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ORIGINAL ARTICLE

The role of sphincteroplasty in adverse effect of anomalous pancreaticobiliary duct union in an animal model

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Abstract Anomalous union between the pancreatic and biliary systems (APBDU) has been reported to produce choledochal cyst. The aim of this experiment was to evaluate the role of sphincteroplasty to adverse effect of APBDU in an animal model. Twelve mongrel puppies were randomly divided into a control group ($n = 5$) and an experimental group ($n = 7$). A well-established model of APBDU was produced in both groups. Transduodenal sphincteroplasty was performed only on the experimental group. For all animals, serial chemical analyses of serum were performed, and biliary tree sizes were measured by magnetic resonance cholangiography 2.5 months after the experimental surgery. At the time of animal sacrifice, 3 months after the experimental surgery, operative cholangiography was performed, and bile juice and tissues were obtained for chemical analysis and histologic examination. Dilatation of the bile duct and thickening of the wall of the bile duct were observed less frequently in the experimental group than in the control group. There were no significant differences found in pancreatic enzyme activity in the bile juice between the two groups. Denudation of the mucosa was the predominant mucosal change seen in the experimental group, while epithelial hyperplasia was the predominant mucosal change found in the control group. Our experiment shows that sphincteroplasty is not effective to prevent the pancreaticobiliary reflux, but may be effective to reduce the degree of both bile duct dilatation and mural thickening in the APBDU puppy model.

Keywords Choledochal cyst · Anomalous pancreaticobiliary duct union · Sphincteroplasty · Animal model

Abbreviations

APBDU	Anomalous pancreaticobiliary duct union
MRC	Magnetic resonance cholangiography

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Introduction

Many theories exist regarding the etiologies of choledochal cyst formation, but one of widely accepted is that a choledochal cyst is caused by pancreaticobiliary reflux through an anomalous union between the pancreatic and biliary systems (APBDU) [1, 2]. Although choledochal cyst origin is generally believed to be congenital, clinical evidence suggests that a choledochal cyst may be caused by irreversible bile

duct dilatation resulting from long-standing pancreaticobiliary reflux through an APBDU [3]. Other reportedly ill effects of APBDU are hepatobiliary malignancy and recurrent abdominal pain requiring extensive surgery [4–7]. In adults, APBDU management recommendations include resection of the gallbladder to prevent gallbladder cancer [4, 5]. In children, several reports recommended resection of the common bile duct as a treatment of the recurrent abdominal pain in APBDU without bile duct dilatation [6, 7]. Because not all patients with APBDU have a choledochal cyst, hepatobiliary malignancy, or recurrent abdominal pain, the role of APBDU as the sole causative factor of these maladies is doubtful. An early study suggested that sphincter of Oddi dysfunction may play an important role in pancreaticobiliary reflux in an APBDU [8].

In this study, we hypothesized that sphincteroplasty may prevent the adverse effects of pathologies associated with APBDU. To verify this hypothesis, we used a well-established puppy model of APBDU [9–11].

Material and methods

Materials

Twelve mongrel 2-month-old puppies were randomly divided into a control group ($n = 5$) and an experimental group ($n = 7$). In the control group, APBDU was produced by end-to-side choledochopancreatostomy according to a previously described technique [8–10]. In the experimental group, in addition to the formation of an APBDU, a transduodenal sphincteroplasty was performed (Fig. 1).All puppies were sacrificed 3 months after surgery. The use and care of laboratory animals in this experiment were based on the Guidelines and Regulations for the Use and Care of Animals of Yonsei University.

Methods

Surgery to produce APBDU in the control group (Fig. 1b)

Detailed methods of the experimental surgery required for APBDU formation have been previously published and are discussed only in brief here [9–11]. Laparotomy was performed under inhalation anesthesia. The common bile duct was divided at the choledochoduodenal junction, and the duodenal stump of the common bile duct was closed with interrupted absorbable sutures. The dorsal pancreatic duct was identified, and a longitudinal incision was made on its anterior wall for the choledochopancreatic anastomosis. The hepatic stump of the common bile duct was pulled down toward the incision site of the dorsal pancreatic duct, and a choledochopancreatic end-to-side ductal anastomosis was performed using interrupted 6-0 proline sutures. Thus, the anastomosis site was definitely located outside the duodenum, and an APBDU was subsequently produced.

Surgery for APBDU formation and sphincteroplasty in the experimental group (Fig. 1c)

The same procedure as described above for the control group was used to produce an APBDU in the experimental group until the opening of the dorsal pancreatic duct. At this point, a longitudinal incision was made in the dorsal pancreatic duct, and a small silver probe was inserted to identify the duodenal opening of the dorsal pancreatic duct. By transduodenal palpation of the probe, the appropriate site for duodenotomy was selected. After duodenotomy, the duodenal papilla of the dorsal pancreatic duct was opened longitudinally over the probe, and sphincteroplasty was performed with interrupted 5-0 Vicryl sutures. Thus, the sphincter function of the duodenal papilla of the dorsal pancreatic duct was lost. The duodenotomy was closed with interrupted sutures. Choledochopancreatic end-to-side ductal anastomosis was performed in an identical manner as described for the control group.

Biochemical assay

Serum amylase and lipase levels were measured prior to surgery, on the 10th postoperative day, and on the day of sacrifice for all animals. The bile juice was collected from the gallbladder and the common bile duct by direct puncture at the time of sacrifice. The activity of amylase and lipase in the bile juice was measured to estimate the degree of pancreatic juice reflux into the bile duct.

Roentgenologic examination

X-ray studies were performed by magnetic resonance cholangiography (MRC) 2.5 months after the experimental surgery, and operative cholangiography was performed at the time of sacrifice. The diameter of the bile ducts was measured by MRC only. Operative cholangiography was not used to determine the diameter of the bile ducts because of potential errors that

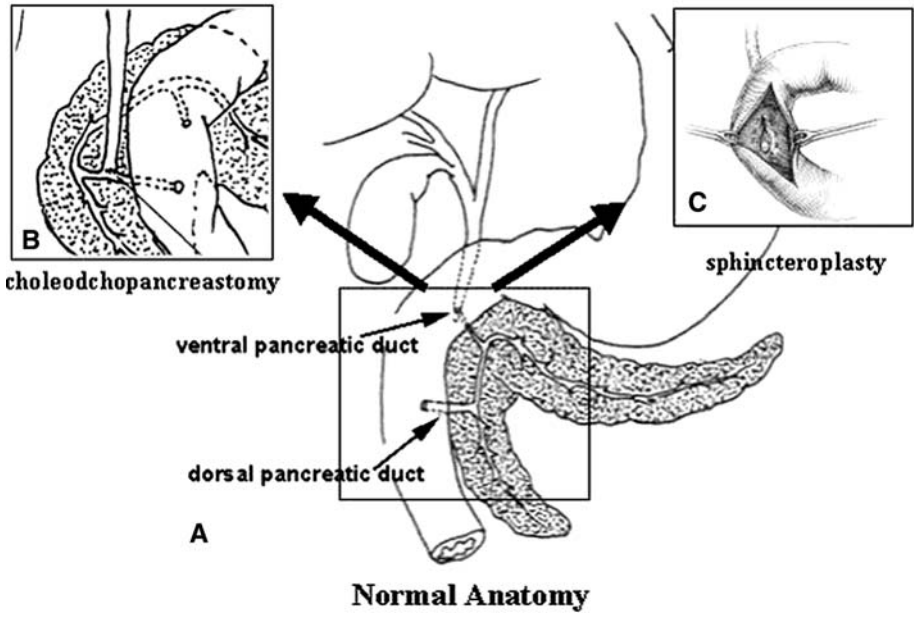

Fig. 1 A schematic drawing of the normal anatomy of the pancreaticobiliary system (**a**), experimental surgery for anomalous pancreaticobiliary duct union (**b**), and transduodenal sphincteroplasty (**c**)

may result from magnification of the films and the pressure variation of the injected dye.

Histologic examination

Liver, gallbladder, bile duct, pancreatic, and duodenal specimens were fixed in formalin solution immediately after animal sacrifice. Specimens were stained with hematoxylin–eosin for microscopic examination and reviewed by a pathologist (Hogun Kim, Yonsei University College of Medicine, Seoul, South Korea). The thickness of each layer in the wall of the common bile duct and gallbladder was measured at three randomly selected sites by using an image-processing program (Image-Pro plus 4.0, Media Cybernetics, USA).

Statistical analysis

Data were analyzed using the Mann–Whitney tests and data were quoted as median and interquartile ranges. *P* values less than 0.05 were considered statistically significant.

Table 1 The activity of pancreatic enzymes in bile juice collected from the common bile duct at the time of animal sacrifice

	Control group (n = 4)	Experimental group (n = 7)	*P* values
Amylase (IU/L)	60,950 (34,900–121,200)	60,600 (145–298,900)	0.705
Lipase (IU/L)	4,000 (300–24,500)	11,800 (49–123,000)	0.450

Results

Final data were available for only 11 animals (four in the control group and seven in the experimental group) because a puppy in the control group died of anastomosis disruption. No statistical differences in sex or body weight were observed at the time of experimental surgery between the two groups (one female vs. three males in the control group and two females vs. four males in the experimental group; 9.25 ± 1.35 kg in the control group and 8.98 ± 0.95 kg in the experimental group).

Biochemical assay

No statistically significant differences in the biochemical serum assays were noted between the two groups. The pancreatic enzyme activity in the bile juice collected from the biliary tree on the day of sacrifice was similar between the two groups (Table 1).

Roentgenologic examination

Although the degree of cylindrical bile duct dilatation was variable, MRC showed that the dilatation of the bile ducts was less definite and less frequently observed in the experimental group than in control group (Fig. 2). This finding was confirmed by operative

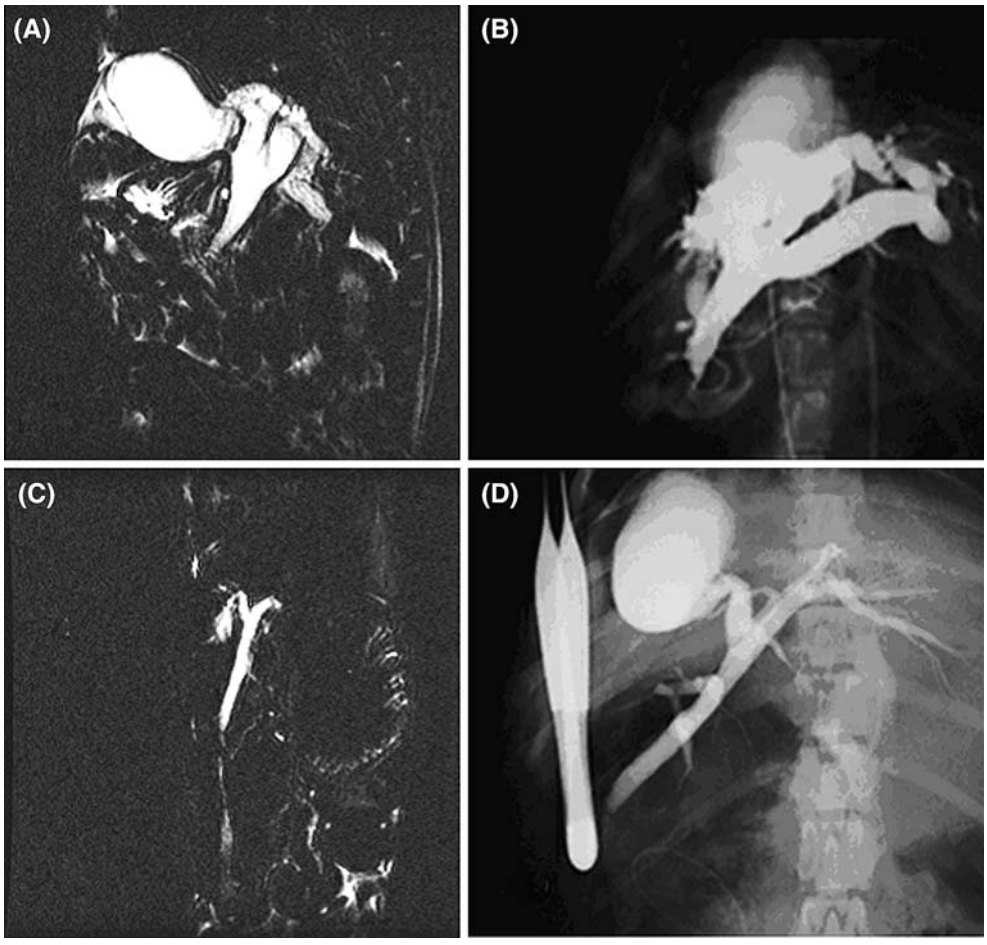

Fig. 2 The magnetic resonance cholangiography (MRC) in the control group shows extensive dilatation of the bile ducts (**a**). However, the MRC in the experimental group does not show such bile duct dilatation (**c**). This finding was confirmed by operative cholangiography (**b** operative cholangiography in the control group; **d** operative cholangiography in the experimental group)

cholangiography at the time of sacrifice. Statistical analysis of the bile duct sizes measured by MRC revealed that the maximum diameters of the common bile duct in the experimental group were significantly smaller than the diameters in the control group (Table 2).

Histologic examination

The mucosal layer of the common bile duct in the experimental group was significantly thinner than the layer in the control group. The muscle layer and the serosal layer of the common bile duct in the experimental group were also significantly thinner than the layers in the control group (Fig. 3; Table 3). Diffuse papillary hyperplasia of the tall columnar epithelium, the most significant mucosal change of bile ducts observed in this APBDU model [9] was less definite and less frequently observed in the experimental group than in the control group (Fig. 3a). Epithelial denudation of the biliary tree was more definite and more frequently observed in the experimental group than in the control group (Fig. 3b). The pathologic findings of acute pancreatitis were not observed in any specimen.

Discussion

The actual incidence of APBDU in humans is unknown, but the estimated incidence of APBDU in the adult population is relatively high, and ranges from 1.5 to 3.2% [4, 5, 12, 13]. However, not all patients with

Table 2 Comparison of maximum bile duct diameters measured by MRC 2.5 months after experimental surgery

Diameter of bile ducts (mm)	Common bile duct	Right intrahepatic bile duct	Left intrahepatic bile duct
Control group (n = 4)	7.9 (5.6–18.7)	8.8 (5.7–14.3)	7.9 (4.9–15.6)
Experimental group (n = 7)	5.3 (4.0–8.0)	6.6 (4.0–11.5)	4.7 (4.1–7.5)
P value	0.038	0.345	0.131

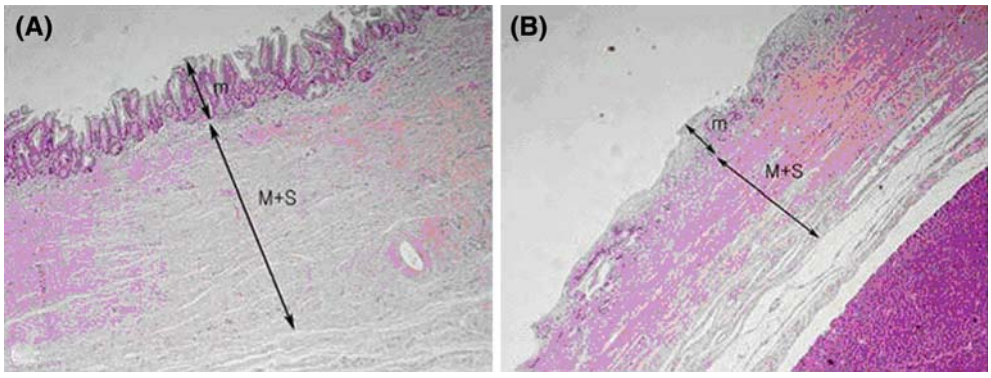

Fig. 3 **a** Microscopic examination of the common bile duct in the control group showing diffuse prominent epithelial hyperplasia without definite epithelial denudation (H & E, ×40). **b** Microscopic examination of the common bile duct in the experimental group showing diffuse epithelial denudation without definite epithelial hyperplasia (H & E, ×40). The wall of the common bile duct in experimental group (**b**) is thinner than that in control group (**a,** *arrows*). Abbreviations: *m* thickness of mucosal layer, *M* + *S* thickness of muscle and serosal layers

Table 3 Comparison of bile duct thickness changes between the control and experimental groups

Thickness of bile ducts (μm)	Common bile duct		Gallbladder	
	M	*M* + *S*	*m*	*M* + *S*
Control group (*n* = 4)	302 (211–425)	656 (368–729)	604 (472–787)	560 (351–800)
Experimental group (*n* = 7)	178 (55–425)	348 (201–499)	448 (205–951)	531 (259–811)
P value	0.003	0.001	0.005	0.477

Abbreviations: *m* thickness of the mucosal layer; *M* + S thickness of muscle and serosal layers

APBDU have an associated choledochal cyst, hepatobiliary malignancy, or pancreatitis, which suggests that other factors are involved in these conditions occur with APBDU. The distal obstruction of the APBDU is expected to be an important factor in the development of the adverse effects associated with APBDU [14]. Although it has been postulated that stones, protein plugs, and stenosis can cause the distal obstruction of APBDU, sphincter of Oddi dysfunction was recently reported to play an important role in the production of symptoms and other complications of APBDU in humans [8, 15–17].

We hypothesized that the adverse effects of APBDU could be prevented by sphincteroplasty to release distal obstruction of bile duct. To verify this hypothesis, we used a well-established animal model of APBDU [9–11]. We had previously confirmed that this model produced APBDU in a similar form as is found in humans and that it produced a dilatation of the bile duct that resembled a human choledochal cyst [11].

Our experiment demonstrates that sphincteroplasty could reduce the degree of both the dilatation and the pathologic thickening of the common bile duct in a 2.5 months period in this animal model. However, the concentrations of pancreatic enzymes in bile in the experimental group after sphincteroplasty were still very high. The sphincteroplasty in this experiment might simply prevent the mechanical drainage effect, but could not prevent pancreatic enzyme reflux since the biliary concentration of pancreatic enzymes was not different between the two groups.

We propose a new concept for the explanation of our results: that the pancreaticobiliary reflux of APBDU can be divided into two processes, ''the chemical pancreaticobiliary reflux'' and ''the physical pancreaticobiliary reflux.'' Chemical pancreaticobiliary reflux is defined as the pancreaticobiliary reflux of chemical substances, such as amylase, lipase, and other pancreatic enzymes. Physical pancreaticobiliary reflux is defined as the transmission of the intraluminal pressure of the pancreatic duct into the biliary tree. It is definitely true that the chemical pancreaticobiliary reflux can occur by simple diffusion without a pressure gradient in the APBDU. Based on this concept, the sphincteroplasty in our animal model may be ineffective in preventing chemical pancreaticobiliary reflux and may be the reason why there was no significant difference found in the levels of pancreatic enzyme activity in the bile juice between the control group and the experimental group (Table 1). It is also a basic physical law that high intraluminal pressure is absolutely required to dilate a tubular structure, even if the wall of tube is

weak. If the sphincteroplasty prevented the transmission of pressure from the pancreatic duct into the bile duct, the bile duct would not dilate despite the presence of a weakness in the bile duct. Based on this concept, the sphincteroplasty in our animal model may be effective in preventing physical pancreaticobiliary reflux and may be the reason why there was a significant difference found in the diameter of the bile duct between the control group and the experimental group (Table 2).

Significant differences in the histologic findings of mucosa existed between the two groups; the denudation of mucosal epithelium was frequently observed in the experimental group while epithelial hyperplasia was the prominent pathologic finding in the control group. Miyano et al. [9] reported that diffuse papillary hyperplasia of tall columnar epithelium was the most significant mucosal change of bile ducts in this APBDU model. The differences in the histologic findings between our two groups are difficult to interpret simplistically. However, we suggest the following hypothesis to explain this difference. The denudation found in the experimental group may be solely due to the irritation effects of the chemical pancreaticobiliary reflux without the pressure effect. This chemical irritation may be more severe in the experimental group than in the control group because the sphincteroplasty in experimental group may promote a reflux of duodenal content into the APBDU, and the refluxed duodenal content may activate the pancreatic enzymes. The mucosal hyperplasia observed more frequently in the control group may be the combined result of both pressure effects and chemical irritation. Mechanical stretching or strain has been suggested to play an important role in the enhancement of cell proliferation in a number of different tissues [18]. The well-known phenomenon of the stretching-enhanced cell proliferation may explain the mucosal hyperplasia in the control group. However, we believe that a long-term experimental study with molecular analysis will be necessary to prove this hypothesis.

Take et al. [19] found elevated basal sphincter of Oddi pressure in nine patients with choledochal cyst and APBDU and other studies have reported that biliary-pancreatic reflux may occur when the common channel is obstructed temporarily by sphincter of Oddi dysfunction [15]. Guelrud et al. [8] reported that recurrent pancreatitis in APBDU is associated with sphincter of Oddi dysfunction in children, and stated that endoscopic sphincterotomy is beneficial in these patients. The results of our animal study definitely support the clinical experience of Guelrud et al. However, long-term animal experiments should be performed to prove the clinical usefulness of sphincteroplasty in APBDU, since the duration of our experiment was relatively short, and because our experiment did not support that sphincteroplasty can prevent all adverse ill effects of pancreaticobiliary reflux.

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References

1. Stringer MD (2002) Choledochal cysts. In: Howard ER, Stringer MD (eds) Surgery of the liver, bile ducts and pancreas in children, 2nd edn. Arnold Publishers, London, pp 149–164
2. Babbitt DP (1969) Congenital choledochal cyst: new etiological concept based on anomalous relationship of the common bile duct and pancreatic bulb. Ann Radiol 12:231–235
3. Han SJ, Hwang EH, Chung KS, Kim MJ, Kim H (1997) Acquired choledochal cyst from anomalous pancreatobiliary duct union. J Pediatr Surg 32:1735–1738
4. Kimura K, Ohto M, Saisho H, Unozawa T, Tsuchiya Y, Morita M, Ebara M, Matsutani S, Okuda K (1985) Association of gallbladder carcinoma and anomalous pancreaticobiliary union. Gastroenterology 89:1258–1265
5. Yamauchi S, Koga A, Matsumoto S, Tanaka M, Nakayama F (1987) Anomalous junction of pancreaticobiliary duct without congenital choledochal cyst: a possible risk factor for gallbladder cancer. Am J Gastroenterol 82:20–24
6. Ando H, Ito T, Nagaya M, Watanabe Y, Seo T, Kaneko K (1995) Pancreaticobiliary maljunction without choledochal cyst in infants and children: clinical features and surgical therapy. J Pediatr Surg 30:1658–1662
7. Miyano T, Ando K, Yamataka A, Lane G, Segawa O, Kohno S, Fujwara T (1996) Pancreaticobiliary maljunction associated with nondilatation or minimal dilatation of the common bile duct in children: diagnosis and treatment. Eur J Pediatr Surg 6:334–337
8. Guelrud M, Morera C, Rodriguez M, Jaen D, Pierre R (1999) Sphincter of Oddi dysfunction in children with recurrent pancreatitis and anomalous pancreaticobiliary union: an etiologic concept. Gastrointest Endosc 50:194–199
9. Miyano T, Suruga K, Suda K (1981) Choleodcho-pancreatic end to side anastomosis in a dog as an experimental model of choledocho-pancreatic long common channel disorders. Jpn J Pediatr Surg 13:525–531
10. Ohkawa H, Sawaguchi S, Yamazaki Y, Ishikawa A, Kikuchi M (1982) Experimental analysis of the ill effect of anomalous pancreaticobiliary ductal union. J Pediatr Surg 17:7–13
11. Han SJ, Chang H, Kim J, Han J, Kim H, Hwang EH (1998) An experimental animal model of anomalous pancreaticobiliary duct union. J Korean Assoc Pediatr Surg 4:100–109
12. Misra SP, Gulati P, Thorat VK, Vij JC, Anand BS (1989) Pancreaticobiliary ductal union in biliary diseases: an endoscopic retrograde cholangiopancreatography study. Gastroenterology 96:907–912
13. Kato O, Hattori K, Suzuki T, Tachino F, Yuasa T (1983) Clinical significance of anomalous pancreaticobiliary union. Gastrointest Endosc 29:94–98
14. Alonso-Lej F, Rever WB, Pessangne DL (1959) Congenital choledochal cyst, with a report of 2 and analysis of 94 cases. Surg Gynecol Obstet 108:1–30

15. Mori K, Nagakawa T, Ohta T, Nakano T, Kayahara M, Kanno M, Akiyama T, Ueno K, Konishi I (1991) Acute pancreatitis associated with anomalous union of the pancreaticobiliary duct system. J Clin Gastroenterol 13:673–677
16. Krishnamurthy S, Krishnamurthy G (1997) Biliary dyskinesia: role of the sphincter of Oddi, gallbladder and cholecystokinin. J Nucl Med 38:1824–1830
17. Yokohata K, Tanaka M (2000) Cyclic motility of the sphincter of Oddi. J Hepatobiliary Pancreat Surg 7:178–182
18. Vandenburgh HH (1992) Mechanical forces and their second messengers in stimulating cell growth in vitro. Am J Physiol 262:R350–R355
19. Take S, Okabe I, Morita K (1998) Endoscopic manometric study of sphincter of Oddi for congenital bile duct dilatation. Jpn J Pediatr Surg 20:303–309