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EDITORIAL

Pancreaticobiliary reflux in patients with a normal pancreaticobiliary junction: Pathologic implications

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Abstract

Knowledge on pancreaticobiliary reflux in normal pancreaticobiliary junction and its pathologic implications has experienced tremendous progress during the last few years. This editorial reviews the current knowledge on this condition and its pathological implications on gallbladder diseases. The following aspects were defined appropriate for discussion: (1) Evidence of carcinogenesis associated with pancreaticobiliary reflux; (2) Evidence of pancreaticobiliary reflux in normal pancreaticobiliary junction; and (3) Evidence of sphincter of Oddi (SO) dysfunction as a cause of pancreaticobiliary reflux in normal pancreaticobiliary junction. The articles reviewed were selected and classified according to five levels of evidence: Level Ⅰ, meta-analysis double-blind randomized clinical trials, Level Ⅱ, cohort non-blinded studies and non-randomized clinical trials, Level Ⅲ, good quality case-control studies and non-randomized cohort studies, Level Ⅳ, case series and poor quality case-control studies, and Level Ⅴ, case report articles and experts' opinion. Evidence levels Ⅱ, Ⅲ, Ⅳ and Ⅴ were found to support biliary carcinogenesis associated with pancreaticobiliary reflux in normal and abnormal pancreaticobiliary junction. The same levels of evidence were found to support the common occurrence of pancreaticobiliary reflux in normal pancreaticobiliary junction, and SO dysfunction as the most plausible cause of this condition. Although an important body of research has been published regarding pancreaticobiliary reflux in normal pancreaticobiliary junction and its clinical significance, the current evidence does not fully support what has been suggested. Studies with evidence level Ⅰ have not been undertaken. This is a fascinating subject of study, and if finally supported by evidence level Ⅰ, the importance of this condition will constitute a major breakthrough in biliary pathology.

Key words: Biliary tract diseases; Biliary tract motility disorders; Pancreaticobiliary junction; Pancreaticobiliary reflux; Sphincter of Oddi

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INTRODUCTION

The reflux of pancreatic enzymes into the biliary tract has been associated with proliferative changes of the biliary epithelium, hyperplasia and carcinoma[1-3]. This sequence of events, caused by pancreaticobiliary reflux (PBR), has been extensively studied in patients with biliary tract anomalies such as anomalous pancreaticobiliary junction and choledochal cysts[4-19]. Recently, it has been recognized that PBR is a phenomenon occurring in normal pancreaticobiliary junction (NPBJ)[20-29]. It has also been associated with gallbladder carcinoma[30-38], and it has been suggested that it could play a role in gallstone formation through inflammatory changes in the gallbladder mucosa[20,29,36]. The

pathophysiology and clinical importance of this phenomenon is now being elucidated, and attributes the occurrence of PBR to biliary tree motility disorders involving the sphincter of Oddi (SO), a theory which remains to be definitively proved[27,29,36]. The purpose of this review is to discuss the current knowledge on PBR in NPBJ and its pathologic implications.

SUBJECTS OF DISCUSSION

The following aspects were defined appropriate for discussion on the current knowledge and pathologic implications of PBR in NPBJ: (1) Evidence of carcinogenesis associated with PBR; (2) Evidence of PBR in normal NPBJ; and (3) Evidence of SO dysfunction as a cause of PBR in NPBJ.

LEVELS OF EVIDENCE

The articles reviewed for this report were selected and classified according to five levels of evidence[38,39]: (1) Level Ⅰ, meta-analysis double-blind randomized clinical trials; (2) Level Ⅱ, cohort non-blinded studies and non-randomized clinical trials; (3) Level Ⅲ, good quality case-control studies and non-randomized cohort studies; (4) Level Ⅳ, case series and poor quality case-control studies; and (5) Level Ⅴ, case report articles and experts´ opinion.

OTHER SOURCES

In order to provide an adequate background, a PubMed search with the MeSH terms: pancreaticobiliary reflux in normal pancreaticobiliary junction and SO pathophysiology and dysfunction was performed. Articles in English language concerning pancreaticobiliary reflux in pancreaticobiliary maljunction, diagnosis and implications of a long common channel, and SO dysfunction were identified and reviewed regardless of their level of evidence.

CARCINOGENESIS ASSOCIATED WITH PANCREATICOBILIARY REFLUX: WHAT IS CURRENTLY KNOWN?

Most of the current knowledge on gallbladder carcinogenesis associated with PBR comes from studies on patients with anomalous pancreaticobiliary junction; consequently this part of the review deals with patients with gallbladder cancer and anomalous pancreaticobiliary junction. A good level of evidence has been collected on this subject and is detailed in Table 1. The reflux of pancreatic juice plays an important role in gallbladder carcinogenesis, and this fact has been recognized for more than 60 years[2,9]. Gallbladder carcinoma associated with PBR was first described in patients with choledochal cysts[2,4,40]. In these cases, the presence of an anomalous pancreatico biliary junction in more than 96% of these patients[42-46]; causes reflux of pancreatic juice into the dilated or non-dilated cystic common bile duct and into the gallbladder, where active pancreatic enzymes concentrate leading to chronic inflammation with associated mucosal malignant changes: hyperplasia, metaplasia, dysplasia, and eventually carcinoma *in-situ* and invasive carcinoma[6,9,12,16-19,40,43-45]. In patients with gallbladder cancer, an incidence of 8.7% to 16.7% of anomalous pancreaticobiliary maljunction has been found[17,45]. Gallbladder carcinoma has an incidence of between 8.4% and 24.6% in patients with anomalous pancreaticobiliary junction[14,17]. Gallbladder carcinoma has been reported to be more frequently associated with gallbladder carcinoma in pancreaticobiliary maljunction without dilatation of the biliary tract than in pancreaticobiliary maljunction with dilatation of the biliary tract, with a reported incidence ranging from 41% to 90%[6,9,14,17,19], whereas in the dilated type the incidence is about 10%[14]. In addition, the incidence of epithelial hyperplasia is significantly higher in gallbladders without common bile duct dilatation (91%) than in those with common bile duct dilatation (38%)[7,19]. A plausible explanation for the differences between dilated and non-dilated common bile duct in anomalous pancreaticobiliary junction, is that refluxed pancreatic juice stagnates in the gallbladder in the non-dilated type consequently injuring the biliary mucosa, and in the case of the dilated bile duct, pancreatic juicy stagnates in the dilated common bile duct injuring the ductal biliary mucosa. Pancreaticobiliary maljunction outside the duodenum causes two-way regurgitation: pancreatic juice refluxes into the bile duct, or bile regurgitates into the pancreatic duct[44], however, pancreatic duct hydrostatic pressure is higher within the pancreatic

Table 1 Articles from "Carcinogenesis associated to pancreaticobiliary reflux: What is currently known?" – Levels of evidence in anomalous pancreaticobiliary junction

Author	Level of evidence
Kimura et al[2], 1985	Ⅳ
Kinoshita *et al*[4], 1984	Ⅳ
Mizuno *et al*[6], 1996	Ⅱ
Chao *et al*[7], 1999	Ⅳ
Matsumoto *et al*[8], 2003	Ⅳ
Funabiki *et al*[9], 2009	Ⅳ
Kamisawa *et al*[10], 2009	Ⅳ
Tsuchida *et al*[12], 2003	Ⅳ
Hanada *et al*[13], 1999	Ⅲ
Obara *et al*[14], 1999	Ⅳ
Hanada *et al*[15], 1999	Ⅲ
Seki *et al*[16], 2005	Ⅲ
Funabiki *et al*[17], 1997	Ⅳ
Wistuba *et al*[18], 1999	Ⅳ
Hanada *et al*[19], 1999	Ⅳ
Iwai *et al*[40], 1992	Ⅳ
Lipsett *et al*[41], 1994	Ⅳ
Lenriot *et al*[42], 1998	Ⅳ
Singham *et al*[43], 2009	Ⅳ
Todani[44], 1997	Ⅳ
Todani *et al*[45], 1994	Ⅴ
Akiyama *et al*[46], 1998	Ⅴ
Yamato *et al*[47], 1999	Ⅲ
Ichikawa *et al*[48], 2004	Ⅲ

duct[10,42], consequently the reflux of pancreatic juice into the common bile duct is a more frequent phenomenon[10]. Refluxed bile, undergoes stasis in the gallbladder accumulating and causing inflammation of the mucosa which suffers multiple cellular and molecular changes finally leading to carcinoma[6,13,15,16,47,48].

Epithelial changes

Metaplasia and hyperplasia are often found in gallbladder epithelia of patients with PBR, while they are seldom found in control epithelia[7,14,17]. Hyperplastic and metaplastic changes in the gallbladder of these patients are commonly found[9,17], and papillary hyperplasia, mainly low-grade hyperplasia, is commonly seen in patients with PBR, and has been associated with gallbladder cancer[16]. However, others suggest that high-grade hyperplasia is more frequent in patients with pancreaticobiliary maljunction and a non-dilated common bile duct[9,14]. Epithelial hyperplasia of the gallbladder is an early characteristic change of the gallbladder mucosa in patients with PBR, with an incidence ranging from 38.5% to 87%; higher than the incidence of epithelial hyperplasia in patients without PBR. Moreover, epithelial hyperplasia is more frequent in patients with non-dilated common bile duct and pancreaticobiliary maljunction (91%) than in patients with a dilated choledochus and pancreaticobiliary maljunction (38%)[9,14]. Gallbladder carcinoma in patients with PBR is habitually found in people over 50 years-old[36,46], more frequently in women than in men, occurring mainly in the gallbladder fundus, and with the atypia of the gallbladder epithelium becoming less marked nearest the common bile duct[46]. The pathogenesis of epithelial hyperplasia of the gallbladder mucosa in PBR can be explained by an increased hydrostatic pressure in the biliary tract secondary to the reflux of pancreatic juice, and an increased concentration of bile cholesterol secondary to biliary stasis in the gallbladder acting as a stimulus for the development of hyperplastic epithelium[14].

Cellular kinetics

The mixture of bile acids and pancreatic activated enzymes present in PBR contains carcinogens, which are mutagenic, and cause injury to DNA which shows aneuploid or polyploid patterns, accelerating the cell cycle of the biliary epithelium of patients with PBR[14,17]. Other specific molecular changes demonstrated in the mucosal cells of patients with PBR related to increased cellular kinetics are: elevation of the labeling indices of bromodeoxyuridine demonstrating a significantly greater nuclear S-phase fraction; elevation of the proliferative cell nuclear antigen; elevation of Ki-67 labeling index (Ki-67LI); an increase in the mean number of argyrophilic nucleolar organizer regions per nucleus; an increase in the activity of the ornithine decarboxylase, the rate-limiting enzyme in the biosynthesis of polyamines; and increased expression of the transforming growth factor-α (TGF-α) which regulates cell proliferation[9,14,17-19]. Of interest, was the finding of overexpression of mucin core protein (MUC1) in hyperplastic and dysplastic epithelia of the gallbladder mucosa and carcinomatous lesions in PBR, this anomaly was found even in noncancerous epithelium reflecting an altered phenotype of epithelial cells, and suggesting that the PBR itself might be related to carcinogenesis[9,47].

Gene mutations: K-ras, p53, and others

The prevalence of K-*ras* mutation ranged from 0% to 58% in gallbladder hyperplasia, and from 5% to 100% in gallbladder carcinoma in patients with PBR[12-14,18,19]. Point mutations of the K-*ras* oncogene in codon 12 (specific point mutation of GGT -Gly- to GAT -Asp- transition, only found in gallbladder carcinoma with PBR), and codon 13 of exon 1 in the gallbladder epithelium have been identified[7,9,13,14,17,19]. Gene mutations of K-*ras* range from 5% to 100% in invasive gallbladder carcinomas and from 15% to 73% in dysplastic gallbladder lesions associated with PBR[13,18]. In addition, the overexpression of *p53* tumor suppressor gene located on the short arm of chromosome 17 (17p) in 57.1% patients with gallbladder carcinoma has been identified[9]. Specific point mutations were found at codons 207, 212 and 217 on exons 5 to 8 in 31% to 80% patients with gallbladder cancer and PBR[8,9,15,17,18]; p53 overexpression or mutations have not been detected in the noncancerous hyperplastic or dysplastic region adjacent to the cancer region, these observations suggest that *p53* overexpression or mutations may be related to the transition from premalignancy to malignancy in the carcinogenesis of gallbladder mucosa[12,17-19]. However, *p53* mutations have also been detected in noncancerous biliary epithelium (38.5%) in patients with PBR, supporting the involvement of p53 gene mutations in biliary epithelium carcinogenesis in PBR[15]. Finally, loss of heterozygosity of p53 has been detected in 72% patients with gallbladder carcinoma and PBR[8,9,15]. In patients with PBR, p53 overexpression was detected in cancer but not in hyperplasia, indicating that it may be a late event in gallbladder carcinogenesis[9,14]. Within the gallbladder, and along the biliary tract, chronic severe inflammation from PBR destroys the protective mucin-producing epithelial cells, and this repeated process of destruction/regeneration of biliary epithelium, leads to the known sequence of hyperplasia, metaplasia, dysplasia and carcinoma[16]. K-*ras* mutations and overexpression of *p53*, are present in malignant, precancerous dysplastic and chronically inflamed bile ducts in patients with choledochal cysts and abnormal pancreaticobiliary junction without choledochal dilatation, this suggests that the reflux of active pancreatic enzymes causes these cellular and molecular alterations and that the biliary epithelium of patients with PBR should be considered as an epithelium with high carcinogenic potential[7,9,15,16,44]. Mutations in tumor suppressor genes *p14ARF, p16INK4a*, and *p16INK4/CDK2* have been frequently found in gallbladder cancer in PBR, and also in PBR without gallbladder cancer[9]. Other genetic changes described in patients with gallbladder cancer and PBR involve inactivation of the

CDKN2 gene (80%), allelic loss at 8p22 locus (44%), DCC (18q21) deletions (31%), allelic loss at 17q13 at the *TP53* gene, allelic loss at 9p21 at the *p16*^{Ink4}/*CDKN2* gene, and 5q21 loss of heterozygosity near the APC gene (22%); these mutations are considered early events in the pathogenesis of gallbladder carcinoma[18]. Overexpression of Bcl2, an inhibitor of apoptosis found in mitochondria membranes, has been found in the noncancerous portion of gallbladders in PBR and in the gallbladder carcinoma of these patients; this was regarded as an early event causing carcinogenesis in PBR[9,48]. Telomerase activity is increased in gallbladder cancer only in patients with PBR, but is also increased in the noncancerous gallbladder mucosa of these patients, and in the epithelia of noncancerous patients with PBR[48]. Microsatellite instability (MSI) was detected in 80% of gallbladder cancerous lesions, in 87.5% of dysplastic lesions and 0% of hyperplastic lesions in patients with PBR, contributing to carcinogenesis in these patients[9]. Finally, mRNA indices in metaplastic and hyperplastic gallbladder epithelia in PBR are significantly increased, signaling metaplasia and hyperplasia as precancerous lesions[9].

Carcinogens

Some bile acid fractions such as lithocholic and deoxycholic acids, deconjugated bile acids and β-glucoronidase, and active pancreatic enzymes in bile such as amylase, lipase, trypsin, elastase I, and phospholipase A2 promote carcinoma under conditions of infection, inflammation, bile stasis, decreased trypsin inhibitors, and the presence of enterokinase[6,7,9,17,19]. Moreover, phospholipase A2 hydrolyzes lecithin into lysolecithin which is harmful to the mucosal barrier injuring the cell membrane[6,7,9,17,47]. Other secondary bile acids, mainly taurodeoxycholic acid, may play a role in carcinogenesis, although this hypothesis is not completely accepted[9]. Also, some amino acids acting as mutagenic substances, such as glycine, tyrosine and phenylalanine, have been found in large quantities in PBR[6,9,17].

Cytokines and growth factors

Akiyama reported high levels of serum interleukin-6 (IL-6), TGF-α, and hepatocyte growth factor in a patient with gallbladder carcinoma and pancreaticobiliary maljunction. Also high levels of IL-6 and TGF-α were found in the same patient, suggesting a relationship between these molecules and chronic inflammation of the biliary tract in PBR [46]. Other investigators have found overexpression of TGF-α, β-catenin, cyclinD1 and COX-2 in hyperplastic gallbladder mucosa of patients with PBR[8,9,12].

Gallbladder cancer in normal pancreaticobiliary junction

Recently, PBR in patients with NPBJ has been identified and has been associated with gallbladder cancer. A good level of evidence was found in published articles dealing with this subject, and is detailed in Table 2. The first reports on patients with NPBJ and PBR associated with gallbladder cancer were almost anecdotal case reports or case series[22,30,33,35]. The reported incidence of gallbladder cancer in reports of series of cases, ranged from 6.5% to 50%[22,26,31,34,36]. The levels of pancreatic enzymes, mainly amylase, have been reported to be extremely high in patients with gallbladder cancer and PBR in NPBJ, compared to patients with benign gallbladder diseases within the same study[22,26,31,34,36]. Although there has been no explanation as to why amylase levels were highly elevated in these cases, a strong correlation was found between gallbladder cancer and higher levels of amylase in the gallbladder bile of patients with PBR and NPBJ [26,30,33,36]. In patients with higher amylase levels, thickening of the gallbladder mucosa was a significant manifestation, and histological examination showed a high incidence of metaplastic changes compared to patients with lower levels of amylase[26,36,37]. Markers of increased cellular kinetics, such as Ki-67LI, were found to be highly elevated in patients with higher amylase levels compared to those with lower amylase levels in patients with gallbladder cancer[26,36]. Moreover, studies on non-cancerous epithelium of patients with PBR and NPBJ, have found an increased Ki-67LI, COX-2 expression and overexpression, and mutations of K-*ras* gene at codon 12 with an increased and statistically significant frequency in patients with higher amylase levels[31,32]; and increased Li-67LI in patients with hyperplastic or dysplastic gallbladder epithelium without gallbladder cancer, with a higher mean Li-67LI in dysplastic epithelium compared to metaplastic epithelium[31]. Dysplasia and hyperplasia are frequently found in patients with PBR and NPBJ, the incidence has been reported to be between 46% and 50%[31,34]; also, intestinal metaplasia has been found in 16.8% of patients with extremely high levels of amylase and NPBJ[37]. These findings suggest that besides PBR, higher levels of pancreatic enzymes in bile constitute a risk factor for gallbladder cancer, and that the sequence of hyperplasia–metaplasia-dysplasia–carcinoma seen in patients with abnormal pancreaticobiliary junction might be similar for patients with NPBJ and PBR[30-34,36]. Overexpression of *p53* gene has not been frequently found in gallbladder cancer of patients with PBR associated with NPBJ[32], while only a few articles have reported this genetic marker as positive in patients

Table 2 Articles from "Carcinogenesis associated to pancreaticobiliary reflux: What is currently known?" – Levels of evidence in normal pancreaticobiliary junction

Authors	Level of evidence
Sai *et al*[22], 2002	III
Horaguchi *et al*[26], 2008	III
Sai *et al*[30], 2005	V
Sai *et al*[31], 2005	III
Itoi *et al*[32], 2005	III
Sai et al[33], 2006	V
Sai *et al*[34], 2006	III
Inagaki *et al*[35], 2005	V
Beltrán *et al*[36], 2007	II
Sakamoto *et al*[37], 2009	III

with gallbladder cancer[30]. This fact is in contrast with the frequent identification of *p53* abnormalities in gallbladder carcinoma of patients with pancreaticobiliary maljunction. It has been assumed that the pathophysiological changes leading to gallbladder carcinoma in patients with NPBJ and PBR parallel the pathophysiology described in studies on patients with anomalous pancreaticobiliary junction and gallbladder carcinoma[22,31,33,34,36].

Summary

Gallbladder carcinogenesis, in patients with PBR, is a multifactorial and multistage dynamic process involving multiple genetic changes and proliferative inductions of the gallbladder mucosa induced by the reflux of active pancreatic enzymes into the biliary tree and pooling in the gallbladder lumen. After many years of investigation and multiple publications on the subject, the precise processes of gallbladder carcinogenesis secondary to PBR are not yet clear. Gallbladder cancer in anomalous pancreaticobiliary junction is associated with a non-dilated common bile tract, in which stasis of the refluxed pancreatic juice occurs in the gallbladder and causes the variety of changes previously reviewed, leading to gallbladder cancer. The pathophysiology of gallbladder cancer in NPBJ probably resembles this well-known condition.

PANCREATICOBILIARY REFLUX IN NORMAL PANCREATICOBILIARY JUNCTION: WHAT IS CURRENTLY ACCEPTED?

The reflux of pancreatic enzymes into the biliary tract and its role in gallbladder diseases has been studied since the first half of the twentieth century[50]. However, despite some early elegant published studies[20], PBR occurring in NPBJ has only recently become accepted [21-24,27,29,36]. Normal pancreaticobiliary junction was defined as the union of the common bile duct and the main pancreatic duct inside the duodenal wall where the SO surrounds them with muscular fascicles which regulate the flow of bile and pancreatic juice[50,51]. According to this definition, reflux of pancreatic juice into the biliary tract in patients with a NPBJ can only be explained by an improper functioning of the SO[29,34,36,52]. Most studies on PBR in NPBJ have addressed the potential relationships with gallbladder cancer and have been previously discussed. In this section we will discuss other aspects referring to benign gallbladder diseases related to PBR, diagnostic methods and normal values (Table 3).

Table 3 Articles from "Pancreaticobiliary reflux in normal pancreaticobiliary junction: What is currently accepted?"

Author	Level of evidence
Anderson *et al*[20], 1979	IV
Sai *et al*[21], 2002	V
Sai *et al*[22], 2003	III
Vracko *et al*[23], 2003	III
Itokawa *et al*[24], 2004	III
Kamisawa *et al*[25], 2006	III
Horaguchi *et al*[26], 2008	III
Xian *et al*[28], 2009	II
Beltrán *et al*[29], 2010	II
Sai *et al*[30], 2005	V
Sai *et al*[31], 2005	III
Itoi *et al*[32], 2005	III
Sai *et al*[33], 2006	V
Sai *et al*[34], 2006	III
Beltrán *et al*[36], 2007	II
Hjorth E.[49], 1947	IV
Paulsen *et al*[50], 2002	II
Toouli *et al*[51], 1999	IV
Vracko *et al*[52], 1994	II
Vracko *et al*[53], 2000	III
Vracko *et al*[54], 2006	II
Ko *et al*[55], 2005	II

Methods employed to diagnose pancreaticobiliary reflux in normal pancreaticobiliary junction

A variety of methods has been employed to demonstrate PBR. Some investigators have used radioimmunoassay of biliary trypsin in bile samples taken directly from a T tube inserted directly into the common bile duct after surgery[20,52]. A widely used method has been the bile sample taken directly from the gallbladder at the time of cholecystectomy and pancreatic amylase and lipase determination by colorimetric or enzymatic methods [20,23,24,29,36,53]. Many researchers have measured pancreatic enzymes in bile using samples taken directly from the bile duct in patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) and analyzing the samples with enzymatic methods or Western-blotting tests[24-28,30-34,36,54]. Others have employed magnetic resonance cholangiopancreatography (MRCP) with secretin injection to stimulate the SO to indirectly show reflux of pancreatic juice into the biliary tract[21,22,24,25,27]. However, of these methods, the sampling of bile by ERCP seems to be an inadequate method to demonstrate PBR because in order to take the sample of bile, the SO must necessarily be disrupted, potentially causing reflux of enzymes into the bile duct and gallbladder and consequently invalidating the sample and the method. Secretin injection MRCP is an indirect and unspecific method with low sensitivity and specificity to prove PBR[22,24], and in patients who should be submitted to surgery, it seems to be a rather unnecessary preoperative study. Taking bile samples from an indwelling T tube is probably an inadequate method to prove PBR because in order to insert the T tube, the biliary tract must have been manipulated and have suffered surgical trauma with instrumentation of the SO, consequently invalidating the method. We believe that the most accurate method to measure PBR is directly sampling the bile from the gallbladder during cholecystectomy, before any manipulation in the triangle of Calot area or over the common bile duct has been performed[29,36].

WHICH ARE "NORMAL" LEVELS OF PANCREATIC ENZYMES IN BILE?

No one has determined and validated normal values or levels of pancreatic enzymes in bile[1,27,36]. However, most researchers use as a reference the normal values of pancreatic enzymes in plasma to discriminate patients with PBR from those without the considered anomalous reflux of pancreatic juice into the biliary tract [22-24,27,29,36,52,53]. The argument favoring this assumption is that any patient with bile levels of pancreatic enzymes within the normal plasma value does not have PBR, considering that the presence of pancreatic enzymes in bile within the normal plasma value depends on the normal hepatic filtration ratio which was determined at 0.30 to 0.70 for trypsin[23,52]. In a recent investigation, we determined that patients without gallstones have minimal or no levels of pancreatic enzymes in their gallbladders[29]. Consequently, we may argue that "normal" values of pancreatic enzymes in bile should be close to zero.

Acute cholecystitis

Anderson *et al*[20] determined the concentration of pancreatic enzymes, amylase and lipase, in bile obtained from the gallbladder in 70 patients and found highly elevated levels of amylase in 87% cases and lipase in 66%, they concluded that the reflux of pancreatic enzymes may initiate chronic inflammatory changes in the gallbladder and could play a role in gallstone formation and in the pathogenesis of some cases of acute cholecystitis . This premise, was also followed by Josef Vracko who investigated the role of PBR in acute cholecystitis [23,54]. Vracko *et al*[23,54] postulated that endoscopic sphincterotomy releasing the common channel outlet obstruction could initially improve the course of acute cholecystitis in elderly patients, reducing the risk of biliary sepsis, and delaying surgery until their conditions were improved in order to undergo elective surgery at a later time and avoid emergency surgery. Pancreatic enzymes were found to be extremely elevated in patients with initial edematous acute cholecystitis compared with patients with late gangrenous cholecystitis and patients with chronic symptomatic cholelithiasis; suggesting that in some patients, acute cholecystitis could be initiated by the reflux of pancreatic enzymes into the gallbladder due to SO dysfunction or an obstructing gallstone in the papilla of Vater or both[23]. Another interesting finding was that in patients with gangrenous cholecystitis, pancreatic enzymes were lower and comparable to patients with chronic cholecystitis; this phenomenon was explained as a consequence of the consumption of pancreatic enzymes by extensive damage of the gallbladder wall, including vascular damage, coagulation, fat necrosis, and intramural hemorrhage[23]. Consequently, although not absolutely proven, the role of PBR in patients with acute cholecystitis might be related to a sudden functional or mechanical obstruction of the SO leading to an excessive reflux of active injurious pancreatic enzymes into the common bile duct and gallbladder, initiating a cascade of events ultimately leading to acute cholecystitis. This theory might find its clinical application in the proposal of endoscopic sphincterotomy in the early course of acute cholecystitis within the first 72 h after the onset of symptoms, as an alternative to more invasive surgery in elderly frail patients, improving the clinical course and allowing time for conservative treatment or delayed elective surgery[23,55].

Gallstone formation

Pancreaticobiliary reflux causes chronic inflammation and injury of the biliary tract mucosa, particularly of the gallbladder mucosa where bile concentrate and pancreatic enzymes pooling reaches high levels[22,24,25,29,31,36]. High levels of pancreatic enzymes have been found within the whole spectrum of gallbladder diseases, benign and malignant, including acute and chronic cholecystitis, common bile duct stones, gallbladder polyps, and gallbladder cancer[20,22,24,26,29,36,37]. This suggests that PBR has a role in the whole spectrum of gallbladder diseases[20,24,27,29,36]. Initial and chronic inflammatory changes of the gallbladder mucosa induced by active pancreatic enzymes could play a role in gallstone formation[20,27,29,36]. The reflux of pancreatic enzymes causes chronic inflammation of the gallbladder mucosa[20-29,36,53,54]. Chronic inflammation of the gallbladder mucosa modifies the hepatic bile in ways other than the reabsorption of fluids and electrolytes, with the addition of total proteins such as mucin and albumin which increase the nucleation time leading to the formation of biliary sludge, microlithiasis and ultimately gallstones[20,27,55]. It has been suggested that motility disorders of the gallbladder and biliary tree; including spastic episodes of the SO that could be influenced by gender, hormones and genetic predisposition, and associated with PBR leading to biliary tract and gallbladder mucosa chronic injury; could play a role in the etiology of gallstones, chronic gallbladder disease and ultimately gallbladder cancer, constituting only different continuous stages of a common pathologic entity[29].

Summary

The occurrence of PBR in patients with a NPBJ seems to be a pathologic condition occurring in benign and malignant biliary diseases such as acute and chronic gallstone cholecystitis and gallbladder cancer. This reflux plays a role in acute and chronic inflammation of the gallbladder epithelium, gallstone formation, acute cholecystitis and carcinogenesis. To study PBR in normal pancreaticobiliary junction, a variety of indirect and direct methods have been employed; however, none seem to be the ideal method. A sample of bile directly from the gallbladder during surgery would be the best currently available method for this purpose. The so-called normal levels of pancreatic enzymes in bile would be close to zero.

Table 4 Articles from "Pancreaticobiliary reflux in normal pancreaticobiliary junction: What is currently suspected?"

Author	Level of evidence
Sai *et al*[22], 2003	III
Vracko *et al*[23], 2003	III
Kamisawa *et al*[27], 2008	IV
Sai *et al*[34], 2006	III
Beltrán *et al*[36], 2007	II
Iwai N, *et al*[40], 1992	IV
Paulsen *et al*[50], 2002	II
Vracko *et al*[51],1994	II
Todani *et al*[52], 1994	IV
Kamisawa *et al*[53], 2009	III
Kamisawa *et al*[58], 2010	II
Kamisawa *et al*[59], 2002	II
Kamisawa *et al*[60], 2007	III
Toouli *et al*[61], 1982	II
Carr-Locke *et al*[62],1981	III
Csendes *et al*[63], 1979	II
Boyden EA[64], 1937	IV
Yokohata *et al*[65], 2000	IV
Tanaka M[66], 2002	IV

PANCREATICOBILIARY REFLUX IN NORMAL PANCREATICOBILIARY JUNCTION: WHAT IS CURRENTLY SUSPECTED?"

This section will deal with the most plausible cause of PBR in patients with NPBJ (Table 4).

What is a normal pancreaticobiliary junction?

Pancreaticobiliary maljunction has been defined as a union of the pancreatic and biliary ducts which is located outside the duodenal wall forming a markedly long common channel[56,57]. Consequently, NPBJ must, necessarily, be located inside the duodenal wall where the sphincteric mechanism provided by the muscular fascicles, of which the SO is composed, can influence the normal antegrade flow of bile and pancreatic juice.

Long common channel and its implications

A long common channel has been regarded as an intermediate variant of pancreaticobiliary maljunction, others have also named it a high confluence of pancreaticobiliary ducts[58,59]. Some authors, argument that a long common channel is included within the SO[27,57-60]; however, it is not plausible that this long common channel would be under the complete influence of the SO, consequently this would be the cause of PBR in these cases. Most authors agree that the normal length of the common channel generally tends to be less than 10 mm in adults and 4 mm in infants[50,56], consequently a longer common channel should be considered an abnormal variant. However, other authors have suggested that a common channel should be considered long when it is equal or larger than 5 mm[58]. A long common channel is not under the complete influence of the SO, no high-pressure area has been found in the area of the common channel of the pancreaticobiliary junction in patients with anomalous pancreaticobiliary junction or a long common channel, which confirms that the SO does not fully extend to the area of the pancreaticobiliary junction in these cases[41,60], consequently pancreaticobiliary and biliopancreatic reflux is a common occurrence[60]. The most important clinical significance of a long common channel is that, as well as in patients with anomalous pancreaticobiliary maljunction, patients with a long common channel showed significant PBR related to gallbladder cancer[22,58,59].

SO: Its role in pancreaticobiliary reflux in normal pancreaticobiliary junction

The human bile duct lacks a contractile muscle layer[52]. Therefore, the flow of bile and pancreatic juice towards the duodenum is a consequence of gallbladder activity and intraductal bile and pancreatic duct pressures, which depend on the production of bile and pancreatic juice, respectively, and is regulated by the SO. The normal sphincter activity directs the flow of bile and pancreatic juice towards the duodenum by antegrade phasic contractions[61], consequently, in patients with PBR and NPBJ, the only plausible explanation for reflux of pancreatic juice into the biliary tract is an anomaly in the normal function of the SO causing a functional obstruction to the normal flow and retrograde contractions favoring the reflux of pancreatic juice into the biliary tract[23,27,29,34,36,52-54], because the intraluminal pressure of the pancreatic duct is higher than the intraluminal pressure of the common bile duct[51,62,63].

SO: How it functions?

The SO is a smooth muscle structure measuring approximately 1 cm in length which is situated at the junction of the bile duct, pancreatic duct and duodenum[51,66]. The SO normally produces high-pressure phasic antegrade contractions that are superimposed on a modest basal pressure, the phasic contractions propel small volumes of either bile or pancreatic juice into the duodenum[51,56,65,66]. In man, most flow occurs between the phasic contractions[51,65,66]. The SO function is influenced by a number of neural stimuli, circulating hormones such as cholecystokinin, and duodenal activity secondary to ingestion of food[51]. During fasting, the SO demonstrates regular phasic contractile activity which alternates with the interdigestive motor activity of the duodenum[51,65]. During duodenal phases I and II, the SO contracts at the rate of 2 to 4 contractions per minute without a quiescent phase; approaching duodenum phase III, the SO also increases its contractile activity concluding at the same time, this pattern repeats over the same period as duodenal activity[51,65,66]. The fact that the SO continues to contract during the quiescence of duodenal phase I supports the independent nature of SO motility. This pattern of function demonstrated by the SO, supports indirectly the theory regarding the cause of PBR in NPBJ, which states that spasms of the SO not related to the migrating myo-

electric complex is the cause of this phenomenon[23,27,36,65]. SO dysfunction is suspected clinically based on the established Rome II Diagnostic Criteria[67,68]. To study SO dysfunction, less invasive procedures to rule-out other biliary tract conditions should be considered first, such as liver function blood tests and abdominal ultrasound[67]. Other more specific, although non-invasive methods, such as the Morphine-Prostigmin Provocative Test or Nardi Test which has a low sensitivity and specificity, the Quantitative Hepatobiliary Scintigraphy Test which has not been fully accepted due to controversial and imprecise criteria to diagnose SO dysfunction, and Magnetic Resonance Cholangiopancreatography with secretin stimulation could also be employed[67,68]. Other invasive methods such as endoscopic ultrasonography, intraductal ultrasonography, and endoscopic cholangiography have been used, and although they can give indirect evidence of the presence of SO dysfunction, their results are nonspecific[67,68]. Currently, the gold standard to study and diagnose SO dysfunction is SO manometry[51,61,62,64,66-68]. The cause of SO dysfunction is unknown at the present time; however, we suggest that this is probably due to congenital motility anomaly traits.

Summary

SO dysfunction is the most plausible cause of PBR in patients with NPBJ.

CONCLUSION

Although an important body of research has been published regarding PBR in NPBJ and its clinical significance, the current evidence does not fully support what has been suggested. Studies with evidence level I have not been undertaken. This is a fascinating subject of study, and if finally supported by evidence level I, the importance of PBR in NPBJ will constitute a major breakthrough in biliary pathology.

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