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Gradient inflammation in the pancreatic stump after pancreaticoduodenectomy: Two case reports and review of literature

Wang TG *et al*. Inflammation in the pancreatic stump

Abstract

BACKGROUND

Postoperative pancreatic fistula (POPF) contributes significantly to morbidity and mortality rates after pancreaticoduodenectomy (PD). However, the underlying mechanisms remain unclear. This study explored this pathology in the pancreatic stumps and elucidated the mechanisms of POPF following PD.

CASE SUMMARY

Pathological analysis and 16S rRNA gene sequencing were performed on specimens obtained from two patients who underwent complete pancreatectomy for grade C POPF after PD. Gradient inflammation is present in the pancreatic stump. The apoptosis rate was lower than that in the normal pancreas. Moreover, neutrophil-dominated inflammatory cells are concentrated in the ductal system. Notably, neutrophils migrated through the ductal wall in acinar duct metaplasia-formed ducts. Additionally, evidence indicates that gut microbes migrate from the digestive tract. Gradient inflammation occurs in pancreatic stumps after PD.

CONCLUSION

The mechanisms underlying POPF include high biochemical activity in the pancreas, mechanical injury, and digestive reflux. To prevent POPF and address pancreatic inflammation and reflux, breaking the link with anastomotic dehiscence is practical.

Key Words: Pancreaticoduodenectomy; Postoperative pancreatic fistula; Inflammation; Digestive reflux; Case report

Core Tip: Postoperative pancreatic fistula (POPF) is a major complication of pancreaticoduodenectomy. However, the underlying mechanisms remain unclear. Compared to the relatively simple histological structure of the gastrointestinal wall, pancreatic stump is undoubtedly the crucial factor in the occurrence of POPF. This study systemically investigated the pathology in pancreatic stumps and provided insights into the underlying mechanisms of POPF. Gradient inflammation and digestive reflux are present in the pancreatic stumps. As the understanding of the role of inflammation in POPF increases, effectively managing the side effects of inflammation will bring about a significant possibility of terminating POPF.

INTRODUCTION

Postoperative pancreatic fistula (POPF) is a mechanical issue caused by the leakage of fluid from the pancreatic remnant into the abdominal cavity when pancreatic anastomosis fails[1]. It is a significant contributor to postoperative morbidity and mortality after pancreaticoduodenectomy (PD) and results in high social and financial costs. Currently, no effective strategies have been developed to prevent POPF[2,3].

Long-term clinical observations have suggested that postoperative pancreatitis (POP) correlates with the occurrence of POPF after PD[4,5]. Analysis of drained fluids and animal studies provide indirect evidence linking trypsinogen activation and inflammation around the site of pancreatoenteric anastomosis to the onset of POPF on the first postoperative day[6]. However, the exact mechanisms behind POPF and the pathology in the pancreatic stump remain unclear[7,8]. At present, the limited sample size of pancreatic stumps at each center has stunted the comprehensive analysis of POPF. This study aimed to investigate the pathology and microbiology of pancreatic stumps and elucidate the underlying mechanisms of POPF.

CASE PRESENTATION

Chief complaints

Case 1: A 60-year-old female who reported yellow urine for 20 d.

Case 2: A 66-year-old male who found jaundice for three months.

History of present illness

Case 1: 20 d ago, the patient noticed yellow urine and sought treatment at a local hospital for gastric disease, but there was no improvement. The patient began experiencing poor appetite, accompanied by an aversion to greasy food, upper abdominal bloating, occasional clay-colored stools, and itching skin. There were no symptoms of fever, acid reflux, nausea, vomiting, or chest tightness.

Case 2: The patient first presented with generalized jaundice without any obvious cause three months ago, accompanied by yellowing of the eyes and urine. There was no nausea, vomiting, abdominal pain, or bloating. The patient did not experience fever, chills, diarrhea, urgency after defecation, or loss of appetite.

History of past illness

Case 1: The patient underwent an appendectomy 43 years ago; has a three-year history of diabetes and hyperlipidemia.

Case 2: Two months ago, the patient experienced a lacunar stroke.

Personal and family history

Neither patient had a personal or family history of similar diseases.

Physical examination

Those two patients had similar performance. The patient is lucid and in good spirits. The skin and sclera of the entire body are jaundiced. The abdomen is flat, with no visible peristalsis or wave-like movements. There is no tenderness, rebound tenderness, or muscular tension throughout the abdomen. The liver and spleen are not palpable,

and Murphy's sign is negative. Abdominal percussion produces a tympanic sound, and shifting dullness is negative. There is no percussion pain in the liver area or bilateral renal area. Bowel sounds are present and normal.

Laboratory examinations

The laboratory test results for these two patients both indicate obstructive jaundice, accompanied by elevated levels of carbohydrate antigen 199.

Pathology and apoptosis: Two experienced pathologists independently evaluated the pathology without prior knowledge of clinical information. The specimens were cut into 4-µm-thick sections and stained with hematoxylin and eosin for pathological examination. The presence of pancreatic ducts and centroacinar cells was detected using a mouse anti-cytokeratin 19 (CK19) antibody (ZM-0074; OriGene Technologies), followed by a secondary antibody using the ultraView Universal Diaminobenzidine (DAB) Detection Kit (Roche).

The inflammatory index was calculated using a histological scoring system for acute pancreatitis, including edema, inflammatory cell infiltration, and necrosis[10]. Furthermore, the occurrence of apoptosis was assessed using a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay with a Cell Death Detection Kit from Roche. The cell nuclei were stained with TUNEL to visualize apoptosis. Pancreatic head tissue was used as a control to define the normal level of inflammation and apoptosis.

Microbiome and 16S rRNA bacterial gene sequencing: DNA was extracted from each sample, preserved in formalin, and embedded in paraffin (Norgen BioTek, Thorold, ON, Canada). Thirty nanograms of DNA were obtained from each sample and subjected to polymerase chain reaction (PCR) amplification. The hypervariable V3-V4 regions were targeted using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') for amplification, which consisted of the

following steps: 30 s at 94 °C, 30 s at 50 °C, and 60 s at 72 °C, which were repeated for 30 cycles. The PCR products were evaluated by agarose gel electrophoresis at 170 voltages for 30 min on a 1% (w/v) gel with 600 ng of amplified DNA in each well.

Purified PCR products were sequenced using a MiSeq platform (Illumina, United States), and image analysis, base calling, and error estimation were performed using the Illumina Analysis Pipeline (Version 2.6). Sequences shorter than 230 bp with low-quality scores, containing ambiguous bases, or those that did not match the primer sequences and barcode tags were screened. The qualified reads were grouped into operational taxonomic units (OTUs) with a similarity level of 97%. Alpha diversity indices were calculated and presented as Chao's, Shannon-Wiener's, and Simpson's indices and the number of OTUs[11]. The distances between samples were determined using principal component analysis (PCA)[12]. PCA is a multivariate analysis that reduces data dimensionality while preserving covariance. The most attractive property of PCA is that the distances between clusters reflect their genetic and geographical distances[13]. The evolutionary distances between the microbial communities in each sample were calculated using Bray-Curtis algorithms and observed using an unweighted pair group method with an arithmetic mean (UPGMA) clustering tree to show dissimilarity (1-similarity) at the phylum, class, order, family, and genus levels[14].

Statistical analysis: Statistical analysis was conducted to determine the significance of the results. One sample t-test was employed in the microbial analysis. A P-value of less than 0.05 was considered statistically significant, and all calculations were carried out using IBM SPSS statistics version 26.0.

Pathology in the pancreatic stumps: The pancreatic stumps exhibit widespread inflammation and other pathological alterations. Subcapsular hematoma is common and extends deep into the parenchyma surrounding the suture, whereas it is relatively sparse in the tail. Neutrophils were the dominant inflammatory cells that infiltrated the glandular and interlobular structures (Figure 1A, B, and D). Necrotic foci were scattered

throughout the stump, with the center exhibiting homogeneous staining and surrounded by plasma cells, neutrophils, lymphocytes, and eosinophils (Figure 1C).

Apoptotic activity in the pancreatic head decreased or disappeared after POPF, resulting in cell proliferation throughout the stump (Figure 1H and I). The glandular lobes were swollen with disrupted and dispersed acini, acinar cell nuclei increased in size, and chromatin density was reduced (Figure 1A-C). Gradient inflammation is observed in the stump.

Inflammatory and red blood cells (RBC) were also concentrated in the ductal system, including the main pancreatic duct, interlobular duct, and abnormal ducts formed by acinar duct metaplasia (ADM) (Figure 1D and E). Notably, the concentration of neutrophils in the ductal system was significantly higher than that in the local vasculature. The ducts formed by the ADM are the weakest parts of the entire ductal network, where the blood-duct barrier is destroyed. The increased permeability of the ductal system allows the migration of blood cells, particularly neutrophils, from the blood vessels into the space between the acinar cells and basal membrane and then into the lumen of the ductal system (Figure 1G). ADM could be detected throughout the entire pancreatic stump, exhibiting no discernible pattern in its distribution. Moreover, the ductal system was unfavorable for the survival of blood cells as they decomposed (Figure 1E and F). RBCs lose their membranes, and neutrophils are decomposed, leaving fragmented nuclei.

The expression of CK19 becomes strongly positive in the pancreatic stump compared with that in the normal parenchyma (Figure 1J and K). During ADM, acinar cells lose their normal shape and function and transform into ductal-like cells. Moreover, the ducts formed by ADM differed significantly in appearance from normal ducts and were characterized by duck-like cells and irregular lumens (Figure 1L). The original acinar cells were either pushed aside by the newly formed duct-like cells or incorporated into the newly formed ducts. Furthermore, it seems that the pressure in the lumen increased, resulting in the dilation of the regional ducts formed by the ADM (Figure 1M).

Microbial distribution and digestive reflux in pancreatic stumps: We conducted microbial analyses, including bacterial and fungal analyses, on both patients. Unfortunately, the vast majority of the fungal sequences could not be identified, so we focused solely on the bacteria. The length of the sequence was 400-440 bp. For further analysis, we selected a subset of 26301 tags from each sample in the patient 1 and 31277 tags in the patient 2. The alpha diversity indices are presented in Table 1. Our analysis revealed a statistically significant increase in OTUs in the stump compared to those in the duodenum (P = 0.000 for patient 1, P = 0.042 for patient 2), but no significant difference was observed in the pancreatic head (P = 0.729 in patient 1, P = 0.161 in patient 2).

At the genus level, the most common bacteria found in the patient 1 samples are *Bacillus*, *Bacteroides*, *Escherichia-Shigella*, and *Faecalibacterium*, along with some unidentified species. The most abundant genera are Bacillus, Comamonas, Stenotrophomonas, Bacteroides, and Pelomonas.

Furthermore, the dominant bacterial species exhibited a declining distribution with increasing distance from the transection plane. The dissimilarity and variability of the samples are shown in Figure 2A and B. The distances between the samples demonstrated a much more clustered distribution in the patient 1 than in the patient 2 through PCA. However, the proportion of bacterial DNA and microbiome in the samples varied in the jejunum, pancreatic head, and pancreatic stump. The UPGMA clustering analysis indicated that the microbial community distribution adjacent to the transection plane in these two patients was similar to that in the jejunum (Figure 2C and D). The preoperative distribution of bacterial DNA in the pancreatic head was comparable to that in the pancreatic tail, where the inflammatory response was relatively slow.

Imaging examinations

Case 1: Computed tomography (CT) examination showed that there was a mass in the middle and lower sections of the common bile duct, with dilation of the intrahepatic and extrahepatic bile ducts.

Case 2: Magnetic resonance imaging suggested a duodenal mass, and a biopsy confirmed it as duodenal adenocarcinoma.

FINAL DIAGNOSIS

Case 1: Biliary carcinoma.

Case 2: Duodenal carcinoma.

TREATMENT

Those two patients accepted conventional PDs. The pancreas was transected at the neck in front of the portal vein, and a modified Blumgart anastomosis was performed in all cases. A U-suture was placed 1 cm from the transection plane. Then, 3-0 polypropylene and 5-0 polydioxanone (both from Ethicon) were used in the outer and inner layers. Catheters with a range of 5-8 Fr were placed in the stump and fixed using anchoring sutures.

POPF happened in both cases within 6 d postoperatively. And those two patients accepted total pancreatectomy after life-threatening and repeat abdominal bleeding. We collected two pancreatic stump samples measuring 12 cm × 6 cm × 3 cm and 12 cm × 4 cm × 2 cm. Eight and six slices of the specimens were sequentially obtained with an interval of 1.5-2.0 cm from the transection plane to the pancreatic tail. In addition, patient-matched specimens from the jejunum and pancreatic head, labeled PLJ0, PLP0, PZJ0, and PZP0, were obtained from prior surgeries and used as controls.

The diagnosis and classification of pancreatic fistula in this study followed the guidelines of the International Study Group on Pancreatic Fistula[9]. Grade A POPF is

characterized as a leak with no significant clinical effects, whereas grades B and C POPFs are considered clinically relevant because of their impact on patient health.

OUTCOME AND FOLLOW-UP

Case 1

This patient passed away in the seventh month after surgery due to severe malnutrition and multiple organ disfunction syndrome.

Case 2

This patient passed away on the day 11 after the first surgery due to multiple infections and multiple organ disfunction syndrome.

DISCUSSION

POPF is a major and unresolved complication of PD. Recent studies have indicated that POPF is associated with POP; however, the exact mechanism remains unknown. One reasonable hypothesis suggests that the local activation of pancreatic enzymes triggers POPF, leading to increased damage to acinar cells, ischemia, manipulation of the gland, and blockage of the pancreatic duct[15]. However, no solid evidence for this has been reported to date.

No consensus exists currently regarding the definition of POP. Although serum amylase, lipase, and urinary trypsinogen levels are often used as diagnostic criteria for pancreatitis[16,17], previous studies utilizing CT imaging have found that only 26% of patients (13/50) showed radiological evidence of the condition[18].

Adults' acinar cells are highly plastic and can undergo trans-differentiation into a progenitor-like cell type with ductal characteristics, which has been linked to multiple mechanisms, including ductal ectasia[19], the activation of nuclear factor-kappaB[20], Notch receptors[21], and epidermal growth factor receptor[22]. ADM is a critical feature of the façade that militates pancreatic regeneration after injury and is an essential protective mechanism during pancreatitis[23].

Inflammation may drive ADM in pancreatitis cases[20]. In our study, ADM-induced ducts exhibited distinct morphological features that differentiated them from normal ducts, including increased lumen size and elongated ductal cells. Moreover, no previous studies have reported increased ductal permeability in patients with pancreatitis. However, a case study has documented the presence of neutrophil-predominant inflammatory cell infiltration, ADM, and fibrosis in a patient with pancreatitis induced by pembrolizumab[24]. Our study showed that the blood-duct barrier was destroyed, and the ducts formed by ADM served as sites for the transmigration of neutrophils and RBCs through the duct wall.

The significant accumulation of neutrophil-dominant inflammatory cells within the ductal system and the gradient distribution of bacteria from the intestine suggest that inflammatory agents originate from the intestine, traverse the site of anastomosis, and accumulate in the ductal system. Further evidence of this reflux is the decomposition of blood cells in the ductal system, as the activated enzymes in the system are not conducive to the survival of blood cells. Intra-acinar trypsin activation within the pancreas is enough to cause acute pancreatitis[25]. The reflux of digestive fluid could exacerbate local pancreatitis caused by mechanical injuries, such as sutures or dissection (Figure 3).

Theoretically, intestinal fluid can flow through the anastomosis site or stent into the stump and has the potential to activate trypsin during Blumgart anastomosis. However, the small size of the jejunal loop incision (2-3 mm) minimizes enteric fluid reflux. In contrast, invagination with pancreaticojejunostomy is associated with increased reflux[26]. This difference in reflux may explain why Blumgart anastomosis minimizes severe complications after PD[27]. Additionally, gastric juice performs less biologically than intestinal fluid, making pancreaticogastrostomy a safer alternative to pancreaticojejunostomy[28].

The main distinction between pancreaticojejunostomy and other digestive anastomoses is the high level of biological activity of the pancreas, which is characterized by its enzymatic secretory capacity[29]. Some researchers have suspected

that suture placement is induced by the placement of sutures[6]. Our study found that suture placement can lead to parenchymal damage and the formation of suture-induced hematomas that extend deep into the parenchyma.

Based on these findings, the underlying mechanisms of POPF include high-level biochemical activity in the pancreas, mechanical injury, and digestive reflux. These three mechanisms contribute to the increased incidence and prolonged healing of POPF. The inflammatory response in the stump reached its peak on day 4 after PD and did not heal even 30 d after surgery.

Additionally, various risk factors, including soft tissue texture, small pancreatic duct[30], ischemia, ductal obstruction, excessive blood loss, high intraoperative fluid intake[31], elevated bilirubin level, large body mass index[32], low fibrosis[33], high acinar cell density, and acinar marginal content[34], increase the complexity of surgical maneuvers, resulting in anastomotic failure due to the heightened local tension created by inflammation and reflux.

Regardless of the surgical techniques employed, including stents[35], various methods of pancreaticojejunostomy and pancreaticogastrostomy[26], fibrin sealants, autologous tissue patches, bioabsorbable mesh[36], externally draining of pancreatic fluid[37], and the application of somatostatin[38], mechanical damage to the pancreas and digestive reflux cannot be entirely avoided. Therefore, surgeons have limited flexibility when treating each patient.

Due to the unavoidable suture and POPF, a paradox arises. Preventing pancreatitis in the stump or achieving a flawless and strong anastomosis for each patient is not possible. Nonetheless, implementing measures to eliminate reflux and prevent pancreatic fluid from entering the abdominal cavity in the event of anastomotic failure has great potential to mitigate the incidence and severity of POPF.

CONCLUSION
POPF is a complex condition that is caused by increased biochemical activity, mechanical damage, and digestive reflux. Currently, manipulation of the pancreatic

stump and reflux into the pancreatic duct cannot be avoided. Based on these findings, stopping reflux and reducing inflammation in the pancreatic stump can decrease the occurrence of pancreatic fistulas. However, a more practical approach is to allow for the presence of inflammation and anastomotic dehiscence while controlling the proper flow of pancreatic juice, thereby breaking the logical relationship between anastomotic dehiscence and POPF.

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