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Outcomes of endoscopic papillary large balloon dilatation in comparison to mechanical lithotripsy for large common bile duct stone

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Abstract
Background: The management of common bile duct stone (CBDS) has changed drastically over the past two decades, as open surgery has been supplanted by per-oral endoscopic procedures. Currently, therapeutic endoscopic retrograde cholangiopancreatography (ERCP) is the standard worldwide. After performing an endoscopic biliary sphincterotomy (EBST), endoscopic therapy entails CBDS extraction using conventional procedures. Lithotripsy is an endoscopic procedure used to remove CBDS that are too large to be extracted using conventional techniques.
Aim: The aim of the present work is to compare the results of endoscopic large balloon dilatation and mechanical lithotripsy for large CBDS.
Subjects and Methods: This prospective cohort study was conducted on 60 cases undergoing endoscopic retrograde cholangial pancreatography for large CBDS who were referred to out-case's clinic and in-case's wards of Gastroenterology, Hepatology and Endoscopy unit of Internal Medicine Department at Tanta University Hospitals December 2019 to February 2021. Cases were divided into two equal groups. Group I included cases undergoing endoscopic papillary large balloon dilatation (EPLBD) and Group II included cases undergoing mechanical lithotripsy (ML).
Results: Success of CBDS extraction, Hb, PLT, TLC, albumin, PA, ALT, AST, creatinine and urea and CBDS parameters (diameter, CBDS size, CBDS number) after ERCP were insignificantly different between both groups. Total serum bilirubin, direct serum bilirubin and, Alkaline phosphatase after ERCP were significantly lower in EPLBD. However, Amylase and lipase were significantly higher in EPLBD.
Conclusion: Despite that the endoscopic large balloon dilatation caused significantly higher pancreatitis, it showed lower TSB, DSB and ALP. However, both techniques showed no difference regarding the success of CBDS extraction and laboratory investigations.

Keywords: Endoscopic papillary large balloon dilatation, mechanical lithotripsy, common bile duct stone

Introduction

In the past twenty years, per-oral endoscopic procedures have supplanted open surgery as the preferred method for treating common bile duct stone CBDS. Currently, therapeutic endoscopic retrograde cholangiopancreatography (ERCP) is performed around the world as the initial technique for the treatment of extrahepatic CBDS and is superior to open or percutaneous techniques, despite the fact that it can be difficult in certain situations [1].

After performing an endoscopic biliary sphincterotomy (EBST), endoscopic therapy entails CBDS extraction using conventional procedures. Balloon catheters, Dormie baskets, and mechanical lithotripters (ML) are the common devices used for CBDS extraction. As an alternative, intracorporeal or extracorporeal shock wave lithotripsy (ESWL) can offer adjuvant treatment for specific cases. In the thirty years since 1990, EBST has become the standard technique for biliary canal CBDS removal, and the vast majority of CBDS can be extracted successfully with traditional approaches. In less than ten percent of cases, the removal of large, barrel-shaped, multiple CBDS, a narrowed (CBD), or cases of abnormal anatomy can be problematic and ineffective [2].

In 1982, endoscopic papillary balloon dilation (EPBD) was introduced. EPBD is an alternative to EBST for cases with CBDS in the due to its reduced risk of hemorrhage and perforation, as well as its successful implementation in cases with surgically altered anatomy. EPBD protects the function of the papillary sphincter, but it may be associated with a higher incidence of post-endoscopic complications [3].

In 2003, Ersoz *et al*. [4] invented EPBD with prior EBST for the treatment of failed EBST and conventional basket/balloon extraction of large CBDS. Although EBST with an extensive incision may be effective for CBDS removal and may have a similar success rates as EPBD with prior EBST for large and/or multiple CBDS, it elevates the risk of complications such as bleeding and perforation. EPBD has been shown to require fewer endoscopy sessions and less frequent mechanical lithotripsy (ML) administration in significant and/or multiple CBDS compared to EPBD [4-6].

Lithotripsy is an endoscopic procedure used to remove CBDS that are too large to be extracted using conventional techniques. Endoscopic lithotripsy techniques consist of intraductal electrohydraulic lithotripsy, laser lithotripsy using the "mother-baby" endoscopic system, and ESWL. ML is the most frequently used procedure for CBDS fragmentation among the available endoscopic techniques. ML necessitates the use of a large, durable receptacle to capture the CBDS. Using a mechanical handle, tension is then applied to the wires, compressing the CBDS against the metal coat. Reimann *et al*. first described it in 1985. Since then, numerous distinct lithotripter designs have emerged. [7-9].

To our knowledge, both techniques (large balloon dilatation and endoscopic mechanical lithotripsy) improved the overall success rate of large CBDS clearance and improved morbidity and mortality. However, data comparing both techniques are still insufficient. This research investigation sets out to contrast the results of endoscopic large balloon dilatation and mechanical lithotripsy for large CBDS.

Subjects and Methods

This prospective cohort study was conducted on 60 cases undergoing ERCP for large CBDS (>10 mm) who were referred to out-case's clinic and in-case's wards of Gastroenterology, Hepatology and Endoscopy unit of Internal Medicine Department at Tanta University Hospitals December 2019 to February 2021 after obtaining cases' informed consent and within the approved protocol of Tanta University ethical committee.

Cases were divided into two equal groups. Group I included cases undergoing endoscopic large balloon dilatation and Group II included cases undergoing mechanical lithotripsy.

All cases included were adults, had a definitive diagnosis of large CBDS confirmed by clinical examination, imaging and endoscopic workup. Cases with cancer pancreas, tumors and cases with enlarged porta hepatis lymph nodes were excluded.

All cases were subjected to history taking, general examination, abdominal examination, and laboratory investigations (Complete blood count (CBC), liver function, renal function, prothrombin time, total and direct bilirubin, serum amylase and serum lipase, abdominal ultrasonography, magnetic resonance cholangial pancreatography).

Endoscopic large papillary balloon dilatation (ELPBD)

ELPBD was carried out utilizing a 5-Fr hydrostatic balloon catheter over a 0.035-inch guidewire, with a maximal diameter of 10 to 20 mm and length of 5.5 cm, respectively. When the balloon reaches inside the sphincter, it was gradually distended to 10 mm by an distension device under fluoroscopy observation. ELPBD was aborted if the "waist sign" remained in the distended balloon when the distension pressure reaches 75% of the target pressure. After attaining the desired diameter, the balloon was distended for 1 minute at a constant pressure before being deflated and removed. To prevent perforation, the balloon diameter was chosen based on the size of the CBDS, but did not exceed the distal CBD diameter.

Mechanical lithotripsy (ML)

Endoscopic lithotripsy is a technique used to remove large CBDS from that cannot be removed via sphincterotomy. This procedure fragments large CBDS, making their retrieval with other instruments such as balloons or carriers simpler. One form of ML employs an instrument called a mechanical lithotriptor, while the others use an electrohydraulic lithotriptor.

This is the most widely used technique for CBDS extraction techniques, and all ERCP units should offer it. This technique necessitates the initial basketing of the CBDS. This robust basket is then retracted through a metal coat that transmits shear forces to pulverize the CBDS mechanically. Lithotripters can be utilized through the scope channel or after the endoscope has been removed. The through-the-scope design consists of three layers: the container, an interior plastic coat, and an outer metal coat. The CBDS is captured while the metal coat is in the endoscope channel, and the metal coat is then advanced against the CBDS and pulverized. Depending on the magnitude of the biliary duct CBDS, the efficacy of ML to fragment them varies.

The primary outcome measure was the incidence of hemorrhage, cholangitis, and pancreatitis overall. Secondary outcome measure was the effective execution of complete CBDS extraction during the same ERCP extraction session performed by an endoscopist. During the final occlusion cholangiogram, the absence of any filling defect served as evidence of the procedure's success. Bleeding was defined as clinical evidence of bleeding, such as melena or hematemesis, accompanied by a 2 g/dL decrease in hemoglobin concentration.

Statistical Analysis

The statistical analysis was done using SPSS v26. (IBM Inc., Chicago, IL, USA). Quantitative factors were given as means and standard deviations and compared the two groups using an unpaired Student's t- test. (SD). Qualitative factors were reported as frequencies and percentages when appropriate, and evaluated with Chi-square or Fisher's exact tests. Statistical significance was defined as a two-tailed P value of 0.05 or less.

Results

Demographic data (age, sex, weight, height and BMI) were insignificantly different between both groups [Table 1]

Table 1: Demographic data of large balloon dilatation and mechanical lithotripsy groups

		Large Balloon Dilatation (n=30)	Mechanical Lithotripsy (n=30)	P value
Age (years)	Mean ± SD	55.3 ± 9.35	57.1 ± 11.49	0.508
	Range	31 – 67	32 - 72	
Sex	Male	11 (37%)	8 (27%)	0.579
	Female	19 (63%)	22 (73%)	
Weight (kg)	Mean ± SD	83.07 ± 15.47	83.43 ± 12.82	0.921
	Range	50 – 121	60 - 116	
Height (m)	Mean	1.67 ± 0.065	1.69 ± 0.056	0.293
	Range	1.52 - 1.79	1.56 - 1.79	
BMI (kg/m^2)	Mean	29.78 ± 5.36	29.36 ± 4.45	0.745
	Range	21.6 - 43.2	21.8 - 40.9	

BMI: Body Mass Index

Laboratory characteristics before endoscopic retrograde cholangiopancreatography were insignificantly different between both groups [Table 2].

Table 2: Laboratory characteristics before endoscopic retrograde cholangiopancreatography of large balloon dilatation and mechanical lithotripsy groups

		Large Balloon Dilatation	Mechanical Lithotripsy	P value
Hb (gm/dL)	Mean ± SD	11.41 ± 1.55	11.96 ± 1.46	0.160
	Range	9 – 13.9	9 – 13.9	
PLT (*10^3 cells/dl)	Mean ± SD	274.77 ± 68.83	295.4 ± 74.39	0.269
	Range	149 – 371	144 – 399	
TLC (*10^3 cells/dl)	Mean ± SD	8.17 ± 2.46	7.5 ± 2.20	0.266
	Range	3.9 – 11.9	3.9 – 12	
TSB (mg/dl)	Mean ± SD	21.87± 8.64	18.15 ± 9.37	0.116
	Range	6.4 – 34.6	5.3 – 34.9	
DSB (mg/dl)	Mean ± SD	18.14 ± 8.43	14.98 ± 9.33	0.174
	Range	4.1 – 29.6	2.7 – 32.3	
Albumin (gm/dL)	Mean ± SD	4.15 ± 0.47	4.23 ± 0.60	0.567
	Range	3.2 – 5	3.2 – 5.1	
PA (%)	Mean ± SD	75.33± 12.49	79.4 ± 13.04	0.222
	Range	60 – 100	61 – 100	
ALT (U/L)	Mean ± SD	31.5 ± 9.18	33.57 ± 12.56	0.470
	Range	21 – 59	20 – 66	
AST (U/L)	Mean ± SD	30.9 ± 9.49	34.3 ± 9.48	0.170
	Range	18 – 67	20 – 66	
Creatinine (mg/dL)	Mean ± SD	1.08 ± 0.34	1.029 ± 0.35	0.593
	Range	0.51 – 1.54	0.5 – 1.47	
Urea (mg/dL)	Mean ± SD	32.03 ± 11.32	30.8 ± 9.18	0.643
	Range	15 – 51	17 – 46	
Amylase (IU)	Mean ± SD	64.23 ± 18.33	60.37 ± 14.50	0.369
	Range	30 – 90	32 – 90	
Lipase (IU)	Mean ± SD	48.57 ± 15.64	51.8 ± 17.61	0.455
	Range	21 – 79	25 – 80	
ALP (IU)	Mean ± SD	124.57 ± 34.09	115.60 ± 36.94	0.333
	Range	67 – 179	54 - 177	

HB: haemoglobin, PLT: platelets, TLC: total leucocytic count, TSB: total serum bilirubin, DSB, direct serum bilirubin, PA: prothrombin activity, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase.

TSB, DSB and ALP after endoscopic retrograde cholangiopancreatography were significantly lower in large balloon dilatation compared to mechanical lithotripsy groups (P value= 0.025, 0.031 and <0.001 respectively), amylase and lipase were significantly higher in large balloon dilatation compared to mechanical lithotripsy groups (P value <0.001, = 0.042 respectively). Hb, PLT, TLC, albumin, PA, ALT, AST, creatinine and urea after endoscopic retrograde cholangiopancreatography were insignificantly different between both groups [Table 3].

Table 3: Laboratory characteristics after endoscopic retrograde cholangiopancreatography of large balloon dilatation and mechanical lithotripsy groups

		Large Balloon Dilatation (n=30)	Mechanical Lithotripsy (n=30)	P value
Hb (gm/dL)	Mean ± SD	10.97 ± 1.53	11.46 ± 1.51	0.216
	Range	8.4 – 13.6	8.5 – 13.4	
PLT (*10^3 cells/dl)	Mean ± SD	276.27 ± 71.89	296.73 ± 76.37	0.290
	Range	131 – 385	133 – 403	
TLC (*10^3 cells/dl)	Mean ± SD	8.23 ± 2.56	7.51 ± 2.17	0.246
	Range	3.3 – 12.5	3.9 – 12.4	
TSB (mg/dl)	Mean ± SD	5.89 ± 5.21	10.13 ± 8.38	0.025*
	Range	1.4 – 22.6	1.5 – 29.5	
DSB (mg/dl)	Mean ± SD	3.57 ± 2.58	5.55 ± 4.17	0.031*
	Range	1.2 – 11.5	1.2 – 15.5	
Albumin (gm/dL)	Mean ± SD	3.72 ± 0.50	3.85 ± 0.56	0.334
	Range	2.8 – 4.5	2.8 – 4.7	
PA (%)	Mean ± SD	75.10 ± 13.03	79.37 ± 12.03	0.193
	Range	55 – 97	56 - 98	
ALT (U/L)	Mean ± SD	21.30 ± 9.12	23.27 ± 12.67	0.493
	Range	9 – 49	9 - 56	
AST (U/L)	Mean ± SD	21.10 ± 9.03	24.33 ± 9.44	0.181
	Range	10 – 56	8 - 55	
Creatinine (mg/dL)	Mean ± SD	1.06 ± 0.34	1.01 ± 0.36	0.576
	Range	0.51 – 1.58	0.46 – 1.56	
Urea (mg/dL)	Mean ± SD	32.27 ± 12.36	30.77 ± 10.88	0.620
	Range	12 – 55	12 - 51	
Amylase (IU)	Mean ± SD	48.47 ± 18.64	31.23 ± 14.49	<0.001*
	Range	14 – 75	12 - 58	
Lipase (IU)	Mean ± SD	33.90 ± 16.72	24.83 ± 17.06	0.042*
	Range	6 – 67	1: 52	
ALP (IU)	Mean ± SD	134 ± 34.24	194 ± 39.07	<0.001*
	Range	74 – 184	116 - 250	

HB: hemoglobin, PLT: platelets, TLC: total leucocytic count, TSB: total serum bilirubin, DSB, direct serum bilirubin, PA: prothrombin activity, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase.

CBDS parameters (size, number) after endoscopic retrograde cholangiopancreatography were insignificantly different between both groups. Success of CBDS extraction after endoscopic retrograde cholangiopancreatography was insignificantly different between both groups [Table 4].

Table 4: CBDS parameters of both groups after endoscopic retrograde cholangiopancreatography

		Large Balloon Dilatation (n=30)	Mechanical Lithotripsy (n=30)	P value
CBD Diameter (mm)	Mean ± SD	19.77 ± 2.97	18.73 ± 1.82	0.109
	Range	15 – 25	16 - 21	
CBDS Size (mm)	Mean ± SD	17.8 ± 3.27	18.57 ± 19.5	0.394
	Range	12 – 24	13 - 24	
CBDS Number	Mean ± SD	2.77 ± 1.22	2.5 ± 1.14	0.385
	Range	1 – 5	1 - 5	
Success (%)	Yes	29 (96.67%)	28 (93.33%)	1.00
	No	1 (3.33%)	2 (6.67%)	

CBD

Complications after endoscopic retrograde cholangiopancreatography were insignificantly different between both groups [Table 5].

Table 5: Adverse events of large balloon dilatation and mechanical lithotripsy groups

	Large Balloon Dilatation (n=30)	Mechanical Lithotripsy (n=30)	P value
Total	1 (3.33%)	8 (26.66%)	0.495
Bleeding	0 (0%)	4 (13.33%)	
Pancreatitis	1 (3.33%)	3 (10%)	
Cholangitis	0 (0%)	1 (3.33%)	

Discussion

In recent years, the management of biliary duct CBDS has changed drastically. This has made this condition's treatment more secure and effective [10]. Endoscopic retrograde cholangiopancreatography (ERCP) has become widespread and routine, whereas open cholecystectomy has been largely supplanted by laparoscopic surgery, which may or may not involve laparoscopic exploration of the common bile duct (LCBDE) [11].

Combined with balloon catheters and/or baskets, endoscopic CBDS extraction is now the standard procedure for the vast majority of cases. Large CBDS are typically treated with mechanical lithotripsy (ML), while the procedure's most severe complication, "basket and CBDS impaction," is typically resolved surgically. In cases of problematic, impacted, multiple, or intrahepatic CBDS, more complex techniques have been employed [12].

Endoscopic papillary large balloon dilation (EPBD) has been introduced as an adjunct to endoscopic biliary sphencterectomy (EBST) for the removal of CBDS that are large or difficult to remove. Compared to a massive EBST, EPBD may present a lower risk of adverse events and pancreatitis. Stefanidis *et al.* [10] report that EPBD without EBST is safe and efficacious for the treatment of large CBDS without increasing the risk of pancreatitis in cases with normal anatomy.

In our study, success of CBDS extraction after endoscopic ERCP and CBDS parameters was insignificantly different between both groups.

Similarly, Radwan *et al*. (2019) [13] carried out a randomized prospective study that included 98 Cases with calcular obstructive jaundice were randomized into two groups: group A consisted of 49 cases treated with EPBD and group B consisted of 49 cases treated with ML. All cases initially underwent EBST and were subjected to a comprehensive history and physical examination. The concentrations of pancreatic enzymes were measured four hours before and twenty-four hours postoperative, and CBC and hepatic functions were assessed before and in the following day. Prior to and throughout ERCP, the size and number of the CBDS were determined. Their results demonstrated insignificant difference between both groups in success of CBDS extraction after ERCP at different parameters "Diameter, CBDS size, and CBDS number".

In consistent with our results, Misra *et al*. (2008) [14] conducted a reterospective study on 243 cases that divided into: group A comprising 174 cases treated by EPLBD and group B comprising 96 cases treated by ML. There was no significant difference in success of CBDS extraction after ERCP and all parameters "Diameter, CBDS size, and CBDS number".

Parallel to our findings, Hwang *et al*. (2013) [15] conducted a prospective analysis on 131 cases with large CBDS 12 mm or larger who underwent ML with EPLBD alone (n = 62) or EBST with EPLBD (n = 69). The results revealed that there were no distinctions in terms of age, CBDS size, number, or duct diameter. CBDS withdrawal (96.8% vs. 95.7%, P = 0.738) and total CBDS withdrawal without the need for ML (80.6% vs. 73.6%, P = 0.360), the EPLBD alone and the EBST with EPLBD had comparable outcomes.

Itoi *et al*. (2009) [16] aimed to compare the procedural time and fluoroscopy time of ESLBD with EBST to those of EBST alone for instances of large CBDS, as well as assess the safety, effectiveness, and practicality of EPLBD. In total, 101 cases with CBDS were treated, 53 with ESLBD and 48 with EBST alone. The percentage of effective CBDS removal in the first session was reported to be 85 percent for the EPBD group, which was higher but not statistically significant.

Also, Heo *et al*. (2007) [12] conducted a prospective randomized controlled trial to compare the efficacy of EBST with EPLBD versus EBST alone in 200 consecutive cases with bile-duct CBDS. Cases were randomly assigned to receive either EBST with EPLBD (12 to 20 mm balloon diameter) or EBST alone. In terms of overall successful CBDS removal (97.0% vs. 98.0%), large size (>15 mm) CBDS removal (94.4% vs. 96.7%), and the use of mechanical lithotripsy (8.0% vs. 9.0%), the results were comparable between EBST + EPLBD and EBST alone.

In addition, Teoh *et al*. (2013) [17] conducted a randomized trial on 56 cases with suspected or confirmed CBDS and assigned them to groups that underwent EPLBD or ESBT. In the ESBD group, cases underwent limited EBST (up to half of the sphincter) followed by balloon dilation to the extent of 15 mm, whereas in the EBST, cases underwent complete EBST alone. The CBDS were then eliminated using standard procedures. The percentage of CBDS removed did not differ significantly between the two groups (EBST vs. EPLBD: 88.5% vs. 89.0%). More cases in the EBST (46.2%) required mechanical lithotripsy than in the EPLBD (28.8%) (P =.028), particularly for CBDS 15 mm (90.9% vs. 58.2%; P =.002).

Moreover, Yang *et al*. (2013) [18] carried out a met analysis EPLBD with EBST vs EBST alone for removal of bile duct CBDS. The results showed that about six randomized controlled trials involving 835 cases were analyzed and demonstrated there were no significant differences regarding complete CBDS removal, CBDS removal in the first session, post-ERCP, hemorrhage, infection, and operation time.

In the present study, complications after ERCP were insignificantly different between both groups

In agreement with our results, Afifi *et al*. (2017) [19] who evaluated the efficacy of therapeutic ERCP operations for endoscopic removal of CBDS in 74 cases in a randomized prospective study. In Group I, 31 cases had CBDS removal using the EPLBD method in conjunction with balloon catheters and/or baskets. In group II, 43 cases had the more common endoscopic method of CBDS extraction, EBST, which also includes the use of balloon tubes and/or baskets. No discernible difference in the incidence of minor side effects. However, they found that 9.6% of people experienced bleeding, 3.2% experienced more severe complications like melena, and 6.4% experienced moderate pancreatitis. Mild, self-limiting post-ERCP discomfort occurred in 7% of cases and mild intraprocedural bleeding occurred in 4.6% of cases in Group 2, more severe complications such as mild pancreatitis emerged in 4.6% of cases and post-ERCP cholangitis occurred in 2.3% of cases.

In agreement with our study, Radwan *et al*. (2018) [13] study findings highlighted the incidence of side effects was not significantly different.

Also, Hwang *et al*. (2013) [15] highlighted that Comphlications in the EPLBD alone and EBST with EPLBD were as follows: pancreatitis (6.5% vs. 4.3%, *P* = 0.593), obstruction of basket and CBDS (0% vs. 1.4%, *P* = 0.341), and perforation (0% vs. 1.4%, *P* = 0.341).

Further, Heo *et al*. (2007) [12] highlighted that Overall, there were equal incidence rate of complications (5.0% vs 7.0%, P =.767). Both the EBST and the EBST + EPLBD had 4% for pancreatitis, 1% for cholecystitis, and 2% for delayed hemorrhage.

Moreover, Yang *et al*. (2013) [18] reported that Meta-analysis demonstrated that EBST with EPLBD resulted in fewer overall complications than EBST alone; EBST + EPLBD had a significantly reduced chance of perforation compared to the other subcategories (Peto OR = 0.14, 95%CI: 0.20 to 0.98, P = 0.05). Use of ML in the EBST with EPLBD declined markedly (OR = 0.26, 95%CI: 0.08-0.82, P = 0.02), particularly in cases with a CBDS size bigger than 15 millimeters (OR = 0.15, 95%CI: 0.03-0.68, P = 0.01) where complications where ranged as following: pancreatitis (7% vs. 4.3%, P = 0.593), obstruction of basket and CBDS (0% vs. 2.4%, P = 0.341), and perforation (0% vs. 2.5%, P = 0.341).

Nonetheless, Youn *et al*. (2011) [20] conducted a prospective, multicenter investigation on 98 cirrhotic cases with calcular obstructive jaundice who were arbitrarily divided into two groups: group A, consisting of 49 cases treated with EPLBD, and group B, consisting of 49 cases treated with ML. All cases initially underwent EBST. All cases were subjected to a comprehensive history and physical examination. After EBST, cases treated with balloon dilation experienced fewer adverse events than those treated with ML. This can be justified be larger sample.

In our study, total serum bilirubin, direct serum bilirubin and, Alkaline phosphatase after ERCP were significantly lower in large balloon dilatation compared to ML.

Along with our results, Radwan *et al*. (2018) [13] study findings highlighted higher biliary parameter including alkaline phosphatase, serum bilirubin, and serum lipase were higher in ML compared to EPLBD.

Conclusion

Despite that the endoscopic large balloon dilatation caused significantly higher pancreatitis, it showed lower TSB, DSB and ALP. However, both techniques showed no difference regarding the success of CBDS extraction and laboratory investigations.

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Author's Contribution

Not available

Conflict of Interest

Not available

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