Original Article
Experimental and clinical analysis of a posterolateral lumbar appendicular bone graft fusion

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Abstract: Objective: This study aimed to investigate the animal experimental and clinical results of the bone graft fusion of a posterolateral lumbar appendicular bone. Methods: 1. Sixty rabbits were randomly divided into experimental and control groups. Posterolateral lumbar bone graft with the appendicular bone and iliac bones, respectively, was then performed on these two groups. A lumbar spine X-ray was performed on the postoperative 4th, 8th and 16th weeks, and the gray value changes of the bone graft fusion area were measured to calculate fusion rates. Histology analysis was also performed to observe and count osteoblasts. 2. The appendicular bones of 106 patients who suffered from lumbar disorders were cut during lumbar surgery, and a posterolateral lumbar bone graft was performed. The postoperative follow-up used the Steffee criteria to evaluate clinical efficacy and the White criteria to evaluate fusion conditions. Results: No significant difference was observed in the relative gray values of X-ray bone density, bone graft fusion rates, and osteoblast counts in the bone graft regions between the two groups (P > 0.05). The follow-up duration of the 106 patients were 4-8 years (6.12 years), the clinical efficacy rate was 85.85%, and the fusion rate was 83.02%. Conclusions: The animal experimental and clinical results of posterolateral lumbar bone graft fusion with autologous iliac and appendicular bones were similar.

Keywords: Lumbar, appendicular bone, fusion, experiment, clinical

Introduction
The ideal method of treating lumbar interpyramidal instability is lumbar bone graft fusion. Many fusion methods exist, including anterior lumbar interbody fusion, posterior lumbar interbody fusion, posterolateral lumbar bone graft, lumbar circumferential bone graft, etc. [1]. The lumbar circumferential bone graft and lumbar interbody fusion can obtain a higher fusion rate, reset the maintenance rate, and achieve better functional results [2]. However, both methods have such shortcomings as complex surgical procedures, implanted bone displacement/prolapse, fusion apparatus retropulsion, high costs, and severe trauma, etc. [3]. Posterolateral lumbar bone graft is a method that places the bone graft in the decorticated zygopophysis, transverse process, and paraspinous. The fusion rate and resetting maintenance rate are less than those of the lumbar interbody bone graft. However, the convenience, ease, few complications, and satisfactory efficacy make this method the most widely used [4]. The above bone graft methods usually take the autologous iliac bone as the bone graft material, which may lead to complications such as pain in bone-supply regions and infection that increase the cost of treatment and pain in patients [5]. In recent years, some studies have been conducted on the clinical use of the posterolateral lumbar appendicular bone for bone graft fusion [6, 7]. The spinous process cut during surgery was prepared into small bone pieces or bone strips and combined with the broken bone blocks of the vertebral plate generated during the laminectomy to perform posterolateral lumbar interbody bone graft, including parts of the zygopophysis and transverse process [8]. This fusion method is simple, does not require the iliac bone-taking surgery, can save the patient bone source, reduce the pain and treatment costs, and have few complications. However, whether the appendicular bone graft
has the same fusion effect as the autologous iliac bone graft still lacks comparative studies both locally and abroad.

Materials and methods

Animal research section

Experimental animals and materials: Sixty purebred mature rabbits weighing 2.0-2.5 kg each were randomly divided into two groups. The experimental group was subjected to autogenous appendicular bone graft (n = 30), and the control group was subjected to autogenous iliac bone graft (n = 30). All rabbits were individually bred in cages under centralized management. Grain nutrition diet, free drinking water, and the same lighting and ventilation conditions were provided. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Hunan Provincial Yongzhou Central Hospital.

Animal experiments

Surgical procedures: The rabbits were artificially bred with one rabbit per cage per week, enabling them to adapt to the environment before surgery. The skin was prepared preoperatively, weighed, and anesthetized by ear vein injection with 3% sodium pentobarbital at 30 mg/kg body weight. During surgery, rabbits were fixed in the prone position, disinfected with fortified Lodine solution, and then paved with a sterile towel. The posterior straight midline incision (about 3-4 cm long) was made at the L5/6 vertebral level. The skin and subcutaneous fascia was cut layer by layer and bluntly dissected. The erector spinae was pulled apart, the lamina of both sides was exposed, and the L5 and L6 spinous process was removed. Side fenestration (about 1 × 1 cm²) was then performed, the L5/6 zygapophysis, transverse process, and posterior lateral edge of one side were exposed, and the above parts were decoricated for the bone graft.

Experimental group: The spinous process cut was made into small bone pieces or bone strips and then implanted into the L5/6 zygapophysis, transverse process, and posterior lateral edge, with the combination of the broken bone blocks of vertebral plate.

Control group: The incision was extended to expose the ilium. The autogenous iliac bone was cut as the same length of the appendicular bone. The bone was sectioned into 2 cm × 1 cm² pieces and then implanted into L5/6 zygapophysis, transverse process, and posterior lateral edge.

The two groups were then washed with saline and hydrogen peroxide, and the wound was sutured layer by layer. After surgery, fortified Lodine solution was used again to disinfect the wound before dressing.

Postoperative treatment: The rabbits were bred individually within cages and provided with grain nutrition and free drinking water. The wound was redressed once a day with an intramuscular injection of sodium penicillin 800 000 U once a day for 1 week. The vital signs and general situations of the rabbits were observed. The complications were closely observed and treated in time. Among the 60 rabbits, three cases had diarrhea and treated with norfloxacin capsules 0.1 g once orally, twice a day for 1 week; one case had respiratory infection and treated intramuscularly with benzylpenicillin sodium 1.6 million U a day; and four cases had the formation of subcutaneous mass around the wound 1 week after surgery. However, the skin of the wound healed well, so no special treatment was administered, and the postoperative specimen superscription revealed it to be a limited subcutaneous abscess.

Observation indicators: The two groups were subjected to observations of the following items on the postoperative 4th, 8th, and 16th weeks, respectively.

Gray value of X-ray bone density measurement: Faxitron X-ray Photographer was used to take rabbit lumbar posterior-anterior photographs at each observation time to compare the X-ray gray value changes of the bone graft regions. The photographic conditions were as follows: tube voltage, 35 kV; exposure time, 1 s.

Image J software was used to analyze the X-ray films of the rabbit lumbar bone graft fusion zone taken at each observation time, the standard grayscale was used, and each gray value was the relative gray value for the calculation of the increased relative gray value. The increase in relative gray value was calculated as follows:
relative gray value = gray value after the treatment/standard gray value-gray value before the treatment/standard gray value.

General observation: Before execution, the L5/6 posterior vertebrae of the rabbits were palpated to examine vertebral stability. No loosening was recorded as fusion (+), any loosening was recorded as unfusion (-), and any uncertainty was recorded as unfusion (-) [5]. After sacrificing the animals, the L5/6 lumbar fusion specimens were collected. Fascia and muscles were carefully removed for visual observation of fusion situations.

Histological observation: The specimens of the fusion zones were fixed in 10% neutral buffered formalin for 48 h, followed by decalcification in 10% nitric acid for 12 h. Paraffin-embedded sections were made and subjected to HE staining. An Olympus optical microscope with image analysis system was used to observe the morphological structures in the fusion zones with 200 high-powered ophthalmic lens. Each specimen was subjected to osteoblast counting with six high-powered view fields.

Clinical research section

Clinical cases

From Jan. 2003 to Dec. 2007, a total of 106 patients were subjected to posterolateral lumbar appendicular bone graft during their lumbar surgeries, including 62 males and 44 females aged 15-74 years (average age = 44.45 years). The surgical treatments of the patients included 43 cases of lumbar vertebral fracture, 32 cases of lumbar spondylolisthesis, and 31 cases of lumbar degenerative disease (including lumbar disc herniation and lumbar canal stenosis). This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Hunan Provincial Yongzhou Central Hospital. Written informed consent was obtained from all participants.

Clinical research

Surgical method: For the patients who needed posterior lumbar decompression surgery, the lumbar vertebral lamina, zygopophysis, lumbar posterolateral edge, and transverse process were first revealed, and then the bone graft zone was subjected to decortication. The small bone piece or bone strip made of the spinous process cut during the surgery, combined with the small vertebral small bone blocks, was implanted into the zygopophysis, transverse process, and posterolateral edge. The 89 patients who had lumbar instability or lumbar spondylolisthesis were subjected to posterior resetting and internal fixation with an appropriate pedicle screw system.

Postoperative treatment and follow-up: Conventional postoperative anti-infective therapy was performed, and patients were asked to rest on a hard-board bed. These patients can ambulate with a waist circumference about 3 months later. All 106 patients were followed up for 4-8 years (6.12 years), and re-consultations were performed on the postoperative 1st, 2nd, 3rd, 6th, and 12th months, as well as once a year after a year, to understand the leg pain symptoms and living and working conditions of the patients. During follow-up, lumbar spine X-ray or CT lumbar spine scan were performed when necessary to understand the fusion situations. For the patients who required the removal of inner fixation, some infusion bone tissues were collected when performing the inner fixation-removal surgery. The above procedures were also performed for the histological observation.

Statistical analysis

SPSS18.0 software package was used to set up the database and for statistical analysis. The palpation and visual observation results were subjected to Fisher's exact test. The X-ray gray value change results and the results of osteoblast counting were subjected to the randomized T test with test level $\alpha = 0.05$.

Results

Animal experiment histological observation

Out of the 60 rabbits, 3 rabbits died and 4 rabbits had wound-subcutaneous abscess, among which two died and three had abscesses in the control group. The dead animals were sequentially filled in.

Gray value changes of X-ray bone density

The X-ray photographs of the two bone graft groups on the postoperative 4th, 8th, and 16th weeks all exhibited dense bone tissue shadows.
Analysis of posterolateral lumbar appendicular bone graft fusion

beside the lumbar spine. With prolonged observation time, the densities of the paraspinal bone tissue shadows increased (Table 1). A typical situation of bone graft fusion was observed in the specimens (Table 2). Results of histological observation and osteoblast counting (Figure 1; Table 3) showed that a large number of osteoblasts, new blood vessels, trabecular bone, and some lamellar structures were found in the bone graft fusion zones of the two groups on the postoperative 16th week.

Clinical received treatment and follow-up

The 106 patients who received treatment and follow-up were evaluated for treatment efficacy according to the Steffee standards. Efficacy was categorized as excellent, good, fair, and poor. Results showed that 40 cases were of “excellent” efficacy, 51 were good, 12 cases were fair, and 3 cases were poor. The “excellent” efficacy rate was 85.85%.

X-ray photographs of the normal, lateral, and oblique positions, as well as CT scanning when necessary, revealed that 88 cases possessed a bone bridge connection in the posterolateral lumbar bone graft regions, 14 cases were found to have bone callus, and 4 cases had no callus. According to the X-ray diagnostic criteria of white lumbar instability, 8 patients in this research had lumbar instability during follow-up (patients without bone bridge connection), and the patients were subjected to posterior resetting pedicle screw fixation and bone graft after the lumbar spondylolisthesis, in which the nails of five patients broke. Poor efficacy was mostly concentrated on patients without bone bridges and lumbar instability. The combined X-ray examination and clinical performance tests revealed that the posterolateral lumbar appendicular bone graft fusion rate in this research was 83.02%.

For the patients who required removal of the inner fixation, X-ray examination revealed the existence of a bone bridge connection in the bone graft sites. A small amount of fused bone tissues was collected during the inner fixation removal surgery for histological examination. Results showed that osteoblasts, nourishing blood vessels, and Haversian system existed in the bone graft fusion zones (Figure 2).

Discussion

Lumbar bone graft fusion is a basic technique for the treatment of lumbar instability. This technique is commonly used to assist during the treatment of vertebral fractures, spinal stenosis, degenerative spondylolisthesis, scoliosis, pseudoarthrosis, degenerative disc disease and lumbar facet syndrome, which may require the prevention of lumbar instability [9]. In 1932, Capener proposed anterior interbody fusion based on lumbar interbody bone graft. He proposed that the latter’s fusion rate is significantly higher than the former. Okuyama [10]

Table 1. Relative gray values of the bone density in the 2 bone graft fusion regions (X ± s) in Animal experiments

<table>
<thead>
<tr>
<th>Groups (n = 30)</th>
<th>Postoperative 4th week (n = 10)</th>
<th>Postoperative 8th week (n = 10)</th>
<th>Postoperative 16th week (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendicular bone graft</td>
<td>0.16 ± 0.05</td>
<td>0.33 ± 0.09</td>
<td>0.40 ± 0.12</td>
</tr>
<tr>
<td>Iliac bone graft</td>
<td>0.18 ± 0.06</td>
<td>0.36 ± 0.11</td>
<td>0.42 ± 0.13</td>
</tr>
<tr>
<td>T</td>
<td>4.57</td>
<td>6.34</td>
<td>5.02</td>
</tr>
<tr>
<td>P</td>
<td>0.64 &gt; 0.05</td>
<td>0.73 &gt; 0.05</td>
<td>0.37 &gt; 0.05</td>
</tr>
</tbody>
</table>

There was no significant difference in the relative gray values of the bone density in the bone graft fusion regions on the postoperative 4th, 8th and 16th week (P > 0.05).

Table 2. General observation of bone graft fusion rate in the 2 groups (%) in Animal experiments

<table>
<thead>
<tr>
<th>Groups (n = 30)</th>
<th>Postoperative 4th week (n = 10)</th>
<th>Postoperative 8th week (n = 10)</th>
<th>Postoperative 16th week (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused (unfused)</td>
<td>Fusion rate</td>
<td>Fused (unfused)</td>
<td>Fusion rate</td>
</tr>
<tr>
<td>Appendicular bone graft</td>
<td>4 (6)</td>
<td>40%</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Iliac bone graft</td>
<td>5 (5)</td>
<td>50%</td>
<td>7 (3)</td>
</tr>
<tr>
<td>X²</td>
<td>0.53</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>0.76 &gt; 0.05</td>
<td>1 &gt; 0.05</td>
<td>1 &gt; 0.05</td>
</tr>
</tbody>
</table>

There was no significant difference in the general situation of bone graft fusion rate at each postoperative observation time point (P > 0.05).
Analysis of posterolateral lumbar appendicular bone graft fusion

Figure 1. Histological observation in Animal experiments. A: Morphological structure of the experimental group on the postoperative 16th week. B: Morphological structure of the control group on the postoperative 16th week.

Table 3. Osteoblasts counting in the bone graft fusion zones of the 2 groups (x ± s) in Animal experiments

<table>
<thead>
<tr>
<th>Groups (n = 30)</th>
<th>Postoperative 4th week (n = 10)</th>
<th>Postoperative 8th week (n = 10)</th>
<th>Postoperative 16th week (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendicular bone graft</td>
<td>25.67 ± 12.35</td>
<td>39.94 ± 16.03</td>
<td>32.51 ± 14.33</td>
</tr>
<tr>
<td>Iliac bone graft</td>
<td>28.06 ± 14.22</td>
<td>38.85 ± 17.02</td>
<td>33.12 ± 15.60</td>
</tr>
<tr>
<td>T</td>
<td>3.01</td>
<td>7.67</td>
<td>7.35</td>
</tr>
<tr>
<td>P</td>
<td>0.23 &gt; 0.05</td>
<td>0.85 &gt; 0.05</td>
<td>0.81 &gt; 0.05</td>
</tr>
</tbody>
</table>

Oberved at different postoperative time points, there was no significant difference in the osteoblasts counting in the bone graft fusion zones of the 2 groups (P > 0.05).

proposed the posterior interbody bone graft technique that enables the interbody bone graft fusion to have extensive clinical applications and potential for development. In 1953, Watkins [11] described posterolateral lumbar fusion because of the advantages of ease and less surgical trauma. Since then, this technology has become the most common method of lumbar fusion. A bone graft is placed in the decorticated transverse process and zygapophysis, providing creation conditions for posterolateral fusion. The auxiliary inner fixation can reportedly reduce movement among segments, increase the fusion rate, and increase the fusion rate up to 96% [4].

The autologous iliac bone is usually used to make bone graft material [12], but several shortcomings such as severe surgical trauma, infection of bone donor site, and pain ensue during long-term application [13]. Adopting a safer and easier bone graft material is a research hotspot. The allograft can avoid complications caused by autologous iliac-taking cost [14]. In clinical application, the autologous spinous process cut intraoperatively can be made into small bone pieces or bone strips, together with the broken bone blocks of vertebral plate generated during the laminectomy, and then implanted into the lumbar zygapophysis, transverse process, and posterolateral edge. This method can also achieve the purpose of fusion [15]. Some studies have considered that compared with the bone strip, the bone particles have good plasticity in bone graft and the surface area was huge, thereby providing large contact areas within the body that is conducive to the ingrowth of proliferative vessels and transportation of nutrients. Consequently, the release of bone morphogenetic protein-2 [14] and transforming growth factor β1 increases, which is important toward bone tissue reconstruction [16]. The expression peak time and concentration of the above two osteogenesis-affecting cytokines are positively correlated with the bone contacting area [17]. However, pseudarthrosis can reportedly
Analysis of posterolateral lumbar appendicular bone graft fusion

have a higher incidence if the lumbar cortical bone is used as the posterolateral fusion material after decompression [18].

Although some clinical studies have been conducted on posterolateral lumbar autologous appendicular bone graft fusion [19, 20], no comparative research has been reported between the above technology and posterolateral lumbar autologous iliac bone graft fusion. Whether the above two technologies have the same fusion should be clarified. Through animal experiments, we compared the posterolateral lumbar bone graft fusion situations of rabbit autologous appendicular bone and autologous iliac bone, observed X-ray films of anterior lumbar spine of the two groups on the postoperative 4th, 8th, and 16th weeks, and measured the relative gray value changes of the bone density in the fusion regions. We found that the gray values were 0.16 ± 0.05, 0.33 ± 0.09, 0.40 ± 0.12 and 0.18 ± 0.06, 0.36 ± 0.11, 0.42 ± 0.13, respectively. Through palpation and visual observation of fusion situations, the fusion rates of the two groups were found to be 40%, 70%, and 100%, as well as 50%, 70%, 100%, respectively. Histological observation of the morphological structures of the fusion zones revealed neovessels, trabecular bone, and other structures in the fusion regions of both groups. Osteoblast counting revealed that the results were 25.67 ± 12.35, 39.94 ± 16.03, 32.51 ± 14.33 and 28.06 ± 14.22, 38.85 ± 17.02, and 33.12 ± 15.60, respectively. These results were statistically tested, and no significant difference was observed among them (all P > 0.05), indicating that the posterolateral lumbar autogenous appendicular bone graft achieved the same fusion effect with the autologous iliac bone graft. However, through the complications and death cases, the autologous appendicular bone graft seemed to be safer because the incision need not be expanded and the iliac bone need not be cut.

In clinical application, we made the autologous spinous process cut intraoperatively into small bones piece or bone strips and then implanted them into the lumbar zygapophysis, transverse process, and posterolateral edge together with the broken bone blocks of vertebral plate generated during the laminectomy. This process enabled good fusion results to be achieved. Through the 106 cases of assistive posterolateral lumbar autogenous appendicular bone graft during the lumbar surgeries caused by lumbar fractures, degeneration or spondylolisthesis, as well as the 4-8 years of postoperative follow-up (6.12 years), the patients’ efficacy was found to be 85.85%, and the bone graft fusion rate was 83.02%. This finding was consistent with the usually reported clinical efficacy and bone graft fusion rate of the posterolateral lumbar autogenous iliac bone [21, 22] indicating that the posterolateral lumbar autologous appendicular bone graft fusion was feasible.

Disclosure of conflict of interest

None.

Figure 2. Through clinical observation Histological examination. A: Osteoblasts in the fused bone tissues. B: Newly formed Haversian canal.
Analysis of posterolateral lumbar appendicular bone graft fusion

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References