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ORIGINAL ARTICLE

Retrospective Study

Reconstructing the portal vein through a posterior pancreatic tunnel: New choice for portal vein thrombosis during liver transplantation

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Abstract

BACKGROUND

Thrombectomy and anatomical anastomosis (TAA) has long been considered the optimal approach to portal vein thrombosis (PVT) in liver transplantation (LT). However, TAA and the current approach for non-physiological portal reconstructions are associated with a higher rate of complications and mortality in some cases.

AIM

To describe a new choice for reconstructing the portal vein through a posterior pancreatic tunnel (RPVPPT) to address cases of unresectable PVT.

METHODS

Between August 2019 and August 2021, 245 adult LTs were performed. Forty-five (18.4%) patients were confirmed to have PVT before surgery, among which seven underwent PV reconstruction *via* the RPVPPT approach. We retrospectively analyzed the surgical procedure and postoperative complications of these seven recipients that underwent PV reconstruction due to PVT.

RESULTS

During the procedure, PVT was found in all the seven cases with significant adhesion to the vascular wall and could not be dissected. The portal vein proximal to the superior mesenteric vein was damaged in one case when attempting thrombolectomy, resulting in massive bleeding. LT was successfully performed in

all patients with a mean duration of 585 min (range 491-756 min) and mean intraoperative blood loss of 800 mL (range 500-3000 mL). Postoperative complications consisted of chylous leakage (n = 3), insufficient portal venous flow to the graft (n = 1), intra-abdominal hemorrhage (n = 1), pulmonary infection (n = 1), and perioperative death (n = 1). The remaining six patients survived at 12-17 mo follow-up.

CONCLUSION

The RPVPPT technique might be a safe and effective surgical procedure during LT for complex PVT. However, follow-up studies with large samples are still warranted due to the relatively small number of cases.

Key Words: Liver transplantation; Portal vein thrombosis; Portal vein reconstruction; Retropancreatic tunnel; Computer tomography angiography; Three-dimensional visualization

Core Tip: In the study, we presented a new choice for reconstructing the portal vein through a posterior pancreatic tunnel (RPVPPT) to address the issue of unresectable portal vein thrombosis in adult liver transplantation (LT). Clinical data of seven recipients who had portal vein thrombosis (PVT) and underwent RPVPPT were analyzed. PVT was found in all the seven cases with significant adhesion to the vascular wall and could not be dissected. LT was successfully performed in all patients without serious complications. Six patients survived at 12-17 mo follow-up. The RPVPPT technique may be a safe and effective surgical procedure in LT for complex PVT.

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INTRODUCTION

Liver transplantation (LT) remains the mainstay treatment for end-stage liver disease. However, the incidence of portal vein thrombosis (PVT) in patients on the waiting list for transplantation has been reported to range from 5% to 26%. Due to the complexity of treatment techniques, PVT has long been regarded as a contraindication of LT until the 1980s[1-3]. However, the past decade has witnessed unprecedented progress achieved in surgical techniques, leading to the advent of many surgical approaches for recipients with PVT, including physiological portal reconstruction (such as thrombectomy, interposition venous grafts, and mesoportal jump grafts) and non-physiological portal reconstruction (such as cavoportal hemitransposition, renoportal anastomosis, and arterialization of PV flow)[4,5]. Importantly, physiological reconstruction can restore the anatomical structure of the portal venous system and ensure adequate blood flow to the graft. In contrast, non-physiological reconstruction exhibits limited ability to resolve portal hypertension due to the inability to drain visceral blood into the liver, resulting in a higher incidence of postoperative complications and mortality than physiological reconstruction[6-10]. Most recipients with PVT can undergo thrombectomy and anatomical anastomosis (TAA), yielding satisfactory results. However, in clinical practice, some patients with PVT present with organized thrombi adhering to vascular walls that cannot be completely removed intraoperatively, compromising blood flow to the graft. Non-physiological reconstruction methods are indicated in such cases, including portal-renal vein anastomosis and bypass, portal vena cava semi-transposition, and portal vein arterialization. An increasing body of evidence suggests that this approach is ineffective and might lead to an insufficient blood supply to the portal vein or postoperative hepatic encephalopathy[10-12].

Kasahara *et al*[13] reported a "pullout technique" for portal vein reconstruction in ten pediatric cases of LT. The portal vein was first pulled out from the back of the pancreas and resected. Then the portal vein reconstruction was completed by bridging the back of the pancreas with allograft or autologous blood vessels. However, this technique has not been widely used, and no relevant reports of its application during adult LT have been documented. Therefore, based on the "pullout technique", our center explored the technique of reconstructing the portal vein through a posterior pancreatic tunnel (RPVPPT) in adult LT recipients where PVT could not be resolved.

MATERIALS AND METHODS

General clinical data

A retrospective analysis was performed on 245 cases of LT at Shenzhen Third People's Hospital from August 2019 to August 2021. PVT was documented in 45 cases, of which 7 underwent RPVPPT for PVT and portal vein reconstruction (6 males, 1 female; age 48-65 years, mean 54 years). All patients in this study underwent LT with the approval of the Ethics Committee of Shenzhen Third People's Hospital, and livers were donated after the death of healthy citizens.

Preoperative assessment method

Before surgery, each patient underwent Doppler ultrasound and abdominal computed tomography angiography (CTA) to determine the incidence of complications such as PVT. Three-dimensional (3D) visualization models were reconstructed according to the DICOM format data of CTA, as previously described in the literature[14], and surgery was simulated on the model.

Main surgical methods of RPVPPT technique

Dissection of the hepatic hilum: First, the varicose veins of the hepatic hilum were separated and ligated successively, the common hepatic artery and the proper hepatic artery were dissected, and the left hepatic artery and the right hepatic artery were separated. The main portal vein was dissected from the caudal to the cephalad direction along the trunk to the left and right branches of the portal vein. Finally, the bile duct was isolated and severed near the hilum.

Establishment of the retropancreatic tunnel: First, the main portal vein was dissected from the cephalad to the caudal direction, and the left gastric vein (coronary vein) and the portal vein branch vessels were ligated successively. When the upper edge of the pancreas was reached, dissection started from the lower edge of the pancreas. The superior mesenteric vein (SMV) and splenic vein (SpV) were first separated and lifted with vascular slings. Then, dissection continued from the back of the pancreas to the cephalic side along the main portal vein to establish a retropancreatic tunnel. Subsequently, the pancreas was lifted with a vascular sling or a fine urinary catheter. Finally, the portal vein and its tributary branches behind the pancreas were completely severed and "naked".

Resection of the main portal vein of the recipient: The severed main portal vein was pulled out from the retropancreatic tunnel to the lower edge of the pancreas. The main portal vein containing the thrombus was removed after interrupting blood flow in the SMV and SpV. If the left gastric vein drained into the SpV or the superior mesenteric-portal vein (SMPV) confluence, it was ligated and severed first to avoid insufficient portal venous flow to the graft due to blood shunting.

Portal vein reconstruction: After the donor-recipient inferior vena cava anastomosis was completed, the donor's portal vein was pulled to the lower edge of the pancreas through the retropancreatic tunnel, and the portal vein reconstruction was conducted at the SMPV confluence (Figure 1).

Main evaluation indicators

The clinical data of each LT recipient with PVT were collected, including the medical history and laboratory, imaging, and 3D reconstruction results. The surgical methods and operation-related indicators were analyzed, including the operation time, bleeding volume, amount of blood transfusion, and surgical complications.

RESULTS

General clinical data

Patients with PVT included in the present study were cases with a preoperative diagnosis of decompensated hepatitis B virus (HBV)-related cirrhosis (n = 3) and hepatocellular carcinoma with decompensated HBV-related cirrhosis (n = 4). Five cases had a history of gastrointestinal bleeding before the operation. All patients underwent preoperative 3D reconstructions to visually assess blood vessels and simulate surgery, and LT was successfully conducted. The mean operation time was 585 min (range 491-756 min), and the mean intraoperative blood loss was 800 mL (range 500-3000 mL). More details are provided in Table 1.

Changes in the structure of the portal vein system

Anatomical structure of the PVT: One patient presented with complete portal vein occlusion with thrombosis proximal to the SMPV confluence, four cases with portal vein stenosis greater than 70% and thrombosis extending to the SMPV confluence, and two cases with portal vein stenosis greater than 70% and thrombosis extending to the proximal segment of the SMV. All seven patients with PVT presented with organized thrombi that could be completely removed intraoperatively during surgery. Moreover,

Table 1 Basic demographics and clinical data of cases with portal vein thrombosis (*n* = 7)

	Gender	Age	Diagnosis	Operation time (s)	Anhepatic stage (s)	Intraoperative blood loss (mL)	Transfusion of red blood cell suspension (U)	Cold ischemia time (s)	Outcome
Case 1	Male	65	HBV-related decompensated liver cirrhosis, HCC	648	34	1300	10	510	Survival
Case 2	Male	48	HBV-related decompensated liver cirrhosis	756	31	1000	6	480	Survival
Case 3	Male	38	HBV-related decompensated liver cirrhosis	564	35	800	0	390	Death
Case 4	Female	64	HBV-related decompensated liver cirrhosis, HCC	585	25	600	0	360	Survival
Case 5	Male	57	HBV-related decompensated liver cirrhosis, HCC	583	47	600	0	360	Survival
Case 6	Male	54	HBV-related decompensated liver cirrhosis	491	30	500	0	360	Survival
Case 7	Male	51	HBV-related decompensated liver cirrhosis, HCC	625	34	3000	20	360	Survival

HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma.

the proximal portal vein was damaged near the SMPV confluence in one case when attempting thrombolectomy, resulting in massive bleeding.

Anatomical structure of varicose vessels: The left gastric vein drained into the main portal vein (*n* = 3), SpV (*n* = 3), and SMPV confluence (*n* = 1), and the maximum diameter of the left gastric vein was greater than 1 cm in four cases. All cases presented with esophageal and gastric fundal varices and splenorenal shunt; the maximum diameter of the splenorenal shunt was 24 mm, and an umbilical vein opening was found in two cases. More details are provided in Table 2.

Surgical results and complications

Portal vein reconstruction and LT were successfully conducted in all cases, with patent and sufficient portal vein flow documented by intraoperative color Doppler ultrasonography. Six patients recovered smoothly after the surgery, and one patient died. The liver and coagulation function indicators are shown in Tables 3 and 4. Postoperative complications consisted of chylous leakage (*n* = 3), insufficient portal venous flow to the graft (*n* = 1), intra-abdominal hemorrhage (*n* = 1), pulmonary infection (*n* = 1), and perioperative death (*n* = 1).

Management of postoperative complications included conservative medical treatment for chylous leaks and antibiotics for pulmonary infection. In cases of insufficient portal venous flow, embolization of splenorenal shunt vessels under digital subtraction angiography (DSA) was used to improve portal venous blood flow (Figure 2). An exploratory laparotomy was performed on a patient with post-operative intra-abdominal bleeding (postoperative day 7) that was attributed to multiple blood vessels at the lower margin of the pancreas. Liver ischemia and hypoxia occurred due to hemorrhagic shock after surgery. The patient died 15 d after LT due to liver failure. At 12-17 mo follow-up, six of the seven cases in this study survived.

DISCUSSION

Management of PVT

PVT refers to thrombosis occurring in the main portal vein and its associated venous system (SMV, inferior mesenteric vein, and SpV). It is one of the most common complications of end-stage liver disease, with an incidence of about 5%-26%[1,15,16]. In the present study, the incidence of PVT was 18.4% (45/245). PVT has long been considered a contraindication for LT due to limited surgical techniques and poor understanding of PVT[17]. With significant inroads achieved in recent years,

Table 2 Vascular anatomical changes in the portal vein system of cases with portal vein thrombosis (*n* = 7)

Case	Left gastric vein (coronary vein)				Esophagogastric fundus vein		Superior mesenteric vein		Splenic vein		Shunt situation		
	Drain into the main portal vein	Drain into the confluence of SMV and SpV	Drain into SpV	Maximum diameter of the blood vessel (mm)	Degree of varicose veins	History of upper gastrointestinal bleeding	With or without thrombus	Maximum diameter (mm)	With or without thrombus	Maximum diameter (mm)	With or without splenorenal shunt	Maximum diameter of the shunt (mm)	With or without umbilical vein opening
1	Yes			30	Severe	Yes	No	18.8	No	21.3	Yes	21	No
2			Yes	10.4	Severe	Yes	Yes	17	No	14.2	Yes	24	Yes
3			Yes	24.2	Severe	Yes	No	15.4	No	12.4	Yes	15.7	No
4			Yes	13.8	Severe	Yes	Yes	10.8	No	10.5	Yes	17.3	No
5	Yes			5.9	Severe	No	No	16.4	No	12.5	Yes	11.2	Yes
6	Yes			6.9	Mild	No	No	11	No	18.4	Yes	15.6	No
7		Yes		8.2	Severe	Yes	No	13.1	No	17.1	Yes	7.6	No

Maximum vessel diameter is measured based on contrast-enhanced computed tomography. SMV: Superior mesenteric vein; SpV: Splenic vein.

various innovative surgical approaches are now available.

Hibi *et al*[10] performed LT in 174 cases of PVT, among which 83 (47.7%) and 91 (52.3%) presented with complete and partial PVT, respectively. In terms of portal vein reconstruction, 149 cases underwent physiological reconstruction [thrombolectomy (n = 123), interposition vein grafts (n = 16), and mesoportal jump grafts (n = 10)]. There were 25 cases of non-physiological reconstruction [cavoportal hemitranspositions (n = 18), renoportal anastomoses (n = 6), and arterialization (n = 1)]. The study found that the non-physiological group suffered a significantly increased incidence of rethrombosis of the portomesenteric veins and gastrointestinal bleeding, with a dismal 10-year overall survival rate of 42% (*vs* no PVT, 61%; P = 0.002 and *vs* PVT: Physiological group, 55%; P = 0.043). Rodríguez-Castro *et al*[12] reported that of 25753 liver transplants, 2004 were performed in patients with PVT (7.78%), and complete thrombosis was observed in nearly 50%. TAA was performed in 75% of patients; other techniques included venous graft interposition and portocaval hemitransposition. It was found that PVT significantly increased post-LT mortality at 30 d (10.5%) and 1 year (18.8%) when compared to patients without PVT (7.7% and 15.4%, respectively). Moreover, rethrombosis occurred in up to 13% of patients with complete PVT, whereby no preventive strategies were used, leading to increased morbidity and mortality. In the present study, there was no recurrence of PVT, but one patient had portal venous insufficiency after LT. Accordingly, the optimal approach for portal vein reconstruction is the restoration of the physiological anatomy of the portal vein system while ensuring adequate portal venous flow[10,18].

Table 3 Laboratory examination indicators on postoperative day 7

	ALB (g/L)	TB (μmol/L)	DB (μmol/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	PT (s)	INR
Case 1	32	98	52	111	43	78	20.4	1.72
Case 2	31.4	11.2	4.9	49	24	85	16.6	1.36
Case 3	38.1	27.8	18.3	204	63	236	18.9	1.61
Case 4	35.1	35.1	22.8	224	175	741	16.4	1.30
Case 5	35.3	39.5	23.1	169	41	89	15.9	1.25
Case 6	50	26.8	14.7	329	62	355	14.8	1.19
Case 7	35	20.1	13.5	48	20	328	15.4	1.21

ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; PT: Prothrombin time; INR: International normalized ratio.

Table 4 Laboratory examination indicators on postoperative day 14

	ALB (g/L)	TB (μmol/L)	DB (μmol/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	PT (s)	INR
Case 1	33	66	37	58	21	128	17.6	1.42
Case 2	38.3	12.1	5.2	35	15	75	14	1.09
Case 3	42.1	567	226	246	115	232	52.2	6.0
Case 4	34.3	80	54	135	87	677	15.1	1.18
Case 5	39.1	24.2	13.2	27	21	39	14	1.20
Case 6	39.8	13.8	11.2	57	53	140	13.6	1.12
Case 7	34.5	13.8	8.6	37	15	238	14.7	1.14

ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; PT: Prothrombin time; INR: International normalized ratio.

At present, no consensus has been reached on the optimal reconstruction approach for different types of PVT during LT. Some scholars have formulated surgical methods according to Yerdel classification criteria[19-21]. However, in some cases, this classification criteria cannot be used to guide clinical practice since the Yerdel standard is based on the extent that the thrombus occupies the portal vein lumen and does not take into account adhesion to the blood vessel wall.

Application and precautions of RPVPPT

The RPVPPT technique adopted by our team was mainly applied in patients with PVT contraindicated for routine thrombolectomy during the LT surgery. This approach restores the physiological anatomy of the portal vein system while ensuring adequate portal vein blood flow, which is hypothetically ideal for PVT patients. At 12-17 mo follow-up, six of the seven patients survived, preliminarily validating the feasibility and safety of RPVPPT.

However, severe portal hypertension in this patient population accounts for an increase in varicose vessels around the portal vein, or even cavernous transformation of the portal vein, leading to an increased risk of bleeding during the procedure[22,23]. In addition, the RPVPPT technique requires the establishment of a retropancreatic tunnel behind the pancreas in these patients, increasing surgical risks. Accordingly, this surgical approach requires highly skilled surgeons and a transplant team. During the operation, it is recommended to dissect the hepatic hilum along the portal vein to the upper margin of the pancreas and then successively ligate each branch of the portal vein at the lower margin of the pancreas. When separating the lower edge of the pancreas, the SMV and SpV branches should be dissected first, and vascular slings should be placed to lift them for prompt hemostasis during the establishment of the retropancreatic tunnel or the separation of the surrounding tissues of the portal vein. After a successful retropancreatic tunnel is established, lifting the pancreas with a vascular sling or urinary tube is recommended to facilitate portal vein reconstruction (Figure 1).

Intraoperative traction of the pancreas should be as gentle as possible to avoid pancreatic damage and pancreatitis. Based on our experience, we recommend successfully ligating the branches of the blood vessels that merge into the portal vein behind the pancreas. Given that the blood vessels in this

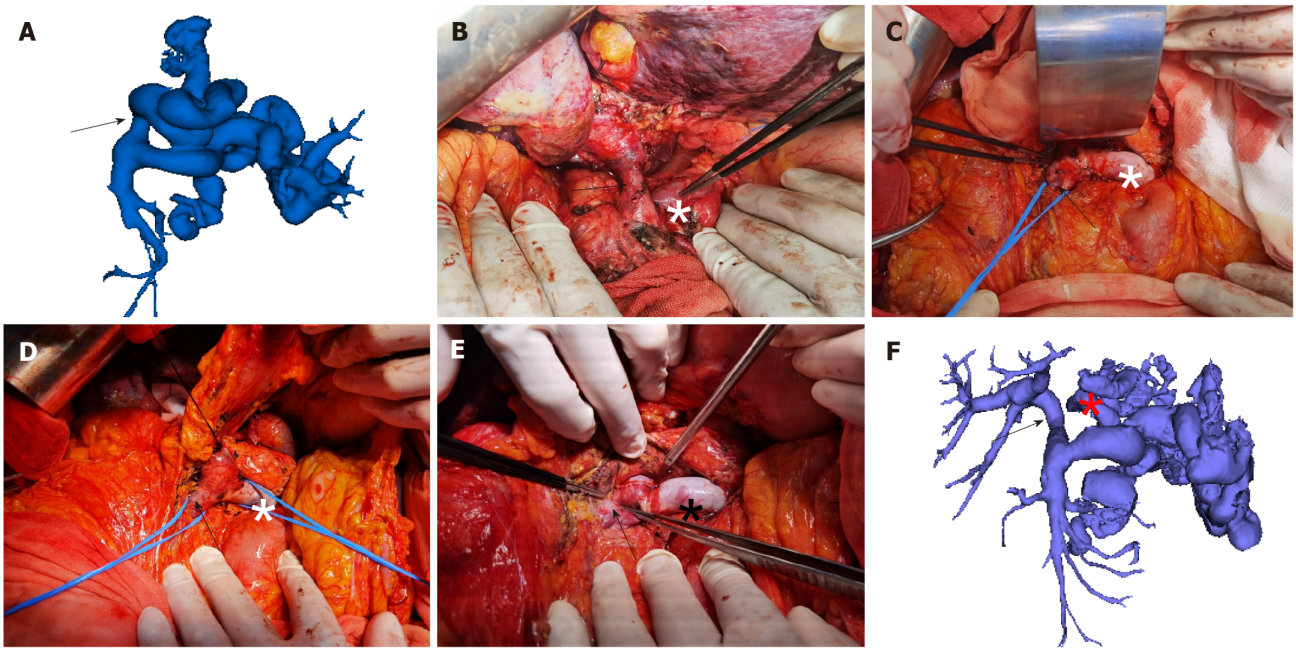

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Figure 1 Main steps of reconstructing the portal vein through the posterior pancreatic tunnel technique during portal vein reconstruction in liver transplantation recipients with complex portal vein thrombosis. A: Three-dimensional (3D) visualization model of the portal vein system constructed before surgery showed that the main portal vein was occluded, and the left gastric veins (coronary veins) were visible (arrow); B: After the varicose vessels were severed, the main portal vein (arrow) was exposed, the portal vein was dissected from the cephalic side to the upper edge of the pancreas, and the coronary varicose was ligated (*); C: Dissection started from the lower edge of the pancreas. The superior mesenteric vein (SMV) (arrow) and splenic vein (SpV) (*) were dissected successively, and the rear of the pancreas was separated towards the cephalic side along the main portal vein to establish a retropancreatic tunnel; D: The main portal vein was pulled out from the retropancreatic tunnel, and the main portal vein, SMV (arrow), and SpV (*) presented a triangular structure; E: Blood flow in the SMV and SpV was blocked. After the portal vein containing the thrombus was resected, the portal vein of the donor was pulled to the lower edge of the pancreas through the retropancreatic tunnel, and portal vein reconstruction was completed at the confluence of the SMV (arrow) and SpV (*); F: 3D visualization model of the portal vein system after surgery showed that the main portal vein was unobstructed (arrow), and the original coronary vein was severed (*).

region are very thin, hemostasis can be challenging once bleeding occurs. In this regard, given the narrow surgical view, it can be challenging to perform suture hemostasis, and the effect of electrocoagulation is often not satisfactory. In such circumstances, we can only resort to compression hemostasis. In addition, due to the brittleness of pancreatic tissue in patients with portal hypertension and the increase of surface varicose vessels, the risk of hemorrhagic shock is relatively high. Therefore, it is advisable to dissect the lower edge of the pancreas during surgery to prevent postoperative abdominal bleeding. In our study, one patient developed intra-abdominal hemorrhage on postoperative day 7. Exploratory laparotomy revealed that the source of the hemorrhage was at the lower edge of the pancreas, with multiple hemorrhagic foci observed. This finding could be attributed to postoperative pancreatitis since the amylase level in drain fluid from the lower edge of the pancreas was 700 U/L. It is highly likely that the extravasation of pancreatic fluid corroded the blood vessel, thus leading to rupture and bleeding. The patient died of liver failure due to hemorrhagic shock resulting in liver ischemia and hypoxia. Based on our experience, we recommend that the drainage tube should be indwelled at the lower margin of the pancreas and properly fixed. Importantly, the drain fluid amylase level should be assessed regularly after surgery.

During the establishment of the retropancreatic tunnel, the varicose vessels around the portal vein were ligated to create the posterior pancreatic tunnel and reduce the blood shunt of the portal vein system to avoid insufficient portal venous flow to the graft after surgery. However, it is often difficult to ligate splenorenal shunt vascular branches intraoperatively due to their deep location. In some cases, postoperative intervention may be required to manage shunt vessels. In this study, one patient developed insufficient portal venous flow to the graft after surgery, mainly due to significant splenic-renal shunting. DSA showed that most splenic venous flow drained into the inferior vena cava through the shunt rather than the portal vein. After shunt embolization, an immediate improvement in portal vein blood supply was observed.

CONCLUSION

With the increased number of LT cases, PVT has become a major conundrum that may be solved by

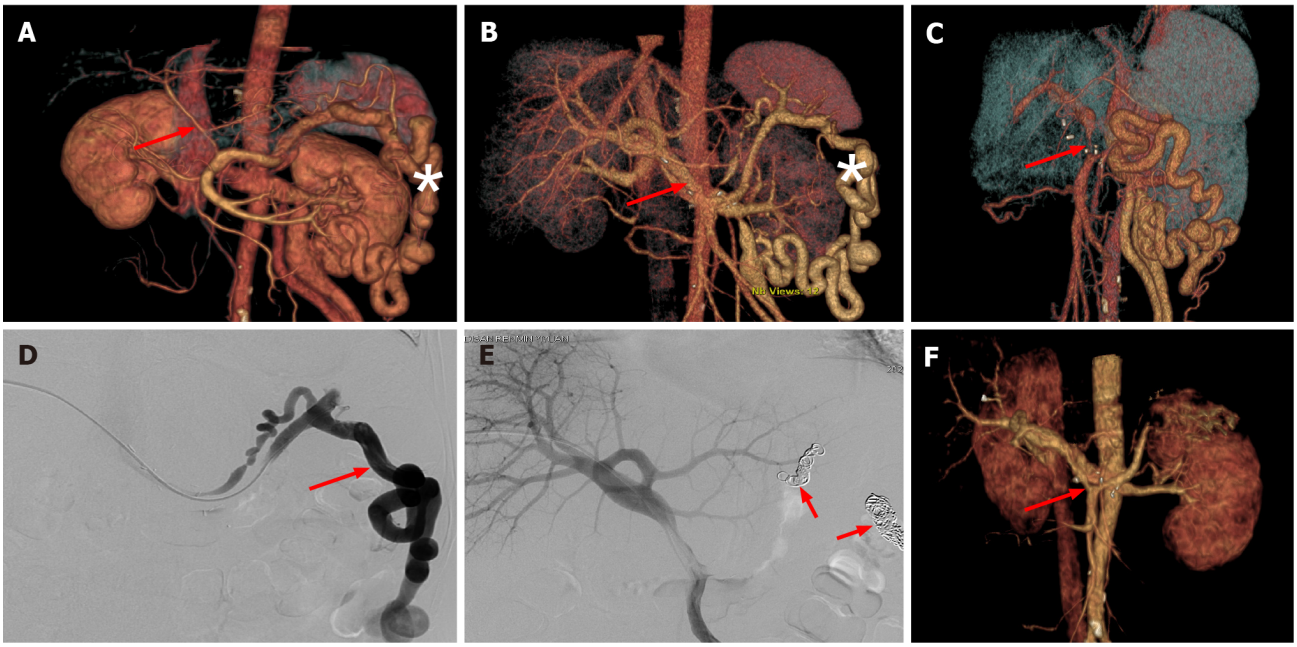

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Figure 2 Embolization of large splenorenal shunt under digital subtraction angiography alleviates portal vein insufficiency after liver transplantation. A: Preoperative three-dimensional (3D) visualization model showed a slender portal vein (arrow) and obvious splenorenal shunt varices (*); B: Postoperative 3D visualization model on day 3 showed a normal portal vein shape and unobstructed blood flow (arrow), and splenorenal shunt varicosity was reduced (*); C: Postoperative 3D visualization model (at 3 wk) showed portal vein stenosis in the initial segment (arrow), and color Doppler ultrasound examination indicated insufficient portal venous blood supply; D: Percutaneous and transhepatic splenic venography showed that most splenic venous flow drained into the inferior vena cava through the splenorenal shunt, but did not drain into the portal vein (arrow); E: After embolization of the splenorenal shunt (arrow), angiography showed that blood flow was mainly present into the portal vein; F: 3D visualization model 1 wk after the vascular intervention showed unobstructed portal vein flow (arrow), and the splenorenal shunt was no longer visible.

portal vein reconstruction. The key point of this technique is to ensure sufficient portal venous blood flow and restore the physiological anatomy of the portal vein system as much as possible. The RPVPPT approach adopted in this study meets the above requirements, and our preliminary assessment yielded good results. We substantiated that the RPVPPT technique is a safe and effective surgical procedure in LT for complex PVT. However, follow-up studies with large samples are warranted due to the relatively small number of cases.

ARTICLE HIGHLIGHTS

Research background

Portal vein thrombosis (PVT) poses a great challenge in liver transplantation (LT). It has been established that thrombectomy and anatomical anastomosis (TAA) can restore the physiological anatomy of the portal vein by complete thrombus excision and has been considered the optimal solution to this problem; however, in some cases, PVT cannot be treated by TAA.

Research motivation

We describe our experience of reconstructing the portal vein through a posterior pancreatic tunnel (RPVPPT) to address the issue of unresectable PVT, which may achieve a similar effect to TAA and provide a new approach to solve this intricate clinical problem.

Research objectives

We sought to describe a new strategy of RPVPPT to address cases of unresectable PVT.

Research methods

A retrospective analysis was performed on 245 adult patients that underwent LT from August 2019 to August 2021. Forty-five (18.4%) patients presented with PVT before surgery, among which seven underwent portal vein reconstruction using RPVPPT. Preoperative clinical data, operation-related indicators, and postoperative complications were statistically analyzed.

Research results

During the operation, PVT was found in all seven cases with significant adhesion to the vascular wall and could not be dissected. LT was successfully performed in all patients without serious postoperative complications. At 12-17 mo follow-up, there were six patients who survived.

Research conclusions

The RPVPPT technique can restore the physiological anatomy of the portal vein system through a retropancreatic tunnel, which might be a safe and effective surgical procedure in LT for complex PVT.

Research perspectives

Due to the relatively small number of cases in the study, follow-up studies with large samples are still required.

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FOOTNOTES

Author contributions: Zhao D and Huang YM were involved in the conception and design of this study; Zhao D provided administrative support in this study; Tang JX, Zhang KJ, Fang TS, and Zeng XC contributed to the provision of study materials or patients; Liang ZM, Yan X, Jin X, and Xie LJ were involved in the collection and assembly of data; Zhang Y and Huang YM analysed and interpreted the data; and all authors approved this manuscript to publish.

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