

Retrospective Study

Varied Low Back Pain Induced by Different Spinal Tissues in Percutaneous Endoscopic Lumbar Discectomy: A Retrospective Study

Nan Ru, MD^{1,2}, Cheng Su, MD³, Jianlong Li, MD³, Yang Li, PhD², Feifei Chen, MD², Guodong Wang, MD², Jianmin Sun, MD², Xingang Cui, MD²

From: ¹Cheeloo College of Medicine, Shandong University, Jinan, Shandong Province, China; ²Department of Spine Surgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No 9677, Jingshi Road, Jinan, Shandong Province, China; ³Shandong First Medical University, Jinan, Shandong Province, China

Address Correspondence: Jianmin Sun, MD
Department of Spine Surgery
Shandong Provincial Hospital
Affiliated to Shandong First
Medical University
No 9677, Jingshi Road
Jinan, Shandong Province, China
E-mail: spine2000@msn.cn

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Background: Percutaneous endoscopic lumbar discectomy (PELD) has become a mature and mainstream minimally invasive surgical technique for treating lumbar disc herniation (LDH). During PELD, various spinal structures, such as ligamentum flavum, dural sac, nerve root, posterior longitudinal ligament, annulus fibrosus, and endplate, were exposed, removed, and decompressed. When we used different endoscopic instruments to touch, remove, and excise different spinal structures, the patient will experience varying degrees of low back pain (LBP). To the best of our knowledge, the differences of the LBP have not been investigated in detail.

Objectives: To evaluate the spinal structures pain variability during PELD.

Study Design: A retrospective study.

Setting: All data were collected from Shandong Provincial Hospital Affiliated to Shandong First Medical University.

Methods: From February 2017 to May 2021, 1,100 patients with LDH underwent PELD surgery. During the operation, the Visual Analog Scale (VAS) was used to assess the pain intensity of each patient, generated by physical stimuli of different endoscopic instruments (i.e., nucleus pulposus forceps, punch forceps, and radiofrequency bipolar coagulator) in different tissue (i.e., posterior longitudinal ligament, nerve root /dural sac, endplate, and ligamentum flavum). Data were analyzed by analysis of variance with Bonferroni post hoc tests.

Results: As for the VAS for LBP among different spinal tissues, the degree of LBP was reduced in each group in the following order (decreasing from most severe to mildest): posterior longitudinal ligament, nerve root/dural sac, endplate, ligamentum flavum, annulus fibrosus ($P < 0.01$). As for the VAS for LBP caused by different endoscopic instruments, we found the most intense LBP always caused by nucleus pulposus forceps, next by punch forceps, then by radiofrequency bipolar coagulator ($P < 0.01$).

Limitations: The retrospective nature of data collection and the educational discrepancies among the trial population may affect data collection to some extent.

Conclusions: During PELD, varied LBP will generate when different spinal tissues are manipulated by different endoscopic instruments, the most severe LBP always came from the posterior longitudinal ligament and nerve root /dural sac. Moreover, compared to incision and thermal stimulus, traction could provoke more severe LBP.

Key words: Low back pain, percutaneous endoscopic lumbar discectomy, visual analog scale, lumbar disc herniation, endoscopic instruments, different spinal tissue

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Lumbar disc herniation (LDH) is a localized displacement of intervertebral disc beyond the normal perimeter of the disc space (1,2) (Fig. 1A-D). It has become a serious social problem, which poses a large burden on society and the health care system (3). Percutaneous endoscopic lumbar discectomy (PELD) has become a mature and mainstream minimally invasive surgical technique for treating LDH (4,5) (Fig. 1E). Compared to traditional lumbar discectomy, PELD has several unique advantages, such as less tissue and muscle dissection, reduced blood loss, reduced hospital stay, early functional recovery, and better cosmesis (6,7).

In our previous study (8), we have identified the patients that experienced different levels of low back pain (LBP) and leg pain when the different tissues were exposed or removed during PELD surgery. During the past 2 decades, various endoscopic instruments were invented along with the development of endoscopic techniques (9-11). We have noticed that even if the same kind of soft tissue was removed, using different instruments to operate can lead to different degrees of LBP. To the best of our knowledge, no such study has yet been conducted.

METHODS

Patients

A total of 1,100 patients with LDH that experienced PELD surgery between February 2017 and May 2021. All operations are undertaken by the same surgeon. Every enrolled patient signed an informed consent form approved by the ethics committee of our institution.

Inclusion Criteria: Patients with single-level symptomatic LDH who received conservative therapy for ≥ 3 months with no remission of the symptoms.

Exclusion Criteria: Patients undergoing multiple levels of discectomy, patients who had a history of spinal surgery, patients with lumbar spinal stenosis, infection, spine fractures, or spinal tumors were also excluded.

Data Collection

All patients were familiarized with testing procedures before PELD surgery, one spinal surgeon explained the questionnaire and the Visual Analog Scale (VAS) scores to enrolled patients.

During the surgery, one surgeon A performed PELD for all enrolled patients. When tissues, such as ligamentum flavum, dural sac, nerve root, posterior longitudinal

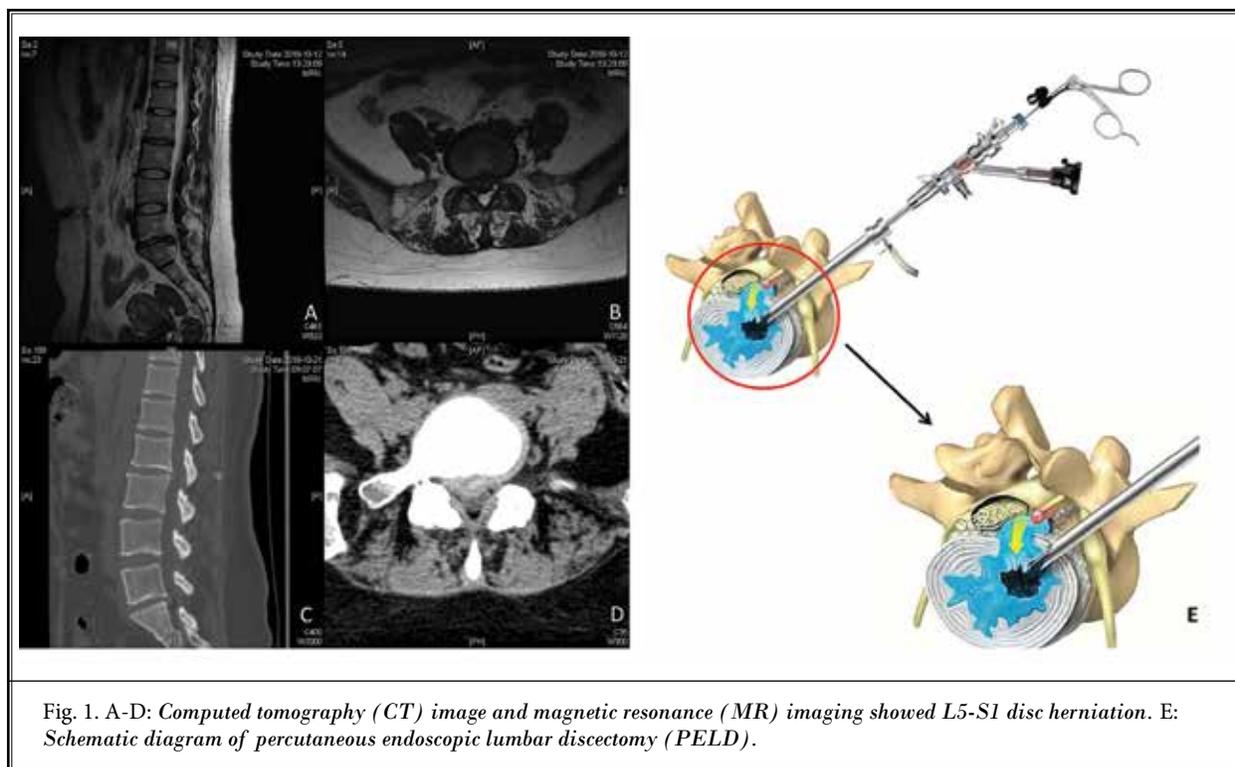


Fig. 1. A-D: *Computed tomography (CT) image and magnetic resonance (MR) imaging showed L5-S1 disc herniation. E: Schematic diagram of percutaneous endoscopic lumbar discectomy (PELD).*

nal ligament (ventral and dorsal), annulus fibrosus, and endplate, were exposed, removed, and decompressed by different instruments (i.e., nucleus pulposus forceps, punch forceps, and radiofrequency bipolar coagulator), the LBPs were clinically assessed with the VAS scores, respectively, the scores were recorded by another surgeon B (Table 1). To prevent dural sac injury and nerve root injury, the use of nucleus pulposus forceps and punch forceps to manipulate dural sac and nerve roots was prohibited.

Statistical Analysis

Statistical analysis was performed using SPSS (Version 21.0; IBM Corporation, Armonk, NY, United States). Analysis of variance and Bonferroni post hoc test were used to investigate the pain differences among different tissue using the same instruments and pain differences among different instruments operating on the same tissue. All data are presented as the means and standard deviations. The statistical significance threshold was $P < 0.05$.

Surgical Technique

Detailed surgical technique route has been reported in several previous studies (6,10).

Step 1: The patients were placed in a prone position under local infiltration anesthesia. The symptomatic disc was localized using C-arm fluoroscopy. The location of the skin incision was marked. Steps for local infiltration anesthesia: 0.5% lidocaine in 10 mL, 0.25% ropivacaine in 4 mL, and 0.9% normal saline in 16 mL were mixed and administered to prevent related pain. The mixed narcotic drug 15 mL to 20 mL was injected layer-by-layer into the skin, subcutaneous tissue, fasciae, muscle, and lumbar facet joint. The mixed narcotic drug would be added intraoperatively, if necessary.

Step 2: Then the percutaneous posterior-lateral approach was conducted under orthogonal radiologic control in 2 planes and the patients were awake for the entire procedure. Epidural anesthesia was administered followed by entering the disc space and injecting a radio-opaque dye (Telebrix, Guerbet, Aulnay-sous-Bois, France). The annulus was then penetrated and discography was done with an indigo carmine (Indigo Carmine, indigotindisulfonate sodium injection) and normal saline mix. A guidewire was inserted into the cannula, and a stab incision

Table 1. *Low back pain questionnaire in PELD.*

Date:

Name: Gender: Age:

Segment: Side: OP Method:

Herniation: ① Central ② Paracentral ③ Foraminal
④ Extreme Lateral

Tissues	Nucleus Pulposus Forceps	Punch Forceps	Radiofrequency Bipolar C
	VAS-B	VAS-B	VAS-B
Ligamentum Flavum			
Dural Sac / Nerve Root	N/A	N/A	
PLL (Ventral)			
PLL (Dorsal)			
Fiber Annulus			
Endplate			

Abbreviations: PELD, percutaneous endoscopic lumbar discectomy; OP, operative; VAS-B, visual analog scale of the back; N/A, not applicable; PLL, posterior longitudinal ligaments.

was made on the skin to pass sequential serial dilators ending with an obturator that entered intradiscally. A multichannel endoscope was then inserted (YESS, Richard Wolf GmbH, Knittlingen, Germany).

Step 3: The decompression was then performed under visual control and gravity-controlled liquid flow. The discectomy was performed first by releasing the intraannular disc attachments to the sequestered disc. The herniated fragment was then removed within the spinal canal with nucleus pulposus forceps slowly while gradually retrieving the working channel and endoscope. Punch forceps was used to incise tough spinal ligaments. A Holmium-YAG side-firing laser (Lumenis Inc, San Jose, CA, United States) was used to vaporize disc fragments that were not removed by the forceps, and a radiofrequency bipolar coagulator (DTF-40, Trigger-Flex® Bipolar System, Elliquance LLC, New York, United States) was used to coagulate bleeding vessels (Fig. 2).

RESULTS

A total of 1,100 patients (620 men and 480 women) received PELD treatment during the study period. There was a wide age range of patients (41.3 ± 12.9 years old), age ranges from 13 to 63 years old. The segments, disc location, disc size, and migration of LDH among 1,100 patients were recorded in Table 2.

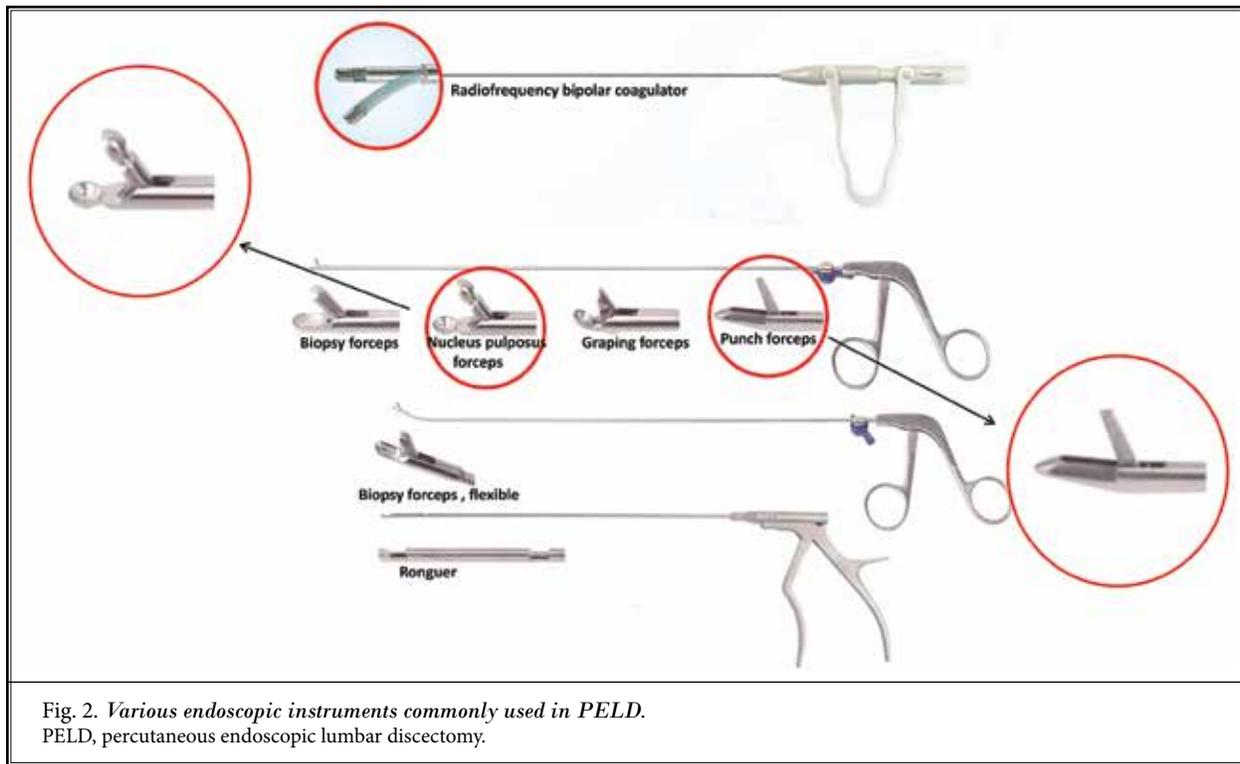


Fig. 2. Various endoscopic instruments commonly used in PELD. PELD, percutaneous endoscopic lumbar discectomy.

As for the VAS for LBP among different spinal tissues, we found that the most severe LBP always came from the posterior longitudinal ligament. The degree of LBP was reduced in each group in the following order (decreasing from most severe to mildest): posterior longitudinal ligament, nerve root/dural sac, endplate, ligamentum flavum, annulus fibrosus ($P < 0.001$) (Fig. 3). The detailed results are shown in Table 3 and Fig. 4.

As for the VAS for LBP caused by different endoscopic instruments, we found the most intense LBP always caused by nucleus pulposus forceps, next by punch forceps, then by radiofrequency bipolar coagulator ($P < 0.001$). The detailed results are shown in Table 3 and Fig. 5.

DISCUSSION

PELD has been used to treat LDH for more than 20 years. This surgical procedure is a current mainstream minimally invasive approach for treating LDH, which is simple and safe, and produces satisfactory results (4,5). It is well known that the manipulation on spinal structures during PELD could provoke LBP (12-15). Our previous study (8) had confirmed the patients experienced different levels of LBP when the different tissues were exposed or removed during PELD surgery. The purpose of this paper is follow-up research based on our previous study (8),

we further explored whether the different properties of the stimulus lead to different degrees of LBP.

In this present study, we found that when we used different instruments to touch, remove, and excise different spinal structures, the most severe back pain always came from the posterior longitudinal ligament, with VAS scores of 3-4. The degree of LBP pain was reduced in each group in the following order (decreasing from most severe to mildest): posterior longitudinal ligament, nerve root /dural sac, endplate, ligamentum flavum, and annulus fibrosus (Table 3).

LBP under endoscope may originate from many spinal structures, for which neuroanatomically feature the structural basis for pain perception (14,16-18). Previous studies (19-21) based on immunohistochemical staining have demonstrated the posterior longitudinal ligament has substantial innervation by sensory nerve fibers and has a structural basis for pain perception in the normal disc. Substantial innervation by nociceptive sensory nerve fibers could also be detectable in the facet joint and the outer third of the annulus fibrosus, whereas do not exist in endplate and ligamentum flavum (14,20-22). Weisskopf et al (13) found the proliferation of nociceptive sensory nerves in the degenerate endplate region, along with the proliferation of vascularity. Others (16,23) held opposing views, they suggested that

the presence of endplate cartilage defects is the source of LBP. Moreover, sensory and autonomic fibers were found at the interior of the annulus fibrosus, as well as at the inner nucleus pulposus (24-26). Persistent disc inflammation may contribute to the regeneration of sensory nerve fibers in these spinal structures.

Table 2. Demographic and clinical characteristics of the patients whom got PELD.

	Parameters	Number
Gender	Men	620
	Women	480
Age(y)	<20	34
	20-30	228
	30-40	383
	40-50	303
	>50	152
Segments	L1-L2	13
	L2-L3	48
	L3-L4	167
	L4-L5	485
	L5-S1	387
Disc Location	Central	347
	Paracentral	316
	Foraminal	296
	Extreme Lateral	141
Disc Size	>50% Canal Compromise	664
	<50% Canal Compromise	436
Migration	Up-migrated	407
	Down-migrated	255
	Low-grade	301
	High-grade	137

Abbreviation: PELD, Percutaneous endoscopic lumbar discectomy.

In addition, we found that when manipulating the ventral and dorsal aspects of the posterior longitudinal ligament, there were significant differences in LBP between the 2. We performed immunohistochemical staining on the ventral and dorsal sides of the posterior longitudinal ligament, respectively. Although immunohistochemical staining is performed in only 6 cases, we found that the density of nociceptive nerves containing sensory neuropeptide substance P on the dorsal side of the posterior longitudinal ligament is higher than that on the ventral side of the posterior longitudinal ligament. Immunohistochemistry staining of substance P in the posterior longitudinal ligament is shown in Fig. 6. This finding has not been reported elsewhere.

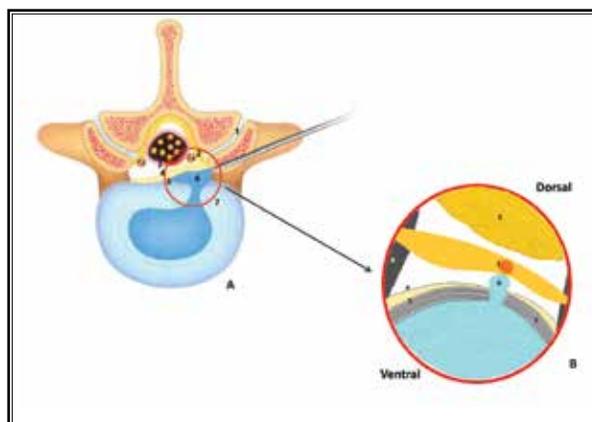


Fig. 3. Concise schematic diagram of tissues seen under endoscope in PELD surgery. 1. Facet Joint. 2. Ligamentum Flavum. 3. Dural Sac. 4. Posterior Longitudinal Ligament (Ventral). 5. Posterior Longitudinal Ligament (Dorsal). 6. Nucleus Pulposus. 7. Fiber Annulus. 8. Endplate. PELD, percutaneous endoscopic lumbar discectomy.

Table 3. Pain variability among different spinal tissues and different instruments in PELD.

	Nucleus Pulposus Forceps	Punch Forceps	Radiofrequency Bipolar Coagulator	P value
PLL (Dorsal)	4.6 ± 0.7	3.9 ± 1.0	3.4 ± 1.1	<0.001*
PLL (Ventral)	2.8 ± 0.9	2.3 ± 1.0	2.0 ± 1.1	<0.001*
Dural Sac/Nerve Root	N/A	N/A	3.8 ± 0.9	N/A
Endplate	1.8 ± 0.6	1.0 ± 0.9	0.9 ± 0.7	<0.001*
Ligamentum Flavum	1.4 ± 0.5	0.6 ± 0.7	0.6 ± 0.5	<0.001*
Fiber Annulus	1.4 ± 0.7	1.0 ± 0.9	0.9 ± 0.8	<0.001*
P value	<0.001*	<0.001*	<0.001*	

The significance of the difference was judged by confidence levels of * $P < 0.05$.

Abbreviations: PELD, Percutaneous endoscopic lumbar discectomy; PLL, posterior longitudinal ligaments.

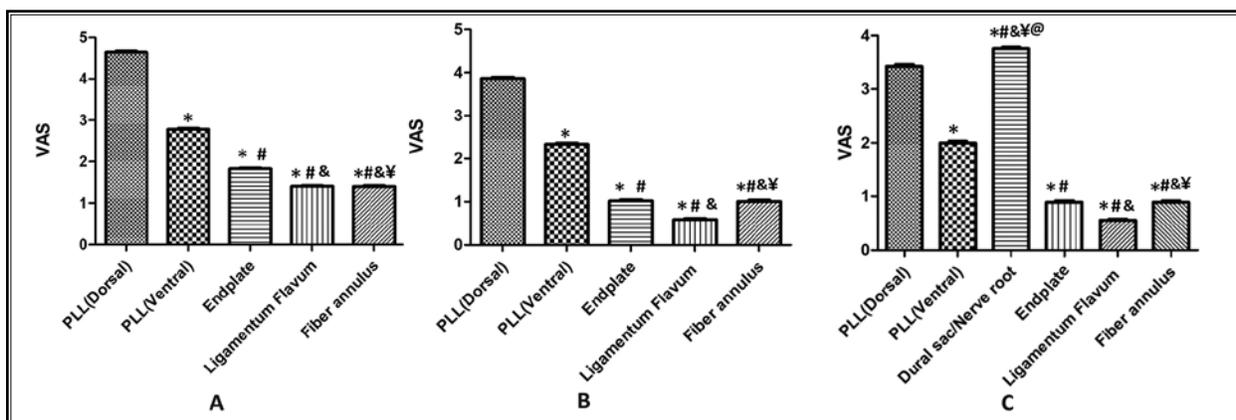


Fig. 4. There were statistically significant Visual Analog Scale (VAS) differences among different spinal tissues. A: Differences in low back pain (LBP) caused by nucleus pulposus forceps. B: Differences in LBP caused by punch forceps. C: Differences in LBP caused by radiofrequency bipolar coagulator. *, $P < 0.05$ (compared with the dorsal aspect of the posterior longitudinal ligaments [PLL]); #, $P < 0.05$ (compared with the ventral aspect of the PLL). &, $P < 0.05$ (compared with endplate). ¥, $P < 0.05$ (compared with ligamentum flavum). @ $P < 0.05$ (compared with fiber annulus). The significance of the difference was judged by confidence levels of $P < 0.05$.

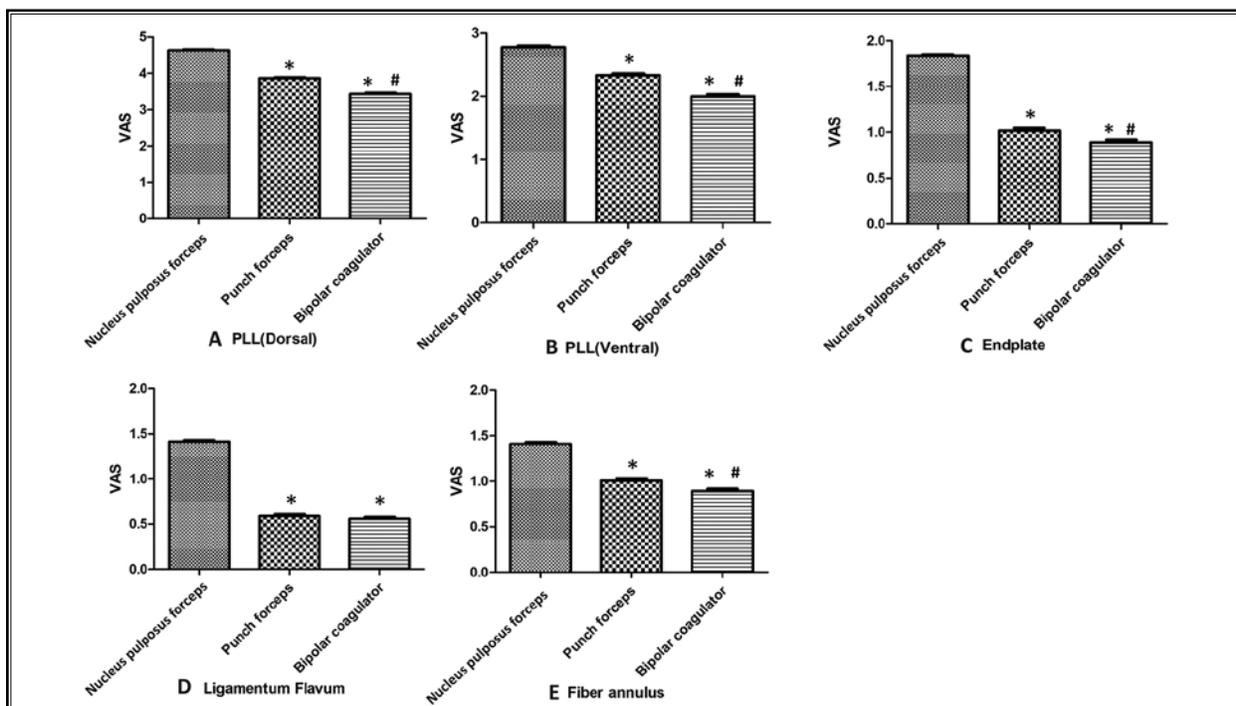


Fig. 5. A-E: There were statistically significant VAS differences among different endoscopic instruments in same spinal structures. VAS, visual analog scale. *, $P < 0.05$ (compared with nucleus pulposus forceps). #, $P < 0.05$ (compared with punch forceps). The significance of the difference was judged by confidence levels of $P < 0.05$.

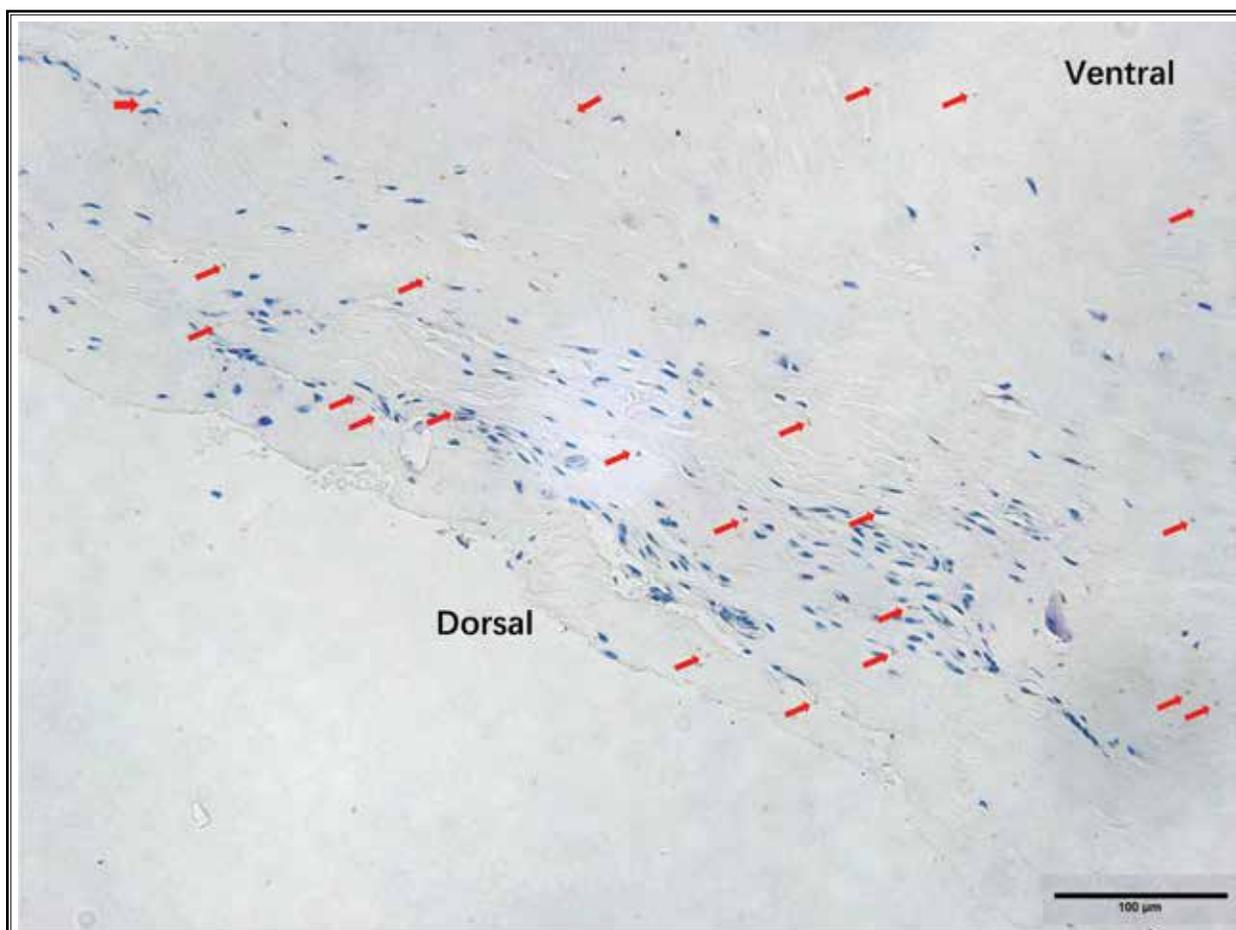


Fig. 6. The density of nociceptive nerves containing sensory neuropeptide substance *P* on the dorsal side of the posterior longitudinal ligament is higher than that on the ventral side of the posterior longitudinal ligament. Red arrows indicate the representative substance *P*-positive area in posterior longitudinal ligament.

Substance *P* is a potent neuropeptide that participates in the sensory, and especially nociceptive, transmission of neural impulses (17,20,27, 28).

As for the pain difference of the same tissue to different stimuli, we chose 3 representative commonly used endoscopic instruments to perform different stimuli.

Nucleus pulposus forceps (Fig. 4A) provides a pulling force to remove the herniated fragment, punch forceps (Fig. 4B) provide a cutting force to incise tough ligament, while a radiofrequency bipolar coagulator (Fig. 4C) provides to coagulate bleeding vessels as well as ablate residual soft tissue. We found that traction causes more severe LBP, relative to incision and thermal stimulus. Previous studies (14,23,28) have demonstrated some of the sinuvertebral nerve terminals act as mechanoreceptors, some as pressoreceptors, and some as thermal receptors.

The data obtained in this study provides a data basis for studying the distribution of these receptors in the spinal structures.

This study is of great clinical significance. First and foremost, patients can be informed of the predicted pain before surgery, which can reduce patients' panic, anxiety, and other negative emotions, and improve surgical fluency. Second, surgeons can preliminarily judge the nature of the tissues according to the patient's intraoperative VAS scores to reduce nerve injury, dural sac tear, and other surgical complications. Third, those results can guide spinal surgeons to use appropriate endoscopic instruments to treat different spinal tissues, which could avoid extra LBP during PELD surgery. Finally, the present research provides a large amount of valuable clinical data on LBP.

Although there are many significant results in this

study, some limitations must be discussed. First, the patient's level of education and cultural differences as well as the differences in understanding the VAS score might have a certain impact on the study results. Second, subtle LBP pain differences are difficult to accurately distinguish by the VAS score. Based on the positive results, we will have a longer-term study in the future and supplement immunohistochemical staining, such as substance P, calcitonin gene-related peptide, as well as enkephalins in various spinal structures.

CONCLUSIONS

During PELD, varied LBP will generate when different spinal tissues are manipulated by different endoscopic instruments, the most severe LBP always came from the posterior longitudinal ligament and nerve root/dural sac. Moreover, compared to incision and thermal stimulus, traction could provoke more severe LBP. The above results provide references and guidelines for the operation, to give humanistic care for patients, and to provide a large amount of valuable clinical data on LBP.

REFERENCES

- Choi G, Lee SH, Bhanot A, Raiturker PP, Chae YS. Percutaneous endoscopic discectomy for extraforaminal lumbar disc herniations: Extraforaminal targeted fragmentectomy technique using working channel endoscope. *Spine (Phila Pa 1976)* 2007; 32:E93-E99
- Ellingson AM, Nuckley DJ. Altered helical axis patterns of the lumbar spine indicate increased instability with disc degeneration. *J Biomech* 2015; 48:361-369.
- Ali A, Khan SA, Aurangzeb A, et al. Lumbar disc herniation in patients with chronic backache. *J Ayub Med Coll Abbottabad* 2013; 25:68-70.
- Kapetanakis S, Giannopoulou E, Charitoudis G, Kazakos K. Health-Related quality of life (HRQoL) following transforaminal percutaneous endoscopic discectomy (TPED) for lumbar disc herniation: A prospective cohort study - early results. *J Back Musculoskelet Rehabil* 2017; 30:1311-1317.
- Häkkinen A, Kautiainen H, Järvenpää S, Arkela-Kautiainen M, Ylinen J. Changes in the total Oswestry Index and its ten items in females and males pre- and post-surgery for lumbar disc herniation: A 1-year follow-up. *Eur Spine J* 2007; 16:347-352.
- Kim HS, Paudel B, Jang JS, Lee K, Oh SH, Jang IT. Percutaneous endoscopic lumbar discectomy for all types of lumbar disc herniations (LDH) including severely difficult and extremely difficult LDH cases. *Pain Physician* 2018; 21:E401-E408.
- Liu X, Yuan S, Tian Y, et al. Comparison of percutaneous endoscopic transforaminal discectomy, microendoscopic discectomy, and microdiscectomy for symptomatic lumbar disc herniation: Minimum 2-year follow-up results. *J Neurosurg Spine* 2018; 28:317-325.
- Chen F, Xin J, Su C, Liu X, Cui X. Pain variability of tissues under endoscope in percutaneous endoscopic lumbar discectomy and its significance: A retrospective study. *Pain Physician* 2021; 24:E877-E882.
- Hurday Y, Xu B, Guo L, et al. Radiographic measurement for transforaminal percutaneous endoscopic approach (PELD). *Eur Spine J* 2017; 26:635-645.
- Yeung AT. The evolution and advancement of endoscopic foraminal surgery: One surgeon's experience incorporating adjunctive technologies. *Sas J* 2007; 1:108-117.
- Chen Z, Zhang L, Dong J, et al. Percutaneous transforaminal endoscopic discectomy compared with microendoscopic discectomy for lumbar disc herniation: 1-year results of an ongoing randomized controlled trial. *J Neurosurg Spine* 2018; 28:300-310.
- Braithwaite I, White J, Saifuddin A, Renton P, Taylor BA. Vertebral end-plate (Modic) changes on lumbar spine MRI: Correlation with pain reproduction at lumbar discography. *Eur Spine J* 1998; 7:363-368.
- Weisskopf M, Birnbaum K, Sagheri M, Lorenzen M, Wirtz DC. [Correlation of low back pain and enhanced vascularization in the vertebral endplate]. *Z Orthop Ihre Grenzgeb* 2004; 142:174-178.
- van Roy P, Barbaix E, Clarijs JP, Mense S. [Anatomical background of low back pain: Variability and degeneration of the lumbar spinal canal and intervertebral disc]. *Schmerz* 2001; 15:418-424.
- Sun CH, Zheng T, Chen Z, et al. [Retrospective and comparative analysis of therapy for degenerative chronic discogenic low back pain with end plate Modic changes with discography and intradiscal injection blockage]. *Zhonghua Yi Xue Za Zhi* 2013; 93:1806-1810.
- Brown MF, Hukkanen MV, McCarthy ID, et al. Sensory and sympathetic innervation of the vertebral endplate in patients with degenerative disc disease. *J Bone Joint Surg Br* 1997; 79:147-153.
- Kontinen YT, Grönblad M, Antti-Poika I, et al. Neuroimmunohistochemical analysis of peridiscal nociceptive neural elements. *Spine (Phila Pa 1976)* 1990; 15:383-386.
- Li W, Gong Y, Liu J, et al. Peripheral and central pathological mechanisms of chronic low back pain: A narrative review. *J Pain Res* 2021; 14:1483-1494.
- Lin W, Ma WT, Xue Y. Low back pain induced by posterior longitudinal ligament incision in percutaneous transforaminal endoscopic lumbar discectomy. *Orthop Surg* 2020; 12:1230-1237.
- Korkala O, Grönblad M, Liesi P, Karaharju E. Immunohistochemical demonstration of nociceptors in the ligamentous structures of the lumbar spine. *Spine (Phila Pa 1976)* 1985; 10:156-157.
- Youssef P, Loukas M, Chapman JR,

- Oskouian RJ, Tubbs RS. Comprehensive anatomical and immunohistochemical review of the innervation of the human spine and joints with application to an improved understanding of back pain. *Childs Nerv Syst* 2016; 32:243-251.
22. Ashton IK, Ashton BA, Gibson SJ, Polak JM, Jaffray DC, Eisenstein SM. Morphological basis for back pain: The demonstration of nerve fibers and neuropeptides in the lumbar facet joint capsule but not in ligamentum flavum. *J Orthop Res* 1992; 10:72-78.
23. Ashton IK, Roberts S, Jaffray DC, Polak JM, Eisenstein SM. Neuropeptides in the human intervertebral disc. *J Orthop Res* 1994; 12:186-192.
24. Palmgren T, Grönblad M, Virri J, Käpälä E, Karaharju E. An immunohistochemical study of nerve structures in the annulus fibrosus of human normal lumbar intervertebral discs. *Spine (Phila Pa 1976)* 1999; 24:2075-2079.
25. Coppes MH, Marani E, Thomeer RT, Oudega M, Groen GJ. Innervation of annulus fibrosis in low back pain. *Lancet* 1990; 336:189-190.
26. Fields AJ, Liebenberg EC, Lotz JC. Innervation of pathologies in the lumbar vertebral end plate and intervertebral disc. *Spine J* 2014; 14:513-521.
27. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. *Cell Mol Life Sci* 2016; 73:4249-4264.
28. Takahashi T, Otsuka M. Regional distribution of substance P in the spinal cord and nerve roots of the cat and the effect of dorsal root section. *Brain Res* 1975; 87:1-11.

