patients with high amylase activity in bile have PBM[10]. Therefore, the incidence of high amylase activity can be utilized as an indirect index to estimate the prevalence of PBM among patients with biliary diseases.

MATERIALS AND METHODS

Amylase activity in gallbladder bile was measured in 297 patients who underwent cholecystectomy at the following 6 hospitals during the period from September 1995 to October 1998. The hospitals that participated in the study were: The Medical Hospital of Nippon Dental University School of Dentistry at Niigata, Saiseikai Niigata Second Hospital, Tashiro Shokakika Hospital, Niigata Prefectural Yoshida Hospital, Niigata Kobari Hospital, and Shirone Kensei Hospital.

Patients who had obstruction of the cystic duct or common bile duct were excluded from the study. Patients with abnormal serum amylase levels and those who had a history of endoscopic sphincterotomy (EST) were also excluded. Amylase activity was determined by enzyme assay. The normal ranges in serum amylase activity of the assay system employed in each hospital are shown in Table 1. According to the assay system used, patients who showed a higher amylase activity level in bile than the upper limit of the normal range in serum (N) were classified into the "high amylase activity" group (>N). An amylase level 25 times higher than the upper limit in serum (N) was labeled as the "very high amylase activity" group (>25 N). The types of gallstones were described according to the Macroscopic Classification published by The Japanese Association of Gastroenterology[11]. Infrared analysis was employed for confirmation in cases where macroscopic diagnosis was difficult. If the major component was other than cholesterol or bile pigment, the stone was classified into the "others" group (mainly inorganic calcium salts).

Statistical analysis was performed by the chi-square test and a p value less than 0.05 was considered significant.

RESULTS

I: Amylase activity in gallbladder bile in patients living in the Niigata area.

Results of examination of amylase activity in gallbladder bile are summarized in Table 2 according to the diagnosis of biliary disease. As a whole, 24.6% of the patients showed "high amylase activ-

Table 1. Method of assay and the normal ranges in each hospital

Hospital	Method of assay	Substrate	Normal range in serum IU/L	No. of Cases Examined
A	Enzyme assay	Ethyliden-p-nitrophenyl-maltopentaoside (G7-PNP)	68～225	167
B	〃	p-nitrophenyl-benzyl-maltopentaoside (BG5-P)	43～116	55
C	〃	Blocked-p-nitrophenyl-α-maltopentaoside (Gal-G5-PNP)	45～130	29
D	〃	β-D-galactocyl-4-nitrophenyl-α-maltopentaoside (Gal-G5-4PN)	23～113	28
E	〃	2-chloro-4-nitrophenyl-β-D-maltopentaoside (Cl-PNP-G7)	～200	11
F	〃	Ethyliden-p-nitrophenyl-maltopentaoside (G7-PNP)	～109	7

A: Medical Hospital: Nippon Dental University School of Dentistry at Niigata
B: Saiseikai Niigata Second Hospital
C: Niigata Prefectural Yoshida Hospital
D: Tashiro Shokakika Hospital
E: Niigata Kobari Hospital
F: Shirone Kensei Hospital

Table 2. Amylase levels in gallbladder bile

Diagnosis	Case No.	Amylase levels in gallbladder bile			
		≦N*(%)	>N(%)	>5N(%)	>25N(%)
Cholecystolithiasis	247	191 (77.3)	56 (22.7)	21 (8.5)	6 (2.4)
Cholecysto-choledocholithiasis	16	10 (62.5)	6 (37.5)	2 (12.5)	1 (6.3)
Choledocholithiasis	7	4 (57.1)	3 (42.9)	2 (28.6)	1 (14.3)
Cholecystitis	2	1 (50.0)	1 (50.0)	1 (50.0)	0
Polypoid lesions	19	16 (84.2)	3 (15.8)	2 (10.5)	2 (10.5)
Gallbladder cancer	6	2 (33.3)	4 (66.7)	2 (33.3)	1 (16.7)
Total	297	224 (75.4)	73 (24.6)	30 (10.1)	11 (3.7)

*N, upper limit of the normal range in serum amylase.

Table 3. Amylase levels and types of gallstone

Types of gallstones	Amylase levels			
	≦N	>N	>5N	>25N
Cholesterol	113	31	11	2
Black pigment	47	16	4	0
Ca. bilirubinate	19	12	6	4*

*, $p<0.01$
compared with cholesterol stones.

ity". Eleven patients among 297 (3.7%) showed "very high amylase activity". Incidence of "high amylase activity" in bile was higher in male patients than in females (36/79 vs 37/145, $P<0.05$). However, that of "very high amylase activity"was not different (6/79 vs 5/145). No statistically significant difference was seen in the incidence of "high" or "very high amylase activity" between biles with positive bacterial culture and those with negative culture. As to the kind of gallstones, the incidence of "very high amylase activity" was significantly higher in patients with calcium bilirubinate stones compared to those with cholesterol stones ($P<0.01$) and to those with black pigment stones ($P<0.05$) (Table 3).

Preoperative endoscopic retrograde cholangiopancreatography (ERCP) was not performed routinely in this series. However, among 78 ERCP cases in whom the pancreaticobiliary ductal junction was clearly discerned PBM was found in two (2.6%). Both of these patients with PBM showed "very high amylase activity" in bile.

Among 11 patients with "very high amylase activity" in bile, PBM was confirmed by ERCP in two cases. PBM was denied in one by ERCP and in another by operative cholangiography. In one patient, PBM was strongly suspected by operative cholangiography even though the X-ray findings were not conclusive. No information concerning the pancreaticobiliary ductal junction was available in the remaining 6 patients.

II: Comparison with results reported from other parts of Japan.

Even though published data on amylase activity in gallbladder bile are rare, two papers were found[12,13] in which materials of the study and assay system of amylase acitivity with its normal range were clearly described (Table 4). One report came from Nagasaki and another from Hiroshima. Nagasaki Prefecture was, according to Kodama[4], designated as a relatively high risk zone for biliary tract cancer next to Niigata, Yamagata and Aomori, while Hiroshima was indicated as a low risk area. Furukawa (Nagasaki) employed the blue starch method for determination of amylase activity and Sasaki (Hiroshima) used the enzyme assay. The upper limit of the normal range in serum was 400 IU/L in both systems. In both papers, amylase activity in bile higher than 10,000 IU/L (25 times higher than the upper limit in serum) was employed as a criterion which showed a high possibility of PBM (41.7% in Furukawa's series). The incidence of "very high amylase activity" group in our series (3.7%) produced the same result as Furuk-

Table 4. Amylase activity in gallbladder bile reports from different areas of Japan

Author		Method	Case	Amylase levels			
				Normal limit(N)	>5N	>25N	
Furukawa (NAGASAKI)	1983	BLUE STARCH	265	400IU/L		12 (4.5%)	CD, CBD obstruction excluded
Sasaki (HIROSHIMA)	1987	ENZYME ASSAY	103	400IU/L	7 (6.8%)	2 (1.9%)	PBM excluded
our series (NIIGATA)	1999	ENZYME ASSAY	297		28 (10.0%)	11 (3.7%)	CD, CBD obstruction excluded

NORMAL LIMIT, upper limit of normal range in serum amylase; CD, CBD, cystic duct, common bile duct; PBM, pancreaticobiliary maljunction.

awa. On the other hand, the incidence of amylase activity higher than 10,000 IU/L in Sasaki's series from a low risk area was lower (1.9%). However, patients with PBM were excluded from Sasaki's study, while cases in both our series and Furukawa's included these patients. If the difference in cases is taken into account, the incidence of the "very high amylase activity" group in Sasaki's series would become quite close to ours.

DISCUSSION

According to the nation-wide survey by the Japanese Study Group on PBM, 20.7% of adult patients with PBM were also afflicted with gallbladder cancer while another 5.2% had carcinoma of the bile duct[9]. Presently, PBM has been accepted as an significant risk factor for biliary tract carcinomas.

Therefore, in the study on high occurrence of biliary tract cancer, it is considered necessary to know regional differences in the incidence of PBM. This paper is the first report from a high risk area for biliary tract cancer relating to the incidence of PBM.

For definite diagnosis of PBM, ERCP is necessary. Up to the present time, several reports on the incidence of PBM among Japanese patients who underwent ERCP have been published. From the results of these studies, the incidence of PBM among Japanese patients with biliary tract disease is considered to be between 1.5 and 3%[10]. However, no study concerning the regional difference in the incidence has been carried out.

Measurement of amylase activity in bile is a technically easier approach for the detection of pancreaticobiliary reflux. However, every patient who shows high amylase activity in bile does not necessarily have PMB. In cases with a damaged sphincter mechanism of Papilla of Vater, as is often seen after the passage of common duct stones, reflux of the duodenal content into the biliary tract can occur. Most previous studies showed coincidentally the incidence of PBM among patients with very high amylase activity in bile (higher than 10,000 IU/L) to be nearly 50%[10]. Another problem with this approach is that the cases of the study are limited to patients who undergo biliary surgery. Despite these limitations, it can be a rough index to estimate the incidence of PBM among patients with biliary disorders. As aspiration of gallbladder bile after cholecystectomy does not add any additional risk for patients, it seems appropriate for prospective investigation in a large number of cases.

Unexpectedly, our result showed almost the same incidence of "very high amylase activity" group among patients with surgical biliary disorders to those among patients in the Nagasaki and Hiroshima districts. The results seem to suggest that the incidence of PBM in the Niigata district is not prominently higher than those in other parts of Japan and does not play a major role in the high occurrence of biliary tract cancer. However, a prospective collaborative study among various areas with different incidence of biliary tract cancer is necessary before reaching a definite conclusion.

Concerning the incidence of PBM among ERCP cases, no report has been published from the Niigata district. However, Narisawa (Third Department of

Table 5. Incidence of PBM in ERCP cases

Author		No. cases	PBM(%)	Location
Kimura K[14]	1985	656	21 (3.2%)	Chiba
Chijiiwa K[15]	1995	1325	29 (2.2%)	Fukuoka
Egami K[16]	1998	1722	52 (3.0%)	Tokyo
Narisawa R	1998	2336	63 (2.7%)	Niigata*

*, unpublished data.

Table 6. The mean age of patients with gallbladder cancer

Author		Materials	Mean age of patients (years)		Location
			with PBM	without PBM	
Yamauchi S[19]	1987	collected	53.5 (n=47)		
Kimura K[14]	1985	ERCP cases	49.8±9.8 (n=16)	61.7±10.3 (n=80)	Chiba
Yoshida T[20]	1999	resected cases	58 (n=9)	68 (n=50)	Nagasaki
Ohta T[21]	1989	resected cases	60.7 (n=112)		Ishikawa
Kubokawa Y[22]	1999	resected cases	65 (n=81)		Tokyo
Shirai Y[23]	1992	resected cases	66.1 (n=40*)		Niigata

*, patients who underwent radical surgery.

Medicine, Niigata University Hospital), has found 65 patients with PBM among 2,332 ERCP cases (2.7%, personal communication). The incidence of PBM among his series of ERCP is favorably comparable to those in other reports from different areas where the risk for biliary tract cancer is lower than Niigata (Table 5)[14~16]. Even though the number of cases studied was very small (78 cases), the incidence of PBM among patients who underwent ERCP in the present series (2.6%) was almost the same to that shown by Narisawa.

In most Japanese surgical series of gallbladder cancer, the age groups involved in the highest incidence are the 7th decade followed by the 6th and the 8 th[17]. On the other hand, the mean age of patients with gallbladder cancer associated with PBM has been reported to be lower by 10 years than that of patients with gallbladder cancer not associated with PBM[18,19]. Therefore, the age distribution of patients with gallbladder cancer can help to estimate indirectly the prevalences of PBM in the series reported from different areas. Table 6 shows the mean ages of patients with gallbladder cancer reported from various parts of Japan[14,19~23]. Even though patients with PBM were not distinguished from those without PBM, the mean age in the series from the Niigata district showed the same level as those of other series. This finding seems not to support the assumption that the prevalence of PBM among patients with gallbladder cancer is higher in the Niigata district than in other parts of Japan.

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