

Anterior Vertebral Reconstruction Surgery using Polymethylmethacrylate and Posterior Spinal Short Fusion through the Posterior Approach for Osteoporotic Vertebral Pseudoarthrosis

Jun Takahashi^{*1}, Mikio Kamimura², Yohei Yuzawa¹, Tetsuya Kinoshita¹ and Hiroyuki Kato¹

¹Department of Orthopaedic Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto-city, Nagano, 390-8621, Japan

²Center of Osteoporosis and Spinal Disorders: Kamimura Orthopaedic Clinic, Kotobuki Toyo-oka 595-17, Matsumoto-city, Nagano, 399-0021, Japan

Abstract: *Background:* Cases of osteoporotic vertebral pseudoarthrosis (PA) with severe back pain and neurologic deficit are being increasingly reported. The lack of anterior structure and osteoporosis are the most serious problems in surgery for vertebral PA. We propose here a surgical procedure with posterior spinal short fusion and anterior support using polymethylmethacrylate through the posterior approach. *Materials/Methods:* PA involved the vertebrae of the thoracolumbar junction in five patients. The mean postsurgical follow-up period was 35.8 months. *Results:* The mean kyphotic angle of affected vertebrae improved from $21.6 \pm 9.4^\circ$ before to $9.8 \pm 7.3^\circ$ after surgery ($P < 0.05$). Severe back pain resolved in all patients postoperatively. The correction loss of kyphosis was only 2° at final follow-up. We encountered no major complications including cement leakage, spinal cord damage, or deep-tissue infection. *Conclusions:* Since this procedure provides spinal stability and allows safe neural decompression, it may be a useful surgical procedure for the treatment of osteoporotic PA. However, to prevent sequential vertebral fractures at the adjacent level, additional countermeasures are necessary.

Keywords: Osteoporotic vertebral pseudoarthrosis, posterior spinal short fusion, polymethylmethacrylate.

INTRODUCTION

Vertebral fractures (VFs) are most the common spinal disorders in elderly patients with osteoporosis. Most VFs heal within a few weeks or months, and it was believed that they do not result in severe clinical problems in most cases [1]. Posttraumatic vertebral collapse and/or vertebral pseudoarthrosis (PA) have rarely been reported in VF until recently, but several pathologic mechanisms have been postulated, such as bone ischemia [2,3]. In cases of vertebral PA with prolonged severe back pain and/or paraplegia resisting conservative treatment, surgical treatment may be necessary.

Established opinion holds that the anterior approach is the most suitable for surgical treatment of VFs, because the main pathology occurs in the anterior part of the spine and that approach allows reconstruction of the anterior area [4]. However, the anterior procedure is generally considered to be more invasive than surgery using the posterior approach and is associated with many problems, especially in elderly patients. Furthermore, spine surgeons are not generally familiar with anterior surgical techniques. There are also unresolved problems concerning the ability of implants to remain fixed to the osteoporotic spine.

In cases of vertebral PA, we performed anterior reconstruction surgery based on Kaneda *et al.*'s [4,5] reports but encountered some cases in which additional posterior fixation was required due to displacement of the anterior instrumentation. Some patients with vertebral PA have complicating disorders such as ossification of yellow ligament (OYL) on radiographic examination, and decompression of both disorders may be required. However, it is not possible to perform decompression of both disorders in the same position or through the same incision using the anterior surgical approach. On the other hand, the major problem associated with posterior surgery of vertebral PA is how to reconstruct the anterior vertebral structure.

The purpose of this study was to report a surgical procedure with posterior spinal short fusion and anterior vertebral reconstruction using polymethylmethacrylate (PMMA) through the posterior approach for PA in elderly osteoporotic patients.

SURGICAL TECHNIQUE AND INDICATIONS

The indications for surgery were vertebral PA with prolonged severe back pain and/or paraplegia resistant to conservative treatment using analgesic agents, bed rest, external fixation, etc. for at least three months. Before surgery, we performed percutaneous transpedicular vertebrography using contrast media under a local anesthetic to confirm the origin of back pain and the potential for cement leakage under fluoroscopic control.

*Address correspondence to this author at the Department of Orthopaedic Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto-city, Nagano, 390-8621, Japan; Tel: 81-263-37-2659; Fax: 81-263-35-8844; E-mail: jtaka@shinshu-u.ac.jp

At surgery, the patient is placed in the prone position to reduce kyphosis. One level above and below the involved vertebra is exposed in the axial direction and up to the transverse process in the lateral direction. A transpedicular space is formed up to the vertebral cavity. PMMA is injected through the pedicle under lateral fluoroscopic control. Furthermore, we always insert pedicle screw into the vertebrae with PA. If decompression is required, laminectomy or circumference decompression through the posterior approach can be performed. After complete decompression of the spinal cord and PMMA injection, in principle pedicle screw fixation is performed one level above and one below the involved vertebra. The rod is bended in situ and fixed with pedicle screws. Finally, posterior or posterolateral bone grafting is performed using iliac bone.

MATERIALS AND METHODS

The analysis is based on five patients (four women and one man) with vertebral PA with osteoporosis who underwent this procedure at Shinshu University. The patients ranged in age from 64 to 81 (mean 74.2) years. All patients had severe back pain (P5 on the Denis Pain Scale [6]) unrelated to any apparent major traumatic episode which had continued for more than three months. None had a history of steroid administration or of other medication related to secondary osteoporosis.

Preoperatively, all patients underwent radiographic examination with plain and stress flexion-extension radiographs, magnetic resonance imaging (MRI), myelography, and myelo-computed tomography (CT) focused on the affected vertebrae. To confirm vertebral instability (VI), we performed cross-table lateral XPs. Kyphosis was measured between the cranial and caudal endplate of affected vertebrae.

The cleft of PA involved the vertebrae of the thoracolumbar junction at T11 in one, T12 in two, and L1 in two patients. The dural sac was compressed anteriorly by the posterior vertebral cortex in all patients. However, we

determined that sufficient decompression was achieved by laminectomy, and anterior decompression was not necessary for the patients in this series. Radiologic examination revealed OYL at T11/12 in one patient, and we thus performed posterior decompression at the same time.

Patients were asked to evaluate their back pain using the standard visual analogue scale (VAS). The VAS consisted of a 10-cm line labeled with “no back pain” at 0 and “most intensive back pain imaginable” at 10. In addition, back pain was evaluated objectively and classified into five stages using the Denis Pain Scale: P1, no pain; P2, occasional minimal pain with no need for medication; P3, moderate pain with occasional medication but no interruption of work or significant change in activities of daily living; P4, moderate to severe pain with frequent medication and occasional absence from work or significant change in activities of daily living; and P5, constant or severe incapacitating pain with the need for chronic medication [6]. Before surgery, all 5 patients had back pain classified as severe (P5).

The data were analyzed by a paired-sample Student t test using SPSS (SPSS Japan Inc., an IBM company, Tokyo, Japan), with p<0.05 defined as significant.

RESULTS

Patient characteristics and surgical results are shown in Table 1. VI was clearly apparent on cross-table lateral XPs in all patients. MRIs usually showed that the cleft was of low intensity on T1-weighted images and of very high intensity on T2-weighted images. Preoperatively, no leakage from the vertebral cavity to the epidural space or vessels was observed on vertebrography, and we confirmed that the cavity of PA was a closure space. The administration of local anesthesia to the vertebral cavity at vertebroplasty resulted in the immediate resolution of back pain.

The mean operative time was 253±72 (165-345) min, and the mean intraoperative blood loss was 502±432 (80-1200) ml. The mean postoperative follow-up period was 35.8 [21-

Table 1. Patients Characteristics and Postoperative Results

| Case | Age | Gender | Level | Area | Follow-up | Time | Bleeding | Prekypho | Postkypho | Final-Kypho | Loss | Pre-BP | Final-BP | BMD | Complication | Other |
|---------|------|--------|-------|--------|-----------|------|----------|----------|-----------|-------------|------|--------|----------|------|--------------------------|------------|
| 1 | 64 | F | L1 | T12-L2 | 36 | 165 | 260 | 26 | 15 | 20 | 5 | P5 | P2 | 0.64 | Loosening of Screw (T12) | |
| 2 | 86 | F | T12 | T11-L1 | 21 | 220 | 80 | 17 | 4 | 6 | 1 | P5 | P2 | | | Dead |
| 3 | 68 | F | T11 | T10-L2 | 37 | 305 | 380 | 10 | 4 | 4 | 0 | P5 | P1 | | | |
| 4 | 81 | F | L1 | T12-L2 | 37 | 230 | 590 | 20 | 6 | 9 | 3 | P5 | P3 | 0.85 | Collapse of T12 | |
| 5 | 72 | M | T12 | T11-L1 | 48 | 345 | 1200 | 35 | 20 | 21 | 1 | P5 | P1 | 0.73 | | T11-12 OYL |
| Average | 74.2 | | | | 35.8 | 253 | 502 | 21.6 | 9.8 | 12 | 2.0 | | | 0.74 | | |

Area: Fusion area, Prekypho: preoperative kyphotic angle, Postkypho: postoperative kyphotic angle, Final-kypho: final follow-up kyphotic angle, Loss: loss of correction, Pre-BP: preoperative back pain, Final-BP: final follow-up back pain, BMD: bone mineral density

48] months. The time required for anterior reconstruction using PMMA was 30 min or less. The intraoperative blood loss during anterior reconstruction was only slight because there was little bleeding from the vertebral cavity and that from inside the pedicle was also slight. Bleeding from the pedicle was easily controlled with the application of bone wax. The mean kyphotic angle of the affected vertebrae improved from $21.6 \pm 9.4^\circ$ before to $9.8 \pm 7.3^\circ$ after surgery ($P < 0.05$). Spinal alignment was maintained postoperatively, and the correction loss of kyphosis was only 2° at final follow-up. Posterolateral fusion have had fused in all cases.

Severe back pain with motion disappeared postoperatively in all patients. Mean VAS of back pain showed significant improvement one month after surgery compared with that before surgery ($1.2 \pm 1.3(0-3)$ and $9.0 \pm 0.4(8.5-9.0)$, respectively; $p < 0.0001$). Mean VAS of back pain also showed significant improvement at the final follow up compared with that before surgery ($1.6 \pm 2.0(0-4.8)$ and $9.0 \pm 0.4(8.5-9.0)$, respectively; $p < 0.0001$). Furthermore, at final follow-up, back pain had objectively improved by at least two grades.

Intraoperatively, the absence of cement leakage was confirmed under fluoroscopy. No other major complications including spinal cord damage or deep-tissue infection occurred in this small patient series. Spinal collapse with back pain occurred at the upper adjacent level six months after surgery in one patient, although bone union was achieved within two months and back pain resolved. In the other four patients, back pain did not increase. At final follow-up no patient reported severe back pain.

In all patients, postoperative VI was not observed on stress and supine cross-table XPs. No complications such as dislodgement had been encountered by the final follow-up visit. Screw loosening was observed at the cranial level in one patient, who complained of dull pain in her back soon after surgery and until final follow-up.

CASE REPORT

A 72-year-old man with a 12-month history of severe back pain with minor trauma was referred to Shinshu University. He was able to sit up and was ambulatory but pain upon motion was severe and evaluated as intolerable (P5). A supine lateral cross-table radiograph showed a T12 VF with an intravertebral vacuum cleft (Fig. 1). Kyphosis at T12 was 35° in the standing position (Fig. 2). MRI showed that the dural tube was compressed by the posterior cortex of the T12 vertebral body and by OYL at the T11/12 level (Fig. 3). Transpedicular vertebrography showed that the contrast media spread from the vertebral cavity through the retroperitoneum, but this cavity was a closure space and there was no further contrast media spread (Fig. 4). As anterior compression by the posterior cortex was slight but posterior compression by T11/12 OYL was severe, we planned only posterior decompression and anterior column support using PMMA. The spinal kyphosis decreased, and the postoperative kyphotic angle at T12 was 20° . The patient was encouraged to sit and walk two weeks postoperatively and soon could walk without back pain. At 48 months, he had no back pain (P1), the spinal sagittal alignment had not changed, and kyphosis at T12 was 21° . Moreover, neither

dislodgement nor loosening of implants was observed (Fig. 5A). Post-operative CT scan showed that the vertebral cavity was filled with PMMA, and the shape of PMMA resembled the form of contrast media in vertebrography (Fig. 5B).



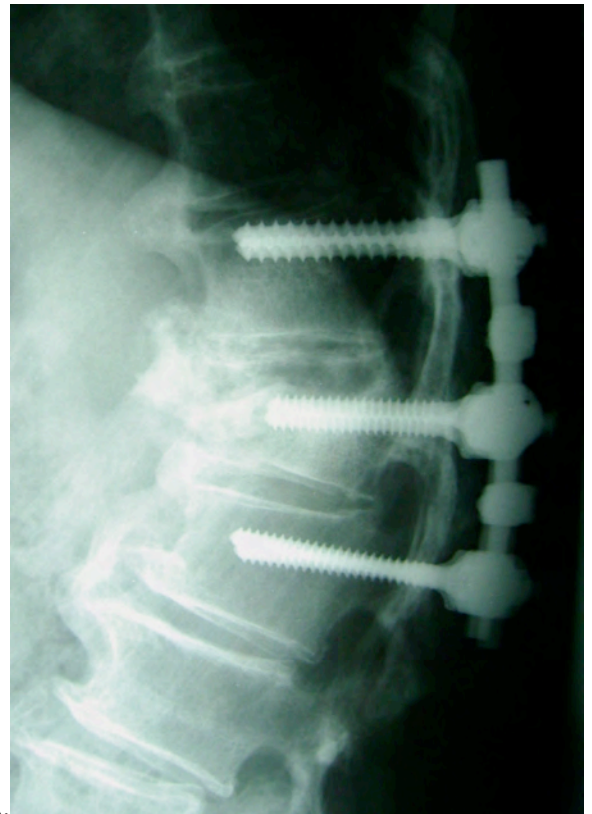
Fig. (1). A vertebral vacuum cleft at Th12 was clearly revealed on supine cross-table lateral radiographs.



Fig. (2). The vacuum cleft shown in Fig. (1) was not visible on conventional standing lateral plain radiographs.



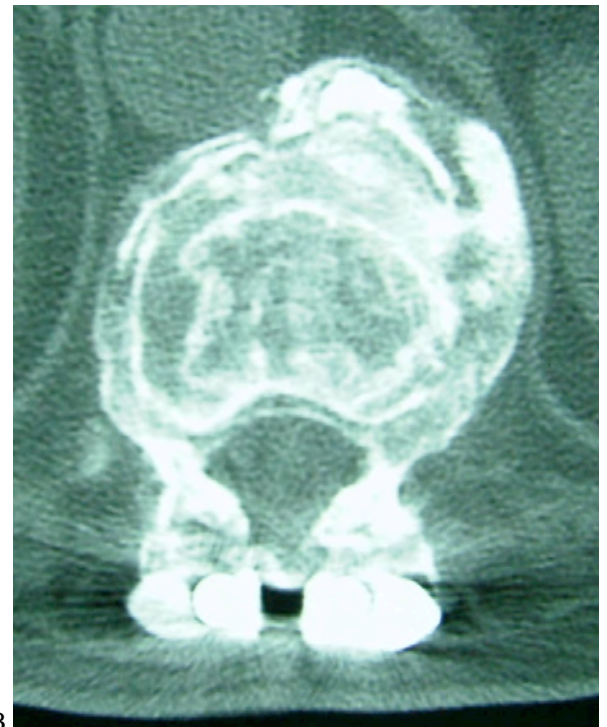
Fig. (3). An intravertebral vacuum cleft at Th12 showed low intensity on T2-weighted MRI and the spinal cord was significantly compressed by OYL at the adjacent Th11/12 level.



A.



Fig. (4). Transpedicular vertebrography. The contrast media spread from the vertebral cavity through the retroperitoneum, but this cavity was a closure space and there was no further contrast media spread.



B.

Fig. (5). A: Plain radiographs and B: CT after surgery. The vertebral cavity was filled with PMMA, and the shape of PMMA resembled the form of contrast media in vertebrography (Fig. 4).

DISCUSSION

The intravertebral cleft is generally considered to be a radiographic sign of avascular necrosis of the vertebral body and so-called Kummell's sign [7]. Recently, the vertebral

cleft has been recognized as a sign of vertebral PA. Many authors noted that bone ischemia may coexist with some VFs, markedly altering their radiologic images, and this might account for the delayed progression of posttraumatic vertebral collapse in some elderly patients [2]. In general, surgical treatment is required for vertebral PA with severe back pain and/or paralysis. VI may be accompanied by severe back pain. Regardless of the type of surgical procedure used, including our method reported here, operative stabilization immediately relieves back pain. This suggests that VI in PA may be the origin of back pain during motion and that it could be treated by solid fixation. In some cases of PA, paralysis of the lower extremities occurs insidiously and gradually worsens to severe paraplegia. Reports of vertebral collapse with neurologic deficits have been increasing recently [8-12].

There are two major clinical problems in osteoporotic PA. One is the absence of anterior vertebral structure causing VI with severe back pain, and the other is compression of the dural sac which may cause neurologic deficits. Therefore, to resolve the clinical problems of PA, a solid spinal fusion and/or surgical decompression procedure is required. Kaneda *et al.* [4] recommended the use of an anterior strut graft with anterior spinal instrumentation in cases of vertebral PA. Surgery through the anterior approach can reconstruct the anterior structure, although this is generally regarded as an invasive procedure in elderly patients. Furthermore, in our experience and that of others [5], additional posterior fixation surgery is required in some cases of anterior reconstruction due to the loosening of screws and anterior strut implants.

In elderly patients, OYL sometimes occurs at the thoraco-lumbar junction and may cause neurologic deficits [13]. In addition, many elderly people have vertebral disc degeneration. Degeneration of the spine accompanied by narrowing of the spinal canal may also cause neurologic deficits. In patients with posterior dural compression, decompression of the posterior part is not possible through the anterior approach. Further, when it cannot be determined whether PA or compression of another spinal region is the cause of neurologic deficits, decompression of both regions may sometimes be advisable. Decompression of both regions cannot be achieved simultaneously using the anterior approach.

The posterior approach has many advantages compared with the anterior approach. Most spinal compression disorders can be decompressed through the posterior approach. Posterior spinal surgery does not pose risks to the chest or abdomen, which can have serious consequences in elderly patients. In addition, spine surgeons are familiar with the posterior approach. A posterior procedure also allows decompression of multiple spinal lesions at the same time and in the same position.

On the other hand, the problem of insufficient implant stability in the osteoporotic spine remains [14-16]. Some authors recommend that posterior instrumentation should be avoided because the range of the application is greater for anterior instrumentation [4]. Further, if posterior decompression is required near the fracture level, posterior decompression destroys posterior spinal elements and may increase VI. In surgery for vertebral PA, posterior reconstruction pro-

cedures may not maintain spinal alignment and may cause loosening of instrumentation.

The lack of anterior structure is the most serious problem in posterior procedures for osteoporotic vertebral PA. It is difficult to reconstruct the anterior spinal architecture through the posterior approach. If adequate reconstruction of the anterior structure is possible through the posterior approach, then posterior surgery may be preferred to anterior surgery. Saita *et al.* [17] proposed the spinal shortening method for anterior decompression and fusion through the posterior approach with short segmental fusion. Such spinal shortening can achieve adequate reconstruction of the anterior structure, although this procedure is technically difficult and neurologic complications due to the compression of nerve roots and the shortening of the spinal cord are a concern.

Vertebroplasty using PMMA was developed in the field of radiology for the treatment of osteoporotic VFs and metastatic vertebral tumors. Many authors reported successful anterior column reconstruction and good clinical results [18-20]. In vertebroplasty of VFs, frequent occurrence of PMMA leakage into the vessels and epidural space was reported [21]. PMMA was also reported to be associated with potential lung injury and polymerization heat problems that might damage the spinal cord or nerve roots [22-24]. We preoperatively confirmed that the cavity of PA was a closure space using vertebrography and concluded that pulmonary complications and cement leakage to the vessels and epidural space would not occur. We therefore first performed anterior column reconstruction using PMMA to treat vertebral PA in 2000.

PMMA is much harder than the osteoporotic spine. The presence of VF is a strong risk factor for subsequent osteoporotic fractures [25,26]. Vertebroplasty may alter the normal loading behavior of the adjacent vertebral body, and there is increased risk of adjacent segment VF [27]. Therefore, since we assumed that anterior reconstruction using PMMA alone might cause the collapse of adjacent vertebra, in principle we added pedicle screw fixation one level above and one below the affected vertebra to our operative procedure. However, in one of our patients, adjacent spinal collapse occurred despite pedicle screw fixation. Recently, Pérez-Higueras *et al.* [21], reported the occurrence of a new VF after percutaneous vertebroplasty. To prevent sequential VFs at the adjacent level, additional countermeasures are therefore necessary.

This procedure may be useful for PA treatment. After our initial patient series, however, it became difficult to use PMMA for spinal reconstruction in Japan based on a notification of pulmonary complications from the Ministry of Health, Labour and Welfare. In this study, we confirmed that the cavity of vertebral PA was a closure space. Therefore, we believe that there is only a slight risk of the complications of cement leakage and pulmonary injury with the use of PMMA and that this procedure may be safe and useful for vertebral PA, especially in elderly patients.

Recently, various biocompatible bone cements have been developed. Biopex (Mitsubishi Materials Corp., Tokyo, Japan) is a biocompatible, injectable, fast-setting in site cement that is stronger than cancellous bone and is replaced

by host bone over time. Biopex is officially approved for use in spinal surgery in Japan. We have thus begun to use Biopex instead of PMMA for this procedure. Further, we revealed that many patients with vertebral PA whose back pain naturally decreased with time despite the presence of vertebral instability [28]. For painful vertebral PA, surgical treatment to improve back pain should be performed after enough conservative treatment.

CONCLUSIONS

Posterior short fusion and transpedicular anterior reconstruction using PMMA resulted in spinal stability, and neural decompression of the whole spine could be performed safely and simultaneously. This procedure may be useful for the treatment of vertebral PA, especially for elderly patients. However, the procedure should be evaluated in a larger patient series in the future.

REFERENCES

- [1] Lee YL, Yip KMH. The osteoporotic spine. *Clin Orthop* 1996; 323: 91-7.
- [2] Maldague BE, Noel HM, Malghem JJ. The intervertebral vacuum cleft: A sign of ischemic vertebral collapse. *Radiology* 1978; 129: 23-9.
- [3] Brower AC, Downey EF. Kummel disease: report of a case with serial radiographs. *Radiology* 1981; 141: 363-4.
- [4] Kaneda K, Asano S, Hashimoto T, *et al.* The treatment of osteoporotic-posttraumatic vertebral collapse using the Kaneda device and a bioactive ceramic vertebral prosthesis. *Spine* 1992; 17: 295-303.
- [5] Kaneda K, Ito M, Taneichi H, *et al.* Osteoporotic post-traumatic vertebral collapse with neurological deficit of the thoracolumbar spine: anterior decompression and reconstruction. *Rinsho-Seikei Geka* 1996; 31: 463-70. (In Japanese)
- [6] Denis F, Armstrong GWD, Searls K, Matta L. Acute thoracolumbar burst fractures in the absence of neurologic deficit. A comparison between operative and nonoperative treatment. *Clin Orthop Relat Res* 1984; 189: 142-9.
- [7] Theodorou DJ. The intravertebral vacuum cleft sign. *Radiology* 2001; 221: 787-8.
- [8] Baba H, Maezawa Y, Kamitani K, *et al.* Osteoporotic vertebral collapse with late neurological complications. *Paraplesia* 1995; 33: 281-9.
- [9] Malghem J, Maldague B, Labaisse MA, *et al.* Intravertebral vacuum cleft: Changes in content after supine position. *Radiology* 1993; 187: 483-7.
- [10] Cho LM, Knight RQ. Idiopathic avascular necrosis of a vertebral body. Case report and literature review. *Spine* 1997; 22: 1928-32.
- [11] Shikata J, Yamamura T, Iida H, Shimizu K, Yoshizawa J. Surgical treatment for paraplesia resulting from vertebral fracture in senile osteoporosis. *Spine* 1990; 15: 485-9.
- [12] Kannus P, Niemi S, Palvanen M, Parkkari J. Continuously increasing number and incidence of fall-induced, fracture-associated, spinal cord injuries in elderly persons. *Arch Intern Med* 2000; 160: 2145-9.
- [13] Payer M, Bruder E, Ficher JA, Benini A. Thoracic myelopathy due to enlarged ossified yellow ligaments. Case report and review of the literature. *J Neurosurg (Spine 1)* 2000; 92: 105-8.
- [14] Havorson TL, Kelly LA, Thomas KA, *et al.* Effects of bone mineral density on pedicle screw fixation. *Spine* 1994; 19: 2415-20.
- [15] Okuyama K, Sato K, Abe E, *et al.* Stability of transpedicle screwing for the osteoporotic spine: An in vitro study of the mechanical stability. *Spine* 1993; 18: 2240-45.
- [16] Soshi S, Shiba R, Kondo H, *et al.* An experimental study on transpedicular screw fixation in relation to osteoporosis of the lumbar spine. *Spine* 1991; 16: 1335-41.
- [17] Saita K, Hohino Y, Kikkawa I, Nakamura H. Posterior spinal shorting for paraplegia after vertebral collapse caused by osteoporosis. *Spine* 2000; 25: 2832-5.
- [18] Lieberman I, Reinhardt MK. Vertebroplasty and kyphoplasty for osteolytic vertebral collapse. *Clin Orthop* 2003; 415 (Suppl): 176-86.
- [19] Rao RD, Singrakhia MD. Painful osteoporotic vertebral fracture. Pathogenesis, evaluation, and roles of vertebroplasty and kyphoplasty in its management. *J Bone Joint Surg Am* 2003; 85: 2010-22.
- [20] Phillips FM. Minimally invasive treatments of osteoporotic vertebral compression fractures. *Spine* 2003; 28: 45-53.
- [21] Perez-Higuera A, Alvarez L, Rossi RE, Quinones D, Al-Assir I. Percutaneous vertebroplasty: long-term clinical and radiological outcome. *Neuroradiology* 2002; 44: 950-4.
- [22] Sarzier JS, Evans AJ, Cahill DW. Increased pedicle screw pullout strength with vertebroplasty augmentation in osteoporotic spine. *J Neurosurg* 2002; 96: 309-12.
- [23] Shapiro S, Abel T, Purvines S. Surgical removal of epidural and intradural polymethylmethacrylate extravasation complicating percutaneous vertebroplasty for an osteoporotic lumbar compression fracture. Case report. *J Neurosurg* 2003; 98: 90-2.
- [24] Lee BJ, Lee SR, Yoo TY. Paraplegia as a complication of percutaneous vertebroplasty with polymethylmethacrylate: a case report. *Spine* 2002; 27: E419-22.
- [25] Gamero P, Sornay-Rendu E, Claustrat G, *et al.* Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. *J Bone Miner Res* 2000; 15: 1526-36.
- [26] Lindsay R, Silverman SL, Cooper C, *et al.* Risk of new vertebral fracture in the year following a fracture. *JAMA* 2001; 285: 320-323.
- [27] Berlemann U, Ferguson SJ, Nolte LP, Heini PF. Adjacent vertebral failure after vertebroplasty. A biomechanical investigation. *J Bone Joint Surg Br* 2002; 84: 748-52.
- [28] Hashidate H, Kamimura M, Uchiyama S, Takahara K, Nakagawa H. Pseudoarthrosis of vertebral fracture: radiographical and characteristic clinical features and natural history. *J Orthop Sci* 2006; 11:28-33.

Received: June 06, 2010

Revised: August 16, 2010

Accepted: August 20, 2010

© Takahashi *et al.*; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.