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Effectiveness and feasibility of injectable *Escherichia coli*-derived recombinant human bone morphogenetic protein-2 on anterior lumbar interbody fusion at lumbosacral junction for adult spinal deformity: A clinical pilot study

Sang-Kyu Im[1], Ki Young Lee[1], Hae Seong Lim[1], Seung-Jin Yoo[2] and Jung-Hee Lee [1,*]

[1] Department of Orthopedic Surgery, Graduate School, College of Medicine, Kyung Hee University, Seoul, Korea; imskyu@naver.com(S.-K.I.); keyng39@hanmail.net(K.Y.L); fofss86@daum.net(H.S.L.); ljhspine@gmail.com(J.-H.L.)
[2] Department of Radiology, College of Medicine, Hanyang University, Seoul, Korea; seungjinyoo@hanyang.ac.kr(S.-J.Y.)
* Correspondence: ljhspine@gmail.com; Tel.: (+82-2-958-8346)

Abstract: Achievement of solid fusion of the lumbosacral junction (L5-S1 level) is an important factor in adult spinal deformity (ASD) surgery. The purpose of this study is to explore the effectiveness and feasibility of injectable rhBMP-2 (a combination of *Eschericia coli*-derived rhBMP-2 and hydrogel type β-TCP carrier; NOVOSIS Inject) as a bone substitute for the fusion of lumbosacral junction. 20 patients (average age 69.1 years) diagnosed with ASD with sagittal imbalance who underwent surgical treatment including anterior lumbar interbody fusion (ALIF) in the L5-S1 level were evaluated. Injectable rhBMP-2 was applied in L5-S1 ALIF and followed-up for 1-year. Solid fusion rates and changes of clinical outcomes (Oswestry Disability Index [ODI], Visual Analog Scale [VAS] of back and leg) were measured and analyzed at 6 and 12 months after surgery. All postoperative adverse events were evaluated about the association with injectable rhBMP-2. Fusion rates were 68.4% and 100% at 6 and 12 months after surgery. Compared to baseline, ODI were improved to 45.8% and 63.7%, VAS(back) were improved to 69.2% and 72.8%, and VAS(leg) were improved to 49.2% and 64.8% at 6 and 12 months after surgery ($p<0.001$, respectively). There were no adverse events associated with injectable rhBMP-2. Thus, injectable rhBMP-2 may be a suitable choice of a bone graft substitute when achieving solid interbody fusion in the lumbosacral junction.

Keywords: adult spinal deformity; lumbosacral junction; anterior lumbar interbody fusion; bone morphogenetic protein; beta-tricalcium phosphate

1. Introduction

Achievement of solid fusion of the lumbosacral junction (L5-S1 level) is an important factor in long-term prognosis and the prevention of complications in long-segment fixation following deformity correction of adult spinal disease (ASD).[1-4] The lumbosacral junction, however, has a high rate of nonunion due to low bone quality, complex anatomy, and application of high biomechanical forces following long-segment fixation.[5,6] Thus, a variety of methods, such as autogenous bone graft, anterior column support, and sacropelvic fixation, have been used to increase fusion rates.[3,6,7] Nevertheless, nonunion of the lumbosacral junction is a major challenge in performing long-segment fixation on ASD patients.

The gold standard for fusion of the lumbosacral junction is an autogenous bone graft, but the amount one can get is limited and there is a potential for complications on the donor site. For these reasons, the use of several other bone substitutes, such as local bone, allogenic bone, demineralized bone matrix (DBM), and bone morphogenetic protein

(BMP), have been preferred as an alternative to autogenous bone grafts. Among these, BMP is a material with greater osteoinductivity than DBM, and recombinant human bone morphogenetic protein-2 (rhBMP-2) of mammalian cell origin has been used in various bone fusion surgeries.[8] Mammalian cell origin rhBMP-2 has widely used because of its excellent fusion outcomes in spinal fusions for ASD. [9-13] However, due to concerns regarding complications from the animal cell origin of mammalian cell origin rhBMP-2, as well as its high cost resulting from low yield,[14,15] *Escherichia coli* (*E.coli*)-derived rhBMP-2 has been developed and confirmed to have equivalent bone fusion performance as mammalian cell origin rhBMP-2.[16-18] Nonetheless, there have not been many reports on the outcomes of interbody fusion in the lumbosacral junction using *E.coli*-derived rhBMP-2.

Meanwhile, since rhBMP-2 is rapidly absorbed in the body, a carrier for its sustained release is needed to maintain its osteoinductive function.[19] Absorbable collagen sponge (ACS), hydroxyapatite (HA) or beta-tricalcium phosphate (β-TCP) can be used as a carrier. Among them, hydrogel type β-TCP has an advantage of having high osteoconductivity, biocompatiblility and fluidity, which enables transplantation of grafts onto irregular surfaces.[20] Thus, combining *E.coli*-derived rhBMP-2 with hydrogel type β-TCP carrier and using it in a form of injectable rhBMP-2 may helpful to increase the fusion rate.

Therefore, this study aimed to explore the effectiveness and feasibility of the injectable rhBMP-2 (NOVOSIS Inject, a combination of *E.coli*-derived rhBMP-2 and hydrogel type β-TCP carrier) as a bone substitute for the fusion of the lumbosacral junctions that have high nonunion rates. For this, injectable rhBMP-2 was applied in the L5-S1 level ALIF in patients who underwent long-segment fixation for ASD diagnosed as lumbar degenerative kyphosis (LDK), and the results were evaluated.

2. Materials and Methods

2. 1. Study design

This study is a prospective single-institution therapeutic exploratory trial involving a 1-year postoperative observation in 20 ASD patients who underwent surgical treatment including ALIF in the L5-S1 level from December 2017.

The inclusion criteria were as follows:

(1) Patients who underwent long-segment fusion[3] to S1 with ALIF L5-S1 as a surgical treatment by a single surgeon at a single institution.

(2) A single etiology of LDK: Patients who clearly showed atrophy of back musculature on magnetic resonance imaging as a diagnostic criterion for LDK and clinical signs including walking difficulty with stooping, inability to lift heavy objects to the front, difficulty in climbing slopes, and need for elbow support when working in the kitchen, resulting in a hard corn on extensor surfaces.[21-23]

The exclusion criteria were as follows:

(1) A history of fusion in the same region

(2) Patients with immunosuppressive or autoimmune disease

(3) Patients with hypersensitivity to rhBMP-2

(4) A history of malignant tumors

(5) Patients with fractures, acute infections, bleeding disorders, active systemic infections, bone formation disorders, or infected surgical sites

(6) Patients with serious conditions that the investigator deems may affect surgery (I.e. heart failure, kidney failure, liver failure, uncontrolled blood pressure, diabetes, blood clotting disorders, etc.)

Informed consent and basic patient information were obtained on screening day. Selection/exclusion criteria and vital signs were also checked. Patients were followed regularly, 6 months and 12 months after surgery. Plain radiographs and lumbar three-dimensional computed tomography (CT) scans were performed along with an evaluation of clinical outcomes in each visit.

Plain radiographs were obtained using lateral 14×36 inch full-spine radiographs obtained with the patients standing in a neutral, unsupported position with "fists-on-clavicle" position.[24] We evaluated SVA, thoracic kyphosis, thoracolumbar junctional angle, LL, lumbosacral junctional angle, PI, PT, and sacral slope (SS). Optimal and suboptimal sagittal balances were defined as SVA ≤ 50mm and > 50mm, respectively.[25] PI, PT, and SS were measured using standing lateral radiographs of the pelvis using methods described in previous reports.[26,27] All radiographs were evaluated using a validated software (Surgimap, Nemaris Inc, New York, NY, USA).[28] All radiological parameters were measured by 3 professional orthopedic surgeons, and the mean measurements were used for analysis.

Clinical outcomes were evaluated using the Oswestry Disability Index (ODI), and a Visual Analog Scale (VAS) for back pain and leg pain.

2.2. Intervention

Approval of the clinical trial plan for the injectable rhBMP-2 (NOVOSIS Inject) made of *E.coli*-derived rhBMP-2 (CG Bio Co. Ltd., Seongnam, Korea) and hydrogel type β-TCP (ExcelOS inject; CG Bio Co. Ltd., Seongnam, Korea) with a pore size of 45~75 ㎛ was obtained from the Ministry of Food and Drug Safety. After dissolving 3mg rhBMP-2 with 1.5ml H2O, it was mixed in-situ with 9g hydrogel type β-TCP. Then final bone graft substitute made of rhBMP-2 loaded β-TCP hydrogel was loaded onto a metal ALIF cage and used for ALIF L5-S1 in a routine manner (Figure 1). Thereafter, posterior column osteotomy (PCO) with multilevel oblique lumbar interbody fusion (OLIF) was performed to restore the sagittal alignment.[29] If multilevel OLIF was not feasible due to previous lumbar spinal fusion, pedicle subtraction osteotomy (PSO) was performed.

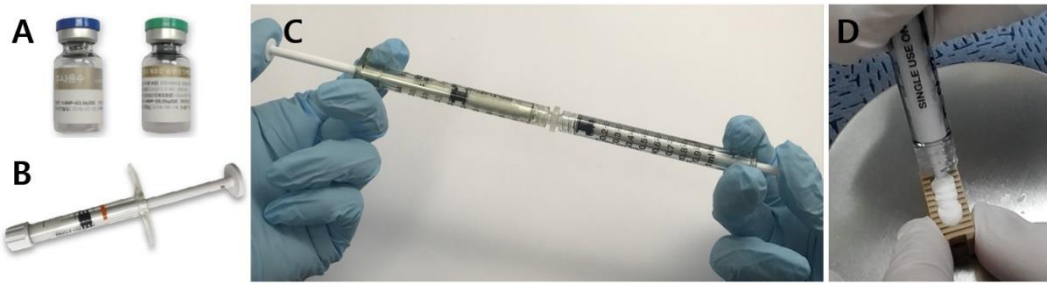

Figure 1. A: 3mg *E.coli*-derived rhBMP-2 dissolved with 1.5ml H2O. B: 9g hydrogel type β-TCP. C: mix A and B in-situ. D: Final bone graft substitute (NOVOSIS Inject) was loaded onto an ALIF cage.

2.3. Outcome measures

For the evaluation of bone fusion, CT-based fusion rates were examined at 6 and 12 months after surgery. Fusion rates were evaluated according to the 4-point grading scale suggested by Whang et al.[30] No evidence of fusion was classified as grade 1, ossification within the disc space but no bridging with the endplate was classified as grade 2, bridging with the end plate under 50% was classified as grade 3, and bridging more than 50% was classified as grade 4. Grades 3 and 4 were defined as fusion. The evaluation of bone fusion was based on the subjective judgement of an independent radiologist who did not participate in the procedure.

The percent change from baseline of ODI and VAS scores were examined at 6 months and 12 months after surgery for the evaluation of clinical symptoms. Percent change from baseline of ODI and VAS scores were calculated as (ODI and VAS scores at each visit – Baseline) / Baseline × 100 (%). ODI score at each visit were calculated as (total score / (number of questions answered × 5)) × 100.

2.4. Safety evaluation

In order to evaluate the safety of the injectable rhBMP-2, the occurrence and severity of all postoperative adverse events were examined. Each event was then evaluated for the association with the injectable rhBMP-2.

3. Results

3.1. Baseline Characteristics

Table 1 presents baseline characteristics. The mean age at surgery was 69.1 ± 5.5 years. Out of 20 patients, loss to follow-up occurred in 1 patient at 6 months after surgery and 1 patient at 12 months after surgery, for a total of 18 patients who were available for follow-up. The average age of all patients was 69.1 years. The upper instrumented vertebra was at T10 in 18 cases and at T9 and at L2 in each case. PCO with multilevel OLIF was performed in 16 patients, while 4 patients underwent PSO. Sacrum 2-alar-iliac (S2AI) screw fixation was performed on all patients.

Table 1. Baseline characteristics (N=20) †

Variables	
Age at surgery (years)	69.1 ± 5.5
Gender	
Female	19
Male	1
Surgical approach	
PCO with multilevel OLIF	16
PSO	4
UIV	
T9	1
T10	18
L2	1
LIV	
S1	20
Fused segments	7.85 ± 0.90
Spinopelvic fixation	
S2-alar-iliac screw fixation	20
BMD spine (gm/cm^2)	0.907 ± 1.53
BMD femur (gm/cm^2)	0.758 ± 0.14

† Data are presented as mean ± standard deviation or number.

PCO indicates posterior column osteotomy; OLIF, oblique lumbar interbody fusion; PSO, pedicle subtraction osteotomy; UIV, uppermost instrumented vertebra; LIV, lowermost instrumented vertebra; BMD, bone mineral density

3.2. Radiographic outcomes

Table 2 lists radiographic parameters of study group. The average SVA before surgery was 187.9 ± 39.6 mm and -14.5 ± 28.0 mm after surgery, and the average LL before surgery was 1.5 ± 16.7° and -70.9 ± 12.3° after surgery, ($p<0.001$, respectively), resulting in restoration of optimal sagittal balance with appropriate lordosis correction. Significant changes were also observed in sacral slope, pelvic tilt and thoracic kyphosis ($p<0.001$, respectively).

Table 2. Radiographic outcomes†

Variables	Preoperative	Postoperative	*p*-value
SVA (mm)	187.9 ± 39.6	–14.5 ± 28.0	<0.001*
TK (°)	6.8 ± 14.7	29.5 ± 10.6	<0.001*
LL (°)	1.5 ± 16.7	–70.9 ± 12.3	<0.001*
PT (°)	30.2 ± 11.2	5.1 ± 7.7	<0.001*
SS (°)	24.1 ± 10.2	49.7 ± 8.3	<0.001*
PI (°)	54.3 ± 8.5		

† Data are presented as mean ± standard deviation

* Statistically significant (p-value < 0.05)

SVA indicates sagittal vertical axis; TK, thoracic kyphosis; LL, lumbar lordosis; PT, pelvic tilt; SS, sacral slope; PI, pelvic incidence

3.3. Fusion rates

6 months after surgery, 13 out of 19 patients (68.4%; disregarding 1 patient lost to follow-up) achieved solid fusion (Figure 2). 12 months after surgery, all 18 patients (100%; disregarding 2 patients lost to follow-up) achieved solid fusion (Table 3).

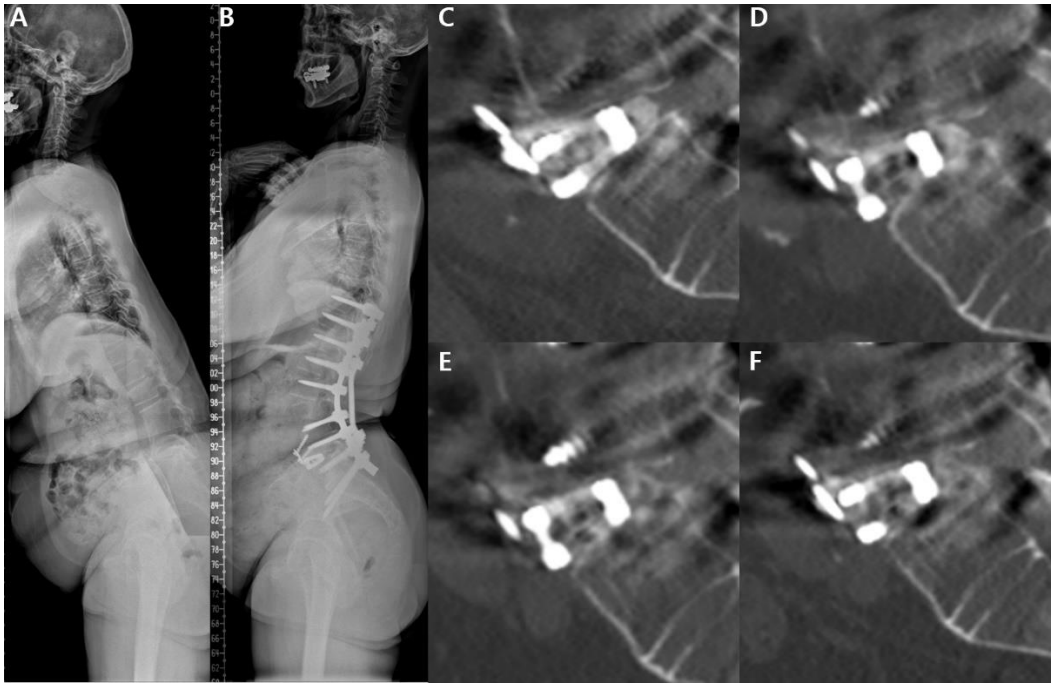

Figure 2. A: This 63-year-old women presented to us with degenerative lumbar kyphosis with sagittal imbalance (SVA 166mm, TK -4°, LL -4°, PT 39°, SS 23° and PI 52°). L3-4 was fused due to old spinal tuberculosis. We performed ALIF on L5-S1 with NOVOSIS Inject, PSO on L3 and posterior spinal fixation from T10 to S1 with sacropelvic fixation. B: Optimal sagittal alignment was maintained until 12 months after surgery (SVA -20mm, TK 13°, LL -68°, PT 16°, SS 52°). C: Immediate postoperative state of lumbosacral junction with NOVISIS Inject loaded onto an ALIF cage. D: 3 months after surgery, grade 3 solid fusion with endplate bridging was confirmed. E: 6 months after surgery, grade 4 solid fusion was achieved. F: 12 months after surgery, fusion proceeded further.

Table 3. Fusion rates †

	6 months after surgery	12 months after surgery
Union, N (%)	13 (68.4)	18 (100.0)
Non-union, N (%)	6 (31.6)	0 (0.0)

† Data are presented as number (percentage)

3.4 Clinical outcomes and adverse events

The patients with follow-up loss were excluded in the analysis of clinical results. ODI score decreased from 68.5 ± 14.0 before surgery to 36.0 ± 20.8 at 6 months after surgery and 24.4 ± 15.7 at 12 months after surgery, improving to 45.8% and 63.7% compared to baseline. VAS (back) score decreased from 7.8 ± 2.1 before surgery to 2.1 ± 1.7 at 6 months after surgery, and 1.7 ± 1.1 at 12 months after surgery, improving to 69.2% and 72.8% compared to baseline. VAS (leg) score decreased from 7.0 ± 1.8 before surgery to 3.4 ± 2.2 at 6 months after surgery, and 2.3 ± 1.8 at 12 months after surgery, improving to 49.2% and 64.8% compared to baseline. All scores demonstrated significant improvements compared to baseline ($p<0.001$, respectively, Table 4).

Table 4. Clinical outcomes and improvement rates after surgery †

	Baseline	Postoperative 6 months		Postoperative 12 months	
		Improvement rate	*p*-value	Improvement rate	*p*-value
ODI	68.5 ± 14.0	36.0 ± 20.8		24.4 ± 15.7	
		45.8 %	< 0.001*	63.7 %	< 0.001*
VAS (back)	7.8 ± 2.1	2.1 ± 1.7		1.7 ± 1.1	
		69.2 %	< 0.001*	72.8 %	< 0.001*
VAS (leg)	7.0 ± 1.8	3.4 ± 2.2		2.3 ± 1.8	
		49.2 %	< 0.001*	64.8 %	< 0.001*

† Data are presented as mean ± standard deviation

* Statistically significant (p-value < 0.05)

ODI indicates Oswestry Disability Index; VAS, Visual Analog Scale

10 cases of adverse events occurred in 5 out of 20 patients (25.0%) (Table 5). All adverse events had no association with NOBOSIS Inject. Otherwise, there were no mechanical complications of ASD surgery, such as proximal junctional kyphosis or pseudarthrosis.

Table 5. Clinical outcomes and improvement rates after surgery †

Pulmonary edema	3 (15%)
Pulmonary thromboembolism	2 (10%)
Wound infection	1 (5%)
Enterocolitis	1 (5%)
Progressive gait disturbance	1 (5%)
Compression fracture	1 (5%)
Colonic tubular adenoma	1 (5%)

† Data are presented as number (percentage)

4. Discussion

Mammalian cell origin rhBMP-2 has demonstrated excellent performance as a bone substitute to promote bone fusion in the surgical treatment of ASD. In previous research involving the use of rhBMP-2 for anterior fusion in ASD, Luhmann et al. reported a fusion rate of 96% upon using a 10.8mg/level rhBMP-2[10], while Mulcorney et al. reported a fusion rate of 91% upon using a 10mg/level rhBMP-2.[11] Furthermore, Annis et al. reported a 97% fusion rate using a low dose (3.2mg) rhBMP-2 for posterolateral fusion of L5-S1 in ASD, similar to what was used in this study.[13] Nonetheless, there is a risk of antibody formation in animal derived rhBMP-2, as well as a risk of disease transmission.[15] It also carries disadvantages of high cost and low yield.[14]

On the other hand, *E. coli*-derived rhBMP-2 is produced through the inclusion body of *E.coli*, eliminating the risk of antibody formation or disease transmission, along with an advantage of a yield up to 99%.[15,31] Cho et al. collectively used an *E.coli*-derived rhBMP-2 with a HA carrier for PLF in spinal stenosis patient, reporting similar bone fusion

capabilities as in an autobone graft.[32] However, to our knowledge, there has not yet been a study that used *E.coli*-derived rhBMP-2 for interbody fusion at lumbosacral junction for ASD patient. Therefore, we combined *E.coli*-derived rhBMP-2 and hydrogel type β-TCP (NOVOSIS Inject) and applied it to the L5-S1 level ALIF in the patients who underwent long-segment fixation for a single etiology of LDK among ASD. And we evaluated the effectiveness and feasibility of this material.

In our study, using an injectable rhBMP-2 made up of a combination of 3 mg rhBMP-2 and 9g hydrogel type β-TCP carrier demonstrated a fusion rate of 100% 12 months after surgery. Although the speed of fusion was slower than demonstrated in the study by Cho et al.[32] where 100% fusion rate was achieved in 6 months, our study showed satisfactory results with achieving 100% fusion rate at 12 months after surgery. While Cho et al. performed single level posterolateral fusion, our study performed long-segment fixation with an average of 7.9 segments which imposing a much greater biomechanical stress on the lumbosacral junction and it may have slowed down bone fusion. In addition, the sacrum has low bone quality and a complex anatomy, making it vulnerable to nonunion.[5,6] Even so, in this study, a 100% fusion rate was obtained using a relatively low dose of 3 mg/level rhBMP-2 in the L5-S1 level ALIF compared to previous studies.[10,11]

S2AI screw fixation was performed in on all patients in this study, along with the use of a titanium cage for L5-S1 interbody fusion to increase the stability of the lumbosacral junction and to achieve solid fixation. Iliac screw fixation in long-segment fixation in ASD has been known to improve fusion rate by increasing the stability of the construct.[3,5,6,13] Additionally, interbody fusion using a titanium cage offers excellent corrosion resistance, low density and an ability to enhance cell adhesion and osseointegration compared to a PEEK cage.[33,34] Lee et al. compared the fusion rates of different cage materials in the lumbosacral junctions, and reported that a titanium cage has advantages over a PEEK cage in interbody fusion.[35] Thus, such methods were used in this study to facilitate fusion progression.

Meanwhile, the main component of the β-TCP used in this study as a carrier is a biodegradable and absorbable ceramic material, which becomes bone within 3 years of being absorbed.[36] The β-TCP has a 95% purity with characteristics of a 45~75um porous circular bead whose porosity is above 68%. Such micro-sized β-TCP beads penetrate into the trabecular bone pores, facilitating initial bone fusion. In this study, a hydrogel type of such β-TCP, composed of thermosensitive polyethylene oxide and polypropylene oxide block copolymer, was used. The hydrogel carrier in a sol state at room temperature changes into a viscous hydrogel by in-situ mixing with rhBMP-2, which enables easy implantation of the graft inside the cage. Upon implantation, the polymeric hydrogel components are gradually bio-degraded or discharged.[37] By using such moldable and injectable β-TCP hydrogel loaded rhBMP-2, the graft was able to fill the irregular cage surface, and its efficiency was increased as the amount of carrier lost during implantation was minimized. Thus, it can be presumed that the injectable nature of the bone graft substitute helped to promote bone fusion, enabling an excellent fusion rate while using a small amount of rhBMP-2.

Along with rapid bone fusion, significant improvements in clinical outcomes were also observed in this study. Many studies have previously demonstrated significant improvements in clinical outcomes with the progression of intervertebral fusion when rhBMP-2 was used in ALIF.[9,38-40] Burkus et al. demonstrated a dramatic improvement in ODI score when using a mammalian cell origin rhBMP-2 in ALIF, from an ODI score of 52.4 before surgery to 21.4 6 months after surgery, and 20.8 12 months after surgery.[9] Although direct comparison is difficult because disease entity was different and long-segment fixation was performed in our study, our study showed similar significant improvement of clinical outcomes after surgery. However, as this study was a single-group study, a comparative analysis compared to a control group will be needed in the future.

We also confirmed the feasibility of injectable rhBMP-2. In this study, 10 adverse effects were occurred in 5 patients (25.0%), but there were no complications directly associated with rhBMP-2. There are some known complications directly associated with rhBMP-

2 using in the spinal surgery such as neuritis, ectopic bone formation, painful seroma formation, vertebral osteolysis, pseudarthrosis, wound infections, and deep vein thrombosis (DVT).[41-43] The incidence of such complications have been reported to increase with the dose of rhBMP-2.[44] Especially, Carragee et al. reported that the incidence of retrograde ejaculation was higher in the rhBMP-2 group (8%) compared to the control group (1.4%) when rhBMP-2 was used in ALIF.[45] But it was impossible to examine the incidence of retrograde ejaculation in this study as there was only 1 male patient. Considering improvements in clinical symptoms, there seems not to have been incidences of neuritis or painful seroma, and although there was 1 case of wound infection, it was due to fat necrosis which was successfully treated using superficial wound irrigation and drainage and healed without deep infection. It is presumed that no adverse effects associated with rhBMP-2 were occurred due to the low dosage of rhBMP-2. But more study is needed to confirm the safety of this material.

This study has following limitations. First, the number of patients was small with a short follow-up period. With a larger number of patients and a longer follow-up period, there may have nonunion cases which lowering the fusion rates, or there may have an increase in complications. Especially, to track the incidence of retrograde ejaculation following ALIF with a greater number of male patients is needed. Furthermore, as this study was a single group study, it was impossible to perform a comparative analysis in fusion rates with another group using autologous iliac bone graft, or a group using mammalian cell-derived rhBMP-2. Therefore, the evaluation of the outcomes of injectable rhBMP-2 through a long-term comparative follow-up study using control groups is needed in the future. Despite such limitations, there are advantages in this study that the study was performed by single surgeon in the same way with the same etiology of LDK among ASD. And this study was the first study analyzing the effectiveness and feasibility of the injectable rhBMP-2, a combination of *E.coli* derived rhBMP-2 with a hydrogel-type β-TCP carrier. The results demonstrated excellent fusion rates without any adverse events in a short 12-month period using a relatively small amount of rhBMP-2 (3 mg) in the lumbosacral junction, where the risk of nonunion is high.

5. Conclusions

Injectable rhBMP-2(a combination of E. coli-derived rhBMP-2 with a hydrogel type β-TCP carrier, NOVOSIS Inject) demonstrated satisfactory fusion rates, clinical outcomes and no adverse events when applied in ALIF at lumbosacral junction. Thus, Injectable rhBMP-2 may be a suitable choice of a bone graft substitute when achieving solid interbody fusion in the lumbosacral junction.

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Institutional Review Board Statement: This study was approved by our Institutional Review Board (KMC IRB 2017-11-033). All patients gave their informed consent before participating in this study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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