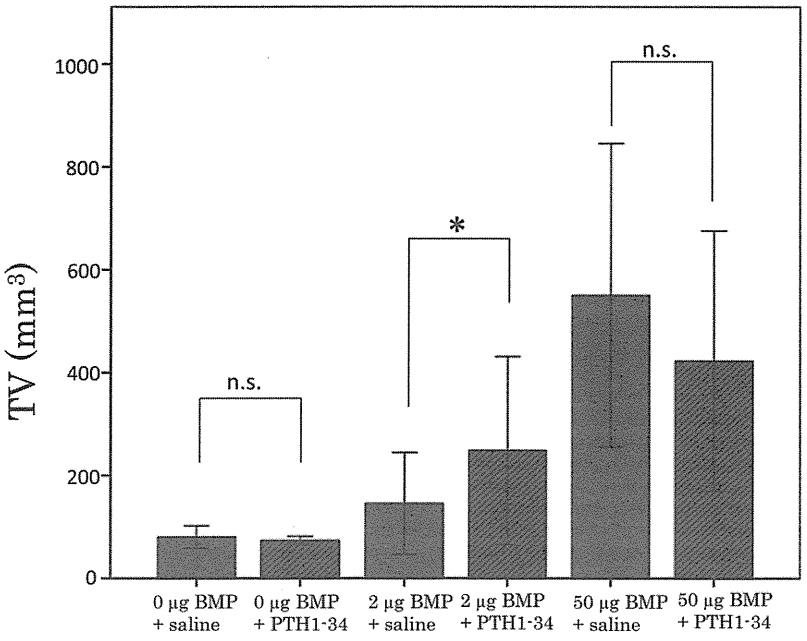

Fig. 2

Teriparatide (parathyroid hormone [PTH] 1-34) administration did not change the tissue volume (TV) in the 0-μg bone morphogenetic protein (BMP) groups. In the groups treated with 2 μg of rhBMP-2, PTH 1-34 administration significantly increased the tissue volume (TV) ($p < 0.05$). In the 50-μg rhBMP-2 groups, PTH 1-34 administration showed a decreasing trend; however, it was not significant (n.s.). The asterisk indicates a significant difference in tissue volume.

2 μg of rhBMP-2 increased from two of seven (29%, Group C) to eight of eight (100%, Group D) with the administration of PTH 1-34 ($p < 0.01$) (Table II).

Micro-CT Analysis

Analysis of the Microstructural Indices of the Fused Spinal Segments

The specimens treated with 2 or 50 μg of rhBMP-2 (Groups C, D, E, and F) were used for this analysis, because the specimens treated with 0 μg of rhBMP-2 (Groups A and B) showed little or no new bone formation.

The trabecular bone volume and structural parameters (bone volume, bone volume density, and trabecular thickness) in the groups treated with 2 μg or 50 μg of rhBMP-2 were significantly increased with the administration of PTH 1-34, and the trabecular number in the group treated with 50 μg of rhBMP-2 was also significantly increased with PTH 1-34 administration ($p < 0.01$). The cortical bone parameters of the groups treated with 2 μg or 50 μg of rhBMP-2 were also increased with the administration of PTH 1-34. Interestingly, the tissue volume in the group treated with 50 μg of rhBMP-2 decreased with the use of PTH 1-34, although the tissue volume in the groups treated with 2 μg of rhBMP-2 was significantly increased with the use of PTH 1-34 (Table III). Micro-CT coronal and axial two-dimensional images and reconstructed three-dimensional images of the newly formed bone clearly demonstrated abundant trabecular bone formation in the groups treated with PTH 1-34 (Fig. 1).

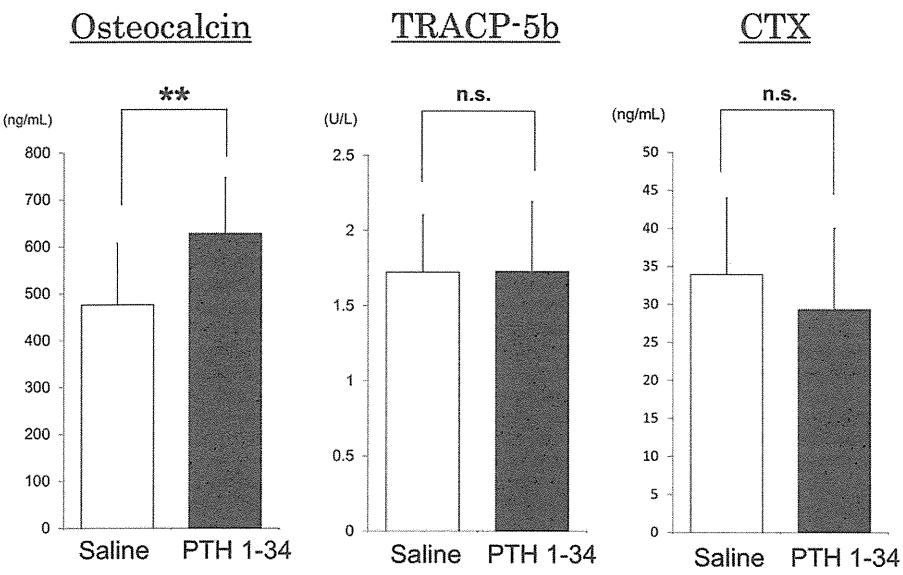

Fig. 3

Osteocalcin (bone-formation marker) levels were significantly increased with the administration of teriparatide (parathyroid hormone [PTH] 1-34) ($p < 0.01$), but tartrate-resistant acid phosphatase (TRACP)-5b (bone-resorption marker) and type-I collagen cross-linked C-telopeptides (CTX) (bone-resorption marker) levels were not significantly altered (n.s.). The double asterisk indicates a significant difference in tissue volume.

Microstructural Analysis of the Fused Spinal Segments

The mean bone volume density (and standard deviation) in Groups A and B, which received 0 μg, was 18.5% ± 3.5% and 27.8% ± 7.4% ($p < 0.01$), respectively. In Groups C and D, which received 2 μg, it was 19.6% ± 3.4% and 23.4% ± 4.8% ($p < 0.05$), respectively. In Groups E and F, which received 50 μg, it was 13.7% ± 4.2% and 24.2% ± 6.0% ($p < 0.01$), respectively. However, the alterations in tissue volume values caused by PTH 1-34 use differed in each group. The tissue volume between the groups treated with 0 μg of rhBMP-2 with or without PTH 1-34 did not differ significantly (Group A, 79.8 ± 10.2 mm^3; Group B, 72.4 ± 5.1 mm^3; $p = 0.14$). The tissue volume in the groups treated with 2 μg of rhBMP-2 significantly increased with the use of PTH 1-34 (Group C, 145.4 ± 49.6 mm^3; Group D, 239.1 ± 88.6 mm^3; $p < 0.05$); the tissue volume in the groups treated with 50 μg of rhBMP-2 decreased with the use of PTH 1-34, although the change was not significant (Group E, 469.1 ± 207.0 mm^3; Group F, 397.3 ± 137.8 mm^3; $p = 0.44$) (Fig. 2). The effect of PTH 1-34 use on tissue volume was anabolic in the 2-μg BMP group and was catabolic in the 50-μg BMP group.

Analysis of the Effect of PTH Administration on the Adjacent Vertebra (L6) and Femur

The bone volume densities of both the distal femoral epiphysis and the L6 vertebral body were significantly increased with the use of PTH 1-34 compared with those treated without PTH 1-34 (femur: 18.5% ± 10.3% compared with 49.4% ± 14.1%, $p < 0.001$; L6 vertebra: 26.1% ± 10.4% compared with 37.7% ± 4.2%, $p < 0.001$). The bone volume density of both the femur and the L6 vertebra did not differ based on the dosage of rhBMP-2.

Analysis of Serum Markers of Bone Metabolism

Enzyme-linked immunosorbent assay demonstrated that serum levels of osteocalcin were significantly higher in the groups treated with PTH 1-34 compared with those treated with saline solution ($p < 0.01$), whereas no differences were observed in serum levels of type-I collagen cross-linked C-telopeptides and tartrate-resistant acid phosphatase-5b between the groups treated with saline solution and those treated with PTH 1-34 (Fig. 3).

Histologic Analysis

Microscopic evaluation of the coronal sections of the treated spinal segments demonstrated that the groups treated with 0 μg of rhBMP-2 (Groups A and B) showed minimal evidence of new bone, and no apparent difference was noted with regard to PTH 1-34 administration (Figs. 4-A and 4-B). In the Group C rats treated with 2 μg of rhBMP-2 and injections of saline solution, the fusion mass between the L4-L5 transverse processes was discontinuous or separated by cartilage tissue (Fig. 4-C). However, in the group with the same 2-μg rhBMP-2 dosage (Group D), the addition of PTH 1-34 clearly improved the osseous continuity between the transverse processes and increased the volume of the fusion mass (Fig. 4-D). In the groups treated with 50 μg of rhBMP-2, a huge fusion mass was found even in the group that received saline solution injections (Group E); however, a majority of the newly formed fusion masses in Group E comprised fatty marrow within thin, eggshell-like cortical bone (Fig. 4-E). PTH 1-34 markedly increased the number and thickness of trabecular bone in the fusion mass (Fig. 4-F).

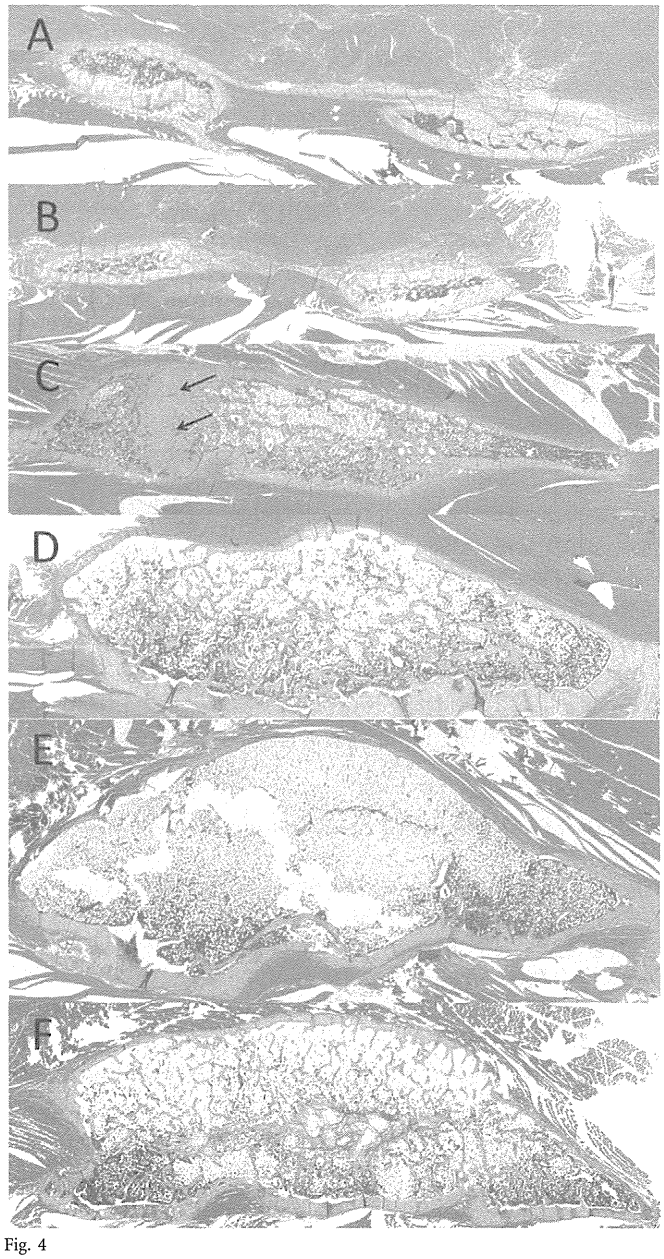

Fig. 4

Low-power photomicrographs (magnification, ×0.5). Coronal sections of the L4-L5 transverse processes of the spines of rats from Group A (Fig. 4-A) and Group B (Fig. 4-B) demonstrate no evidence of bone formation between the transverse processes. Cross-section of the transverse processes of rats from Group C (Fig. 4-C) show fibrocartilaginous union (arrows). In Group D, PTH 1-34 administration clearly improved the osseous continuity and the bone volume of the fusion mass (Fig. 4-D). In Group E (50 μg of rhBMP-2), a huge fusion mass was found; however, the fusion mass comprised fatty marrow within thin, eggshell-like cortical bone (Fig. 4-E). In Group F, PTH 1-34 administration markedly increased the number and thickness of trabecular bone in the fusion mass (Fig. 4-F).

Discussion

Intermittent PTH 1-34 administration significantly increased fusion rates in the low-dose rhBMP-2 treatment group and improved the quality of the newly formed bone in both the low-dose and the high-dose rhBMP-2 groups in a rat model of spinal fusion. The amount in the low-dose rhBMP-2 group (2 μg) is approximately one-fifth of the amount that has been commonly used in previous studies[17,19]. This reduction in dosage facilitates a decrease in the therapeutic dose of rhBMP-2, which can consequently result in a decrease in the adverse effects associated with the uncontrolled release of high-dose rhBMP-2[7,8]. As we did not observe any apparent effect of PTH 1-34 on bone induction when rhBMP-2 was not administered, we believe that rhBMP-2 and PTH 1-34 have a synergistic effect on the volume and quality of the newly formed bone.

PTH 1-34 has a unique mechanism of action in bone; the continuous administration of PTH 1-34 leads to a decrease in bone volume (a catabolic effect), and the intermittent administration of PTH 1-34 leads to the formation of increased amounts of trabecular bone (an anabolic effect)[10-14]. The use of systemic PTH 1-34 therapy has been approved for the treatment of severe osteoporosis. This anabolic effect of PTH 1-34 also provides a rationale for its potential use in the treatment of other skeletal disorders. Recent studies have demonstrated that PTH 1-34 treatment enhances fracture-healing[20,21]. The results of these studies suggest that the anabolic effect of PTH 1-34 is markedly greater in newly forming bone than in bone that is undergoing normal remodeling. We hypothesized that this strong anabolic effect in newly formed bone can be applied to the bone formation that is newly induced by rhBMP-2, and the results of the present study support our hypothesis.

The precise mechanisms through which intermittent PTH administration results in bone formation remain elusive[22-25]. PTH in vitro enhances the differentiation of mesenchymal stem cells into the osteoblast lineage[12]; however, the traditional signaling pathway of PTH does not appear to satisfactorily account for these anabolic effects. Modification of the extracellular BMP antagonist network via the low-density lipoprotein receptor-related protein 6 (LRP6), which functions in the canonical Wnt pathway and forms a complex with both PTH type-1 receptor (PTH1R) and BMP antagonists, was recently identified as one of the mechanisms through which PTH influences the differentiation of mesenchymal stem cells into the osteoblast lineage[15]. PTH stimulates the endocytosis of the PTH1R/LRP6/BMP antagonist complex, thus facilitating the enhancement of BMP signaling by blocking the negative-feedback mechanism of BMP antagonists, including noggin[15]. Another synergism between rhBMP-2 and PTH is typically seen in the groups receiving 50 μg of rhBMP-2. A high dose of rhBMP-2 is reported to induce formation of cyst-like bone voids filled with adipose tissue instead of with normal trabecular bone structure despite complete bone union[26]. Pleiotropic effects (osteoblastogenesis, adipogenesis, and osteoclastogenesis) of BMPs are mainly controlled by the BMP signaling pathway and the canonical Wnt pathway[8,9,27]. Excessive BMP signaling upregulates the expression of *Sost*, leading to the increased translation of sclerostin, which negatively regulates the Wnt pathway[16]. Downregulation of the Wnt pathway results in activation of the transcription of peroxisome proliferator-activated receptor gamma (PPARγ). This activation of PPARγ, which is a key regulator of adipocyte commitment[27], leads mesenchymal stem cells to differentiate into adipocytes rather than into osteoblasts. The activation of the Wnt pathway by PTH can downregulate PPARγ expression and promote osteoblastogenesis by BMPs.

These data suggest that the concomitant administration of PTH 1-34 and rhBMP-2 could be an ideal combination for inducing new bone formation, as PTH can induce a synergistic anabolic effect by accelerating the osteoblastic differentiation of the rhBMP-2-induced mesenchymal progenitor cells and can attenuate the negative-feedback mechanism by blocking the action of BMP antagonists.

Although PTH 1-34 injections were initiated on the day of surgery or a few days after surgery in many previous studies[28-32], our injections were initiated two weeks prior to surgery. This earlier treatment with PTH 1-34 was performed to maximize its effect on rhBMP-2, as PTH 1-34 requires approximately two weeks to exert its anabolic effect on bone metabolism, and rhBMP-2 can only persist locally for a few days after implantation.

The systemic effect of PTH 1-34 was confirmed with the increase in the bone volume density at the adjacent vertebra (L6) and femur.

PTH 1-34 administration is expected to accelerate the remodeling of newly formed bone because PTH 1-34 enhances both osteogenic and osteoclastic activity. In fact, the excessively induced new bone in the 50-μg rhBMP-2 treatment groups showed a decreasing trend in tissue volume at six weeks after surgery, which appeared to be caused by PTH 1-34 administration. Thus, PTH 1-34 may promote the remodeling process of rhBMP-2-induced newly formed bone, depending on the mechanical requirements[33].

The current results of a spinal fusion model with quadrupedal rodents cannot be directly extrapolated to spinal arthrodesis in humans because of the differences in biomechanics and biological reaction to the agents. Another limitation is a lack of biomechanical assessment of fusion because of the size and complex geometry of the rat spine. However, the results of the manual palpation test performed in this study have been shown to correlate with those of biomechanical testing[34].

In conclusion, the present study demonstrated that intermittent PTH 1-34 administration significantly increased fusion rates in the low-dose rhBMP-2 treatment group, thus indicating the potential to reduce the required rhBMP-2 dose and improve the quality of the newly formed bone in both the low and high-dose rhBMP-2 treatment groups in a rat model of rhBMP-2-induced spinal fusion. Our results indicate that the combined administration of rhBMP-2 and PTH 1-34 has a synergistic effect rather than an additive effect. Thus, we believe that PTH 1-34 has potential clinical applications in BMP-induced spinal fusion surgery.

Appendix

(eA) A table showing the six different treatment groups and a figure demonstrating the regions of interest for

the evaluation of the newly formed bone are available with the online version of this article as a data supplement at jbjs.org. ■

Note: rhPTH 1-34 was kindly provided by Asahi Kasei (Tokyo, Japan), and rhBMP-2 was kindly provided by Osteopharma (Osaka, Japan).

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ORIGINAL ARTICLE

New classification system for ossification of the posterior longitudinal ligament using CT images

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Abstract
Background Ossification of the posterior longitudinal ligament (OPLL) is most frequently seen in the cervical spine. The types of cervical OPLL are classified into continuous, mixed, segmental, and other based on plain lateral X-ray. Computed tomography (CT) imaging is often used in clinical practice for evaluating ossified lesions as it can detect their precise location, size, and shape. However, to date, no CT classification of OPLL lesions has been proposed.
Methods One hundred and forty-four patients diagnosed with cervical OPLL by plain radiograph were included in this study. Sagittal and axial CT images of the cervical spine were obtained. We propose three classification systems: A, B, and axial. Classification A comprises two lesion types: bridge and nonbridge. Classification B requires examiners to describe all vertebral and intervertebral levels where OPLL exits in the cervical spine. Axial classification comprises central and lateral lesions identified on axial CT images. Seven observers evaluated CT images using this classification system, and intra- and interrater reliability were examined.
Results Averaged Fleiss' kappa coefficient of interrater agreement was 0.43 ± 0.26 among the seven observers, averaged intrarater reliability for the existence of OPLL was 72.4 ± 8.8 % [95 % confidence interval (CI) 67.5–76.8]. Fifty-four patients (37.5 %) had the bridge type and 90 the nonbridge type according to Classification A; 102 (70.8 %) had central and 42 (29.2 %) lateral OPLL in the axial classification. Four representative cases defined according to the three classification types are reported here.
Conclusion Subcommittee members of the Investigation Committee on the Ossification of the Spinal Ligaments of the Japanese Ministry of Public Health and Welfare propose three new classification systems of cervical OPLL based on CT imaging: A, B, and axial.

Study group of subcommittee members of the Investigation Committee on the Ossification of the Spinal Ligaments of the Japanese Ministry of Public Health and Welfare.

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ERRATUM

Erratum to: Regenerative medicine in orthopedics using cells, scaffold, and microRNA

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The correct name of the fifth author should be given as Elhussein Mahmoud, not Hussein El Mahmoud.

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Introduction

Ossification of the posterior longitudinal ligament (OPLL) is characterized by the replacement of ligamentous tissue by ectopic new bone [1]. OPLL often causes narrowing of the spinal canal and has been recognized as one of the causes of myelopathy and/or radiculopathy [2]. The disease was first reported in Japan in 1960 [1]. Since then, numerous cases of OPLL have been reported, and its existence in the general Japanese population is reported to be 1.9–4.3 % among people >30 years [3]. Although the pathogenesis of OPLL has not been fully elucidated, a genetic background factor related to systemic ossification could be involved [4].

A radiological study revealed that OPLL is frequently observed in the cervical spine [5] and are classified as continuous, mixed, segmental, and other types based on plain lateral X-ray of the cervical spine according to the classification established by the Investigation Committee on the Ossification of Spinal Ligaments of the Japanese Ministry of Public Health and Welfare in 1981 [6]. This classification [6] is very simple and easy to use; however, X-ray-based classification has the following potential limitations:

1. Explicit definition of each type is unclear
2. Agreement ratio between examiners has not been confirmed
3. Precise evaluation of the ossified lesion at each vertebral and intervertebral level is not sufficiently expressed
4. Data collection regarding lesion location might be difficult using X-ray classification

Computed tomography (CT) imaging is often used in clinical practice to evaluate OPLL lesions and can detect the precise location, size, and shape of ossified lesions. Thus, several members of the investigation committee were selected to develop new classifications for cervical OPLL using CT imaging. The purpose of this study was to introduce the new classification system and assess its classification adequacy.

Materials and methods

One hundred and forty-four patients diagnosed with cervical OPLL by plain radiograph were entered into this study. All patients were treated and followed in one university hospital. There were 90 men and 54 women, with an average age of 67.5 years (range 36–86 years). Informed consent was obtained from each patient before enrollment, and the study was approved by the Institutional Review Board of the university hospital. Forty-six patients had a history of cervical laminoplasty, which is a posterior decompression surgery in the cervical spine. Patients who had anterior decompression surgery (ADS) for OPLL treatment were excluded, because ADS might affect OPLL configuration. Lateral radiographs [6] were obtained in all patients; accordingly, 35 were classified with continuous, 66 with mixed, 41 with segmental, and two with other OPLL types. Sagittal and axial multidetector CT images (SOMATOME Sensation 64 Cardiac, SIEMENS Co., Erlangen, Germany) were also obtained. Specific CT parameters were 1 tube rotation/s, 17.28 mm/s table-feed speed, 160 mA, and 120 kV. Image reconstructions were made using a CT console (Wizard, SIEMENS, Co.) at a 1-mm interval from the 0.75-mm scan-slice data. A technique was used to determine the threshold for bone-density measurement. Images were constructed using the bone-window setting (width 1,500, center 200); OPLL lesion classifications were established and then evaluated. Classification analysis was independently performed by seven senior spine surgeons. Classification system details are described below.

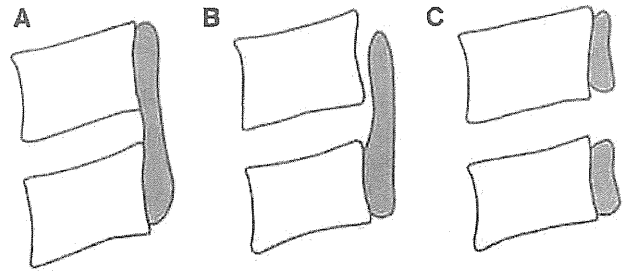

Fig. 1 Typical bridge (a) and nonbridge (b, c) lesions. *Gray areas* ossification of the posterior longitudinal ligament (OPLL) lesions

Classification A

In classification A, ossified lesions were divided into two types: bridge and nonbridge, based on presence or absence of a bony bridge between vertebral bodies on sagittal CT images (Fig. 1). Bony bridge is defined as an OPLL connection to the adjacent posterior margins of vertebral bodies at two or more levels. The observers evaluated the ossification using all of the sagittal CT images. When an ossified lesion connected to the adjacent posterior margin of a vertebral body, even if a small ossification and not necessarily the most extended ossification, it was classified as a bridge type. The number of connected vertebral bodies is included in the classification.

Classification B

This classification requires the examiners to describe all vertebral and intervertebral levels where OPLL >2 mm in

width exist in the cervical spine. Then, connection or disconnection of OPLL is expressed as follows:

① A dot (".") is applied when the OPLL lesion is disconnected, similar to the segmental type in the X-ray classification.
② A slash ("/") is applied when the OPLL lesion is beyond the intervertebral level, without any bridge formation to the adjacent vertebral body.
③ A bar ("-") is applied when the OPLL lesion is beyond the intervertebral level, with bridge formation to the adjacent vertebral body.
④ A circle ("O") is applied at the level of the vertebral body when the OPLL lesion is not attached to the vertebral body (level number is circled). This means that if the OPLL lesion is fused with the vertebral body, the circle is not applied at the level of the vertebral body.

Axial classification

The ossified lesion is divided into two types, central and lateral, on axial CT images at the level where the ossification most significantly occupies the spinal canal. If the posterior prominence of the OPLL is located in the middle third of the spinal canal, it is defined as central; the lateral type is subdivided into left- and right-side types.

Interrater and intrarater reliability and agreement

To evaluate the adequacy of classification A, inter- and intrarater reliability measures were determined with Fleiss' kappa coefficient using a dedicated MATLAB (Mathworks, Paris, France) program. Kappa values of 0.00–0.20 were considered as being slight agreement, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect [7, 8]. As classification B is a complex process, we did not calculate its inter- and intrarater agreement ratio. Likewise, we did not calculate the agreement ratio of the axial image classification.

Results

Interrater and intrarater reliability and agreement in classification A

Averaged Fleiss' kappa coefficient of interrater agreement was 0.43 ± 0.26 among the seven observers. The averaged intrarater reliability for the existence of OPLL was 72.4 ± 8.8 % [95 % confidence interval (CI) 67.5–76.8].

Table 1 Classification A

Ossification of posterior longitudinal ligament (OPLL) lesions	No. of patients
Bridge type	54
2-level	28
3-level	4
4-level	5
4 continuous levels	2
2 + 2 levels	3
>5-level	17
5 continuous levels	3
2 + 3 levels	5
3 + 2 levels	2
7 continuous levels	4
2 + 5 levels	1
8 continuous levels	1
4 + 4 levels	1
Nonbridge type	90

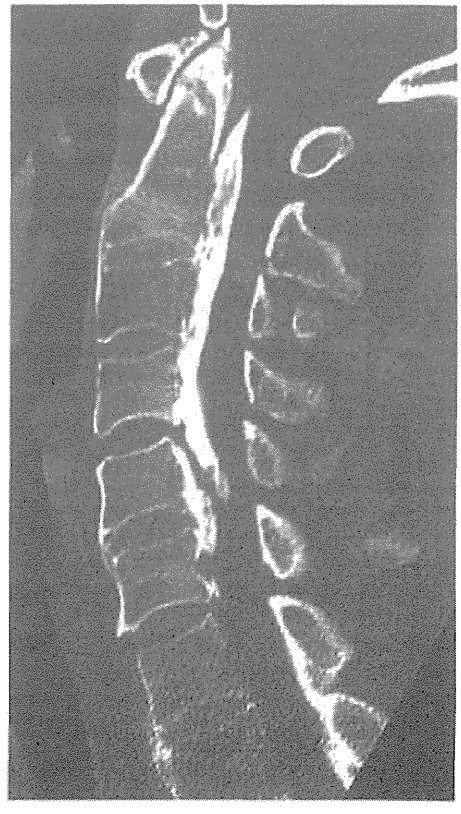

Fig. 2 Typical case with bridge formation in two separate areas. 63-year-old man with three-level bridge at C2–4 and two-level bridge at C5–6 (3 + 2)