INVESTIGATIVE AND CLINICAL UROLOGY
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Expression of bladder α_1-adrenoceptor subtype after relief of partial bladder outlet obstruction in a rat model

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Purpose: Many patients with benign prostatic hyperplasia require treatment for persistent storage symptoms, even when the obstruction is successfully relieved by surgery. Previous studies identified a characteristic increase in α_{1D}-adrenoceptor levels in the bladder in a bladder outlet obstruction (BOO) model. Here, we investigated the expression of α_1-adrenoceptor subtypes in the bladder after relief of partial BOO (pBOO) in a rat model.

Materials and Methods: A total of 60 female Sprague–Dawley rats were randomly divided into three groups (sham-operated, pBOO, and pBOO relief groups), and the expression of α_1-adrenoceptor subtypes in the urothelium and detrusor muscle tissues was examined by western blot.

Results: The expression of the α_{1D}-adrenoceptor was significantly higher in the urothelium and detrusor muscle tissue of the pBOO and pBOO relief groups than in the corresponding tissue of the sham-operated group. Additionally, the α_{1A}-adrenoceptor was predominant in the sham-operated group but significantly decreased in the urothelium in the pBOO group. No significant differences were found in α_{1A}-adrenoceptor levels in detrusor muscle or whole bladder.

Conclusions: Our results showed that α_{1D}-adrenoceptor levels were consistently increased with pBOO, even after relief, suggesting that the α_{1D}-adrenoceptor might be a cause of persistent storage symptoms after relief of pBOO.

Keywords: Adrenergic alpha-1 receptor antagonists; Lower urinary tract symptoms; Urinary bladder neck obstruction; Urinary bladder, overactive

INTRODUCTION

In the treatment of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH), α_1-adrenoceptor antagonists are the most widely prescribed agents and play a primary role in diminishing muscle tension and relieving voiding symptoms [1,2]. The mechanism of action of α_1-adrenoceptor antagonists may explain their

ability to improve voiding symptoms in patients with bladder outlet obstruction (BOO); however, their ability to reduce storage symptoms is not easily determined. Many studies have attempted to define the mechanism of action of α_1-adrenoceptor antagonists in relation to storage symptoms. In animal models, α_1-adrenoceptor antagonists reportedly increase bladder blood flow and function, and human studies have reported upregulation of α_1-adrenoceptors and improvement of urodynamic storage parameters after treatment with α_1-adrenoceptor antagonists [3-6].

Studies examining the distribution of α_1-adrenoceptor subtypes have shown that the α_{1A}-adrenoceptor is the most abundant subtype in the healthy rat bladder; however, whether this is so in humans remains unclear [7]. Expression of the α_{1D}-adrenoceptor is increased in rats and humans with BOO [8,9], and several studies report that selective α_{1D}-adrenoceptor antagonists, such as naftopidil, significantly improve storage symptoms [10-12]. Therefore, these findings suggest that the α_{1D}-adrenoceptor plays an important role in BOO-induced detrusor overactivity.

BPH-induced BOO causes bladder instability that persists in ~40% of patients after surgical removal of the obstruction [13]; however, the cause of detrusor overactivity that persists after relief of BOO is controversial. Several reports suggest that the urothelium of the bladder might play a role in signaling by both efferent and afferent sensory pathways [14,15]. Therefore, it is possible that modulation of α_1-adrenoceptor expression in the urothelium could be related to detrusor overactivity. However, to the best of our knowledge, there have been no studies of the distribution and modulation of α_1-adrenoceptor subtypes in the bladder after relief of partial BOO (pBOO).

In this study, we investigated the expression of α_1-adrenoceptor subtypes in the urothelium and detrusor muscle of rat models of pBOO and pBOO relief.

MATERIALS AND METHODS

1. Animal model and experimental groups

The experimental animals used in this study were female Sprague–Dawley rats (weight: 180–210 g). Rats were placed in separate cages in a room under a controlled environment of 55±5% humidity and 25±1℃ and a 12-hour alternating light-dark cycle. Animals had free access to tap water and were fed standard rat chow. All animal experiments were performed according to guidelines of the ethics committee of Chungnam National University and the Institutional Animal Care and Use Committee (approval number: 201906A-CNU-086). The animals in this experiment were divided into three groups: the sham-operated group (Sham; n=20), the pBOO group (pBOO; n=20), and the pBOO relief group (pBOO+R; n=20).

2. Surgical induction of pBOO and relief of pBOO

Each rat was anesthetized with xylazine and ketamine by intramuscular injection and placed on a servo-controlled operating table to reduce heat loss during surgery. The bladder and urethra were carefully exposed through a midline suprapubic incision.

We followed a previously described surgical method [16] to induce pBOO relief. A 4-0 silk stay suture was placed 3 mm lateral to the urethra in the retrourethral space to allow easy incision of the vaginal epithelium. A small paraurethral incision was made, and a needle with a 3–0 nylon ligature was inserted through the paraurethral incision, passed through the vagina, and then removed on the opposing side of the urethra. A nylon ligature was placed around the dissected urethra and vaginal epithelium and tied in the presence of a steel rod (diameter: 1 mm) in the presence of constant application of ligation tension. The steel rod was removed after suturing, and the ends of nylon from the ligature were pulled down through the paraurethral incision, with the knot positioned in the vaginal space. This process enabled easy removal of the ligature through the vagina. The pBOO and pBOO+R groups were generated as described, and a 3–0 nylon ligature was tied around the dissected urethra and vaginal epithelium with a steel rod. Sham surgery was performed as described, but with the ligature loosely tied to avoid inducing any obstruction, and the end of the nylon also placed in the vaginal space.

After 2 weeks, pBOO group bladders were harvested and immediately stored at -70℃. At the same time, the partial obstruction in the pBOO+R group was resolved by removing the knot through the vagina. Additionally, the loose knot in the Sham group was removed. At 2 weeks after removal of the partial obstruction, bladders from the Sham and pBOO+R groups were harvested.

3. Bladder tissue preparation

The bladder was removed by separating the body and dome from the bladder base at the level of both ureteral orifices, followed by clearance of adhering connective tissue. The weight of the removed bladder tissue was then measured, and the urothelium was separated from the detrusor muscle under a microscope by dissecting the lamina propria layer. We conducted hematoxylin and eosin staining to confirm whether the urothelium layer was separated from the muscle layer, with proper separation of the urothelium and

detrusor muscle confirmed by a pathologist. Each sample was stored for biochemical measurements.

4. Western blot of urothelium and detrusor muscle tissue

Samples of urothelium (n=5) and detrusor muscle (n=5) tissues were homogenized in radio-immunoprecipitation assay buffer, whole-tissue homogenates were centrifuged at 13,000g and 4°C for 20 minutes, and the supernatants were collected. Western blot was conducted according to previously described protocols [17] using the following primary antibodies: glyceraldehyde 3-phosphate dehydrogenase (GAPDH; SC25778; Santa Cruz Biotechnology, Dallas, TX, USA), α-1a (Ab137123; Abcam, Cambridge, UK), α-1b (Ab169523; Abcam), and α-1d (Ab84402; Abcam). Immunoreactive proteins were detected and visualized using a chemiluminescence reagent (Daeillab Service, Seoul, Korea), and the scanned films were quantified using a gel documentation system (Dongjinsa, Seoul, Korea).

5. Statistical analysis

All data are expressed as the mean±standard deviation and were analyzed using SPSS (v.18.0; SPSS Inc., Chicago, IL, USA). Kruskal–Wallis and Mann–Whitney U tests were used for analyses of other parameters. A $p<0.05$ represented statistical significance.

RESULTS

1. Bladder weights

The bladder weights of rats in the Sham, pBOO, and pBOO+R group were 103.3±10.5 mg, 187.1±12.1 mg, and 172.5±14.8 mg, respectively. A significant increase in bladder weight was observed in the pBOO group relative to the Sham group ($p<0.01$), and bladder weight in the pBOO+R group decreased relative to the pBOO group, although this difference was not statistically significant.

2. Western blot analysis of urothelium samples

Levels of the α_{1D}-adrenoceptor in the urothelium were significantly higher in the pBOO and pBOO+R groups than in the Sham group ($p<0.05$) and significantly lower in the pBOO+R group than in the pBOO group ($p<0.05$). Additionally, after relief of pBOO, the α_{1D}-adrenoceptor level in the urothelium remained higher than that of the other α_1-adrenoceptor subtypes. Moreover, among the adrenoreceptors examined, increased levels of the α_{1D}-adrenoceptor was the most dominant effect of pBOO. Furthermore, the α_{1A}-adrenoceptor level in the urothelium was significantly lower in the pBOO and pBOO+R groups than in the Sham group ($p<0.05$), although there was no significant difference in these levels between the pBOO+R and pBOO groups. Additionally, the α_{1B}-adrenoceptor level in the urothelium did not differ significantly between the three groups (Fig. 1).

3. Western blot analysis of detrusor muscle tissue

The α_{1D}-adrenoceptor level was higher in the pBOO and pBOO+R groups than in the Sham group ($p<0.05$), but there was no significant difference in these levels between the pBOO+R and pBOO groups. Additionally, after relief of pBOO, the α_{1D}-adrenoceptor level in the muscle remained higher than the levels of the other α_1-adrenoceptor subtypes. Notably, the observed changes in α_{1A}-adrenoceptor levels in
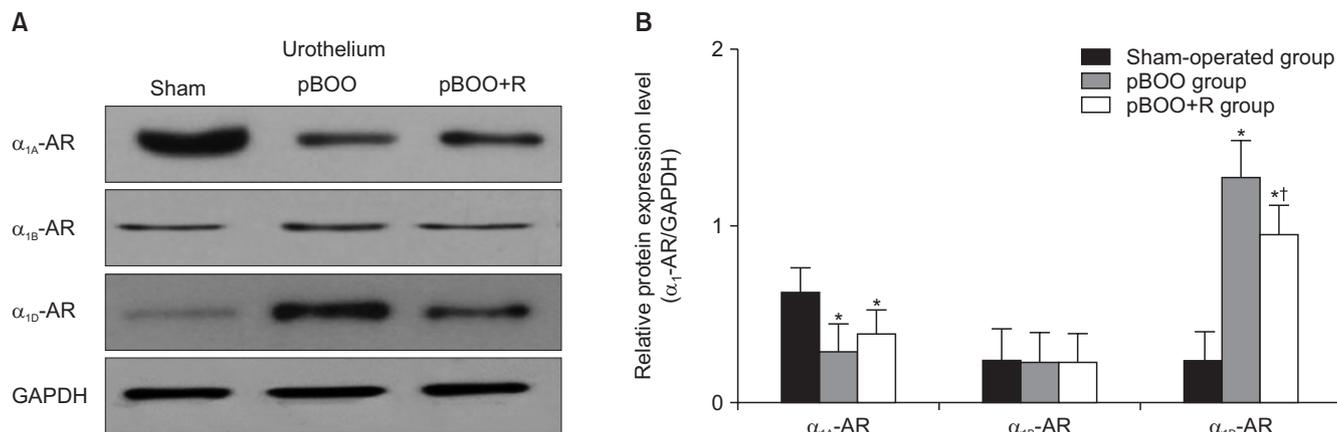
Fig. 1. Western blot analysis of α₁-adrenoceptor (AR) subtypes in the urothelium. (A) Immunoblot of α₁-AR subtypes and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in representative animals. (B) Bar graph showing data for the urothelium from all animals in each group. In the pBOO and the pBOO+R groups, α₁A-AR levels were significantly decreased, and α₁D-AR levels were significantly increased, relative to the Sham group. α₁D-AR levels were significantly decreased in the pBOO+R group relative to the pBOO group. Data represent the mean±standard deviation. *p<0.05 vs. Sham group; †p<0.05 vs. pBOO group. BOO, bladder outlet obstruction; pBOO group, rat model of partial BOO; pBOO+R group, rat model of partial BOO relief.

A

Detrusor muscle

B
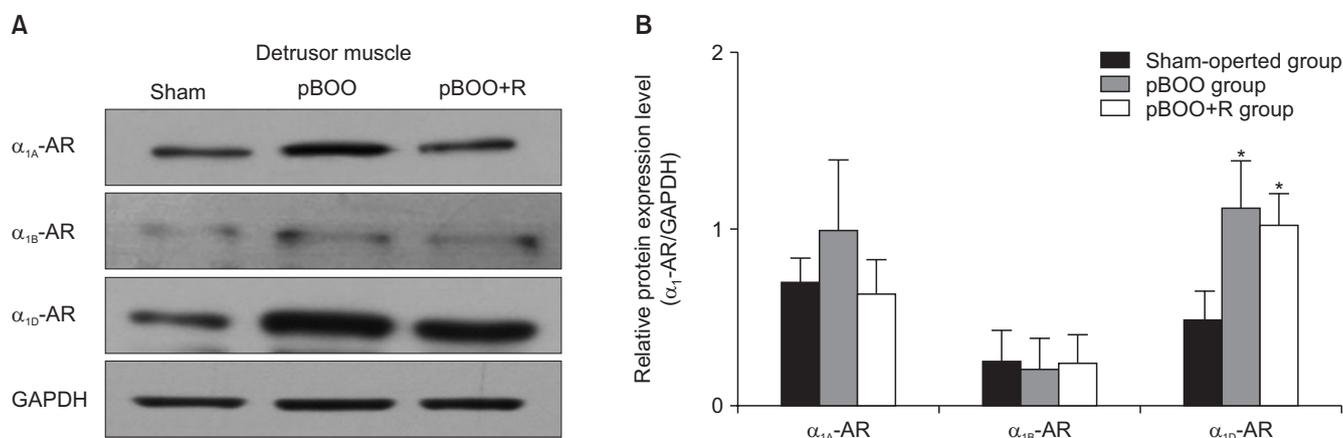
Fig. 2. Western blot analysis of α_1-adrenoceptor (AR) subtypes in the detrusor muscle. (A) Immunoblot of α_1-AR subtypes and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in representative animals. (B) Bar graph showing data for the detrusor muscle from all animals in each group. In the pBOO and pBOO+R groups, α_{1D}-AR levels were significantly increased relative to the Sham group, and differences in α_{1A}- and α_{1B}-AR levels were not statistically significant among the three groups. Data represent the mean±standard deviation. *p<0.05 vs. Sham group. BOO, bladder outlet obstruction; pBOO group, rat model of partial BOO; pBOO+R group, rat model of partial BOO relief.
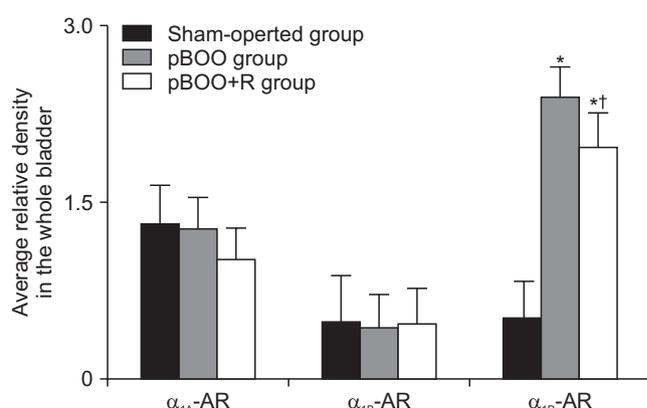
the pBOO and pBOO+R groups in the muscle were opposite of those in the urothelium. Specifically, the α_{1A}-adrenoceptor level increased after pBOO and decreased after relief of the obstruction; however, the changes were not statistically significant, and the α_{1A}-adrenoceptor level in the muscle did not differ significantly between the three groups. As observed in the urothelium, the α_{1B}-adrenoceptor level in the muscle was not significantly affected by pBOO or pBOO relief. Moreover, among the adrenoreceptors examined in the muscle, the α_{1B}-adrenoceptor was expressed at the lowest level (Fig. 2).

4. Comparison of α_1-adrenoceptor subtypes in whole bladder

Fig. 3 shows the levels of α_1-adrenoceptor subtypes in the whole bladder. Levels of α_{1D}-adrenoceptor in the whole bladder were significantly higher in the pBOO and pBOO+R groups than in the Sham group (p<0.05) and significantly higher in the pBOO group than in the pBOO+R group (p<0.05). Additionally, the α_{1A}-adrenoceptor level in the whole bladder decreased slightly after pBOO, but the change was not statistically significant. Moreover, α_{1B}-adrenoceptor levels were unaffected by pBOO. In fact, levels of both α_{1A}- and α_{1B}-adrenoceptors did not significantly differ between the three groups. Furthermore, in the whole bladder, α_{1B}-adrenoceptor levels in the pBOO and pBOO+R groups were lower than those of the α_{1A}- and α_{1D}-adrenoceptor (Fig. 3).

DISCUSSION

Given their therapeutic effects on voiding and storage symptoms, α_1-adrenoceptor antagonists are used as primary

Fig. 3. Average relative density of α_1-adrenoceptor (AR) protein levels in the whole bladder. The bar graph shows data for the urothelium and detrusor muscle tissue represented as the mean±standard deviation. In the pBOO and pBOO+R groups, α_{1D}-AR levels were significantly increased relative to the Sham group and significantly decreased in the pBOO+R group relative to the pBOO group. *p<0.05 vs. Sham group; †p<0.05 vs. pBOO group. BOO, bladder outlet obstruction; pBOO group, rat model of partial BOO; pBOO+R group, rat model of partial BOO relief.

treatment options for LUTS in patients with BPH [1-6]. α_1-Adrenoceptor antagonists are effective at relieving voiding symptoms by reducing smooth-muscle tone in the prostate and urethra, and it is believed that the α_{1A}-adrenoceptor subtype predominant in the human prostate has a functional effect on this mechanism [2]. For storage symptoms, the mechanism of action of α_1-adrenoceptor antagonists has not yet been established but might be associated with the effect of these agents on the bladder [11,18-20].

All α_1-adrenoceptor subtype mRNAs and proteins are expressed in rat urothelium, and both α_{1A}- and α_{1D}-adreno-

ceptors are suggested to play functional roles in this cell layer [21]. The α$_{1D}$-adrenoceptor is expressed at low levels in the normal bladder, but several studies report that its expression is increased during aging and under pathological conditions, such as BOO and overactive bladder [8,9,21,22], suggesting that it might be associated with detrusor overactivity induced by obstruction.

In this study, the α$_{1D}$-adrenoceptor level was significantly higher in the urothelium and detrusor muscle of rats in the pBOO group relative to the Sham group. By contrast, the α$_{1A}$-adrenoceptor level was significantly lower in the urothelium of rats in the pBOO group relative to the Sham group but was not significantly different between the pBOO and pBOO+R groups in the muscle and whole bladder. The urothelium acts as a potential sensory organ, because it can respond to neurotransmitters [15]. Although not directly investigated here, elevated α$_{1D}$-adrenoceptor levels in the urothelium suggest that this α$_1$-adrenoceptor might affect urothelial function when activated. The α$_{1D}$-adrenoceptor is found in smooth muscle and promotes efferent acetylcholine release from the pelvis [23]. Additionally, activation of the α$_{1D}$-adrenoceptor can directly affect muscle contraction [24]. Our findings suggest that pBOO induces α$_{1D}$-adrenoceptor expression in the urothelium and bladder smooth muscle. Although the reasons for this alteration are not yet clear, this activity might be related to hypertrophied bladder masses [8]. Moreover, elevated α$_{1D}$-adrenoceptor expression might contribute to storage symptoms associated with bladder hypertrophy due to increased detrusor-contraction tone and decreased compliance. Additionally, because the affinity of the α$_{1D}$-adrenoceptor for endogenous neurotransmitters is ~10- to 100-fold higher than that of other subtypes, it is possible to initiate responses, such as unstable contractions, at low concentrations of neurotransmitters. These findings imply that in the pBOO model, the urothelial α$_{1D}$-adrenoceptor might play a more significant role than the α$_{1A}$-adrenoceptor in the development of detrusor overactivity.

Selective α$_{1D}$-adrenoceptor antagonists, such as naftopidil, can significantly improve storage symptoms. In a randomized controlled trial, treatments with naftopidil and tamsulosin were associated with significant improvements in International Prostate Symptom Scores and daytime frequency, urgency, and nocturia subscores [10]. Takahashi et al. [11] examined frequency volume charts for 2 days in 82 patients, finding that naftopidil significantly improved urinary frequency and significantly increased mean volume per void. In another study, naftopidil reportedly eliminated overactivity in 21% of BPH patients with detrusor overactivity and increased cystometric capacity in 36% of these patients [12],

with the amplitude of largest overactive detrusor contraction also significantly decreased by naftopidil treatment. These findings support our interpretation of the present results.

In addition to medical treatment of BPH with various α$_1$-adrenoceptor antagonists, surgical approaches, such as transurethral resection of the prostate (TURP), photoselective vaporization of the prostate, and holmium laser enucleation of the prostate, can improve LUTS by reducing prostatic urethral resistance. However, in several studies, some patients undergoing surgical procedures for BPH experienced persistent or newly developed storage symptoms associated with detrusor overactivity on urodynamic investigation [25-27]. According to a study of patients who underwent TURP, ~40% of the patients showed detrusor overactivity, despite successful relief of BOO [13]. This phenomenon can cause stress and anxiety to both patients and surgeons; however, the cause has not yet been established. Chai et al. [28] found that 20% of a rat model displayed persistent hyperactive voiding after urethral delegation and suggested that this might have been related to persistent neuroplasticity mediated by nerve growth factor. Additionally, ischemia-reperfusion injury has been proposed as a cause of postoperative bladder dysfunction in patients with BPH [29]. Our findings suggest that increased α$_{1D}$-adrenoceptor expression could be a cause of this phenomenon.

In this study, we used the animal model surgical method described by Jin et al. [16] to analyze α$_1$-adrenoceptor subtype expression after relief of pBOO. To the best of our knowledge, there have been no previous studies of the distribution and modulation of α$_1$-adrenoceptor subtypes after pBOO relief. We found that pBOO significantly increased α$_{1D}$-adrenoceptor levels in both the urothelium and detrusor muscle and reduced the α$_{1A}$-adrenoceptor level in the urothelium. Moreover, α$_{1D}$-adrenoceptor downregulation induced by pBOO relief was statistically significant in the urothelium but not the muscle. This might be due to the different compositions of urothelium and muscle. Furthermore, this implies that the urothelium might respond more quickly in the recovery phase after pBOO relief and relative to the muscle. Notably, we found that α$_{1D}$-adrenoceptor levels remained elevated, even after pBOO was relieved. Because α$_{1D}$-adrenoceptor reportedly stimulates cellular growth through the extracellular signal-regulated kinase pathway [30], increased α$_{1D}$-adrenoceptor levels associated with pBOO might contribute to bladder hypertrophy. Therefore, α$_{1D}$-adrenoceptor, which is maintained in an elevated state after pBOO relief, might continually contribute to bladder hypertrophy, thereby sustaining storage symptoms. However,

ICUROLOGY

the reason for sustained elevation of α_{1D}-adrenoceptor levels after pBOO relief remains unclear. There are many possible hypotheses, but we suggest that after pBOO relief, bladder hypertrophy is maintained, with the associated hypoxia sustaining increases in α_{1D}-adrenoceptor levels.

Our results suggest that the urothelial α_{1D}-adrenoceptor plays an important role in the persistence of storage symptoms after pBOO relief. If our findings are confirmed in humans, selective α_{1D}-adrenoceptor antagonists, such as naftopidil, might represent an effective treatment strategy for persistent postoperative storage symptoms in BPH patients.

A limitation of our study is that no functional assessment was performed to confirm the physiologic role of α_1-adrenoceptor modulation. In the future, urodynamic studies in animal models or determination of physiologic responses to α_1-adrenoceptor antagonists should be performed to confirm our findings.

CONCLUSIONS

In this study, we observed consistent elevations in the α_{1D}-adrenoceptor level, even after pBOO relief, suggesting that the α_{1D}-adrenoceptor might be a cause of persistence of storage symptoms after relief of pBOO. Further studies are needed to evaluate the physiologic action of the α_{1D}-adrenoceptor and elucidate the associated mechanisms in a pBOO relief model.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Ji Yong Lee, Jong Mok Park, and Ju Hyun Shin. Data acquisition: Ji Yong Lee, Jong Mok Park, and Ju Hyun Shin. Statistical analysis: Ji Yong Lee, Jong Mok Park, Gun Hwa Kim, and Ju Hyun Shin. Data analysis and interpretation: Ji Yong Lee, Jong Mok Park, Gun Hwa Kim, and Ju Hyun Shin. Drafting of the manuscript: Ji Yong Lee, Jong Mok Park, and Ju Hyun Shin. Critical revision of the manuscript: all authors. Obtaining funding: Ju Hyun Shin. Administrative, technical, or material support: Gun Hwa Kim and Ju Hyun Shin. Supervision: Yong Gil Na, Ki Hak Song, Jae Sung Lim, Seung Woo Yang, and Ju Hyun Shin. Approval of the final manuscript: all authors.

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