Original Article

The diagnostic value of glycogen, mucin, carbonhydrate antigen 19-9, and carcinoembryonic antigen in aspirated cyst fluid in cystic lesions of pancreas

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Abstract. The aim of the present study was to evaluate diagnostic value of glycogen and mucin-staining features of aspirated cyst fluid, and carbonhydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) levels in the blood and cyst fluid in differentiating benign from malignant lesions.

A total of 13 patients, who were referred to the General Surgery Clinics due to pancreatic lesion between January 2013 and November 2013, were included in the study. In the preoperative period, CA 19-9 and CEA were obtained from all patients. The cyst fluid was aspirated in the guidance of radiological imaging methods in 6 methods and using laparoscopic or surgical methods in 7 patients. CA 19-9 and CEA levels were evaluated in the aspirated cyst fluids, and immunohistochemical tests were performed using PAS (glycogen) and mucin staining in patients with sufficient cyst fluid. The pancreatic tissue was macroscopically evaluated in patients who underwent an operation, and appropriate procedure was selected depending on the location of the lesion.

The pathological examination of the 13 patients revealed malignant lesion in 5, benign-inflammatory cyst (IC) in 5, serous cystic neoplasm (CSN) in 1, mucinous cystic neoplasm (MCN) in 1, and non-functional cystic neuroendocrine tumor (CNT) in 1. The blood tests performed in 5 patients with malignant findings on pathological examination revealed elevated CEA levels in 1 patient and elevated CA 19-9 levels in 3 patients. In the aspirated cyst fluids, CA 19-9 levels were high in 3 patients with malignant lesion and in patients with MCN and CNT. The immunohistochemical examination revealed positive staining for PAS and mucin in 2 patients with malignant lesion and one patient with MCN, and another patient with malignant lesion showed PAS-positive staining. Immunohistochemical examination involving PAS and mucin could not be performed in two patients one of which had malignant lesion due to insufficient aspiration fluid.

In the present study, CA 19-9 levels in the blood and aspirated cyst fluids were found to be higher in patients with malignant pancreatic lesions. Of patients with malignant lesions, 3 showed positive PAS staining. However, long-term studies on larger series of patients are warranted in order to establish the diagnostic value of PAS-staining and mucin-staining in immunohistochemical examination of the cyst fluids and the value of CEA levels in the blood and aspirated cyst fluid.

Key words: Pancreas, cystic neoplasm, pancreas mucinous neoplasia

1. Introduction

The improvements in diagnostic imaging have increased the detection rates of pancreatic cystic lesions (1); however, there is still no established standard in the diagnosis and treatment of pancreatic cystic neoplasms (PCN). Pancreatic cystic lesions are divided into three groups as real cysts, acquired cysts (pseudocyst, parasite cysts), and cystic tumors. PCNs account for 10-15% of pancreatic cystic lesions and 1-5% of all pancreatic neoplasms (2). Furthermore, particular importance is placed on PCNs from a clinical perspective, due to confusion of these lesions with pseudocysts and the potential for malignant transformation. In most cases, a definite diagnosis may not be possible prior to surgery. Hence, clinical and radiological findings should

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be evaluated together with laboratory data in order to reach a definitive diagnosis (1,3-5). Presently, immunohistochemical parameters and tumor markers are known to be utilized in the differential diagnosis of pancreatic cystic lesions. In particular, there are various reports on the diagnostic value of glycogen-mucin staining, and CA 19-9 and CEA levels in the fluids aspirated from the lesions. These studies evaluated numerous markers in the cyst fluid including CEA, CA 19-9, CA 125, and CA72.4; however, CEA and CA 19-9 are the most important two markers that have proven diagnostic value (6-8).

The aim of the present study was to evaluate the diagnostic value of immunohistochemical PAS and mucin staining patterns and biochemical CA 19-9 and CEA levels in the aspirated cyst fluid, and blood CEA and CA 19-9 levels by comparing the values with definitive pathological diagnoses in PCLs, which are still a matter of controversy regarding the parameters of the diagnosis and treatment.

2. Materials and methods

The current study prospectively evaluated the data of 13 patients who underwent evaluation in the Department of General Surgery between January 2013 and November 2013 due to pancreatic cystic lesions. In the preoperative period, blood samples were obtained from the patients using a standard method for the measurement of CEA and CA19-9 levels. Percutaneous transabdominal pancreatic cyst aspiration was performed in six eligible patients under the guidance of ultrasonography (US) or computerized tomography in the Department of Radiology. In the remaining seven patients, fluid samples from the cystic lesions were obtained during laparoscopic (two patients) or surgical (five patients) procedures that were performed for diagnostic or therapeutic purposes. CEA and CA19-9 levels were measured using biochemical methods, and PAS and mucin staining patterns were evaluated using immunohistochemical methods in the aspirated cyst fluids. Immunohistochemical analysis could not be performed in two patients with insufficient cyst fluid. The treatments and/or follow-up protocols of the patients were determined according to the findings of clinical, radiological, and laboratory assessments. Regular follow-up was recommended in five patients who did not have clinical, radiological, or laboratory findings suggestive of a malignancy, and CT obtained 6 months later revealed regression in two of these patients. The lesion was deemed to be inoperable in radiological assessment of three of the five patients who appeared to have malignant lesions on pathological examination, and a conservative approach was planned accordingly. Of five patients with a high index of suspicion for malignant lesion, two underwent a Whipple operation, two underwent distal pancreatectomy plus splenectomy, and one patient underwent laparoscopic distal pancreatectomy after obtaining consent for the procedure. In these patients, the examination of paraffin-embedded tissue sections revealed malignant lesions in two patients (ductal adenocarcinoma), serous adenoma in one patient (microcystic adenoma), islet cell tumor+chronic pancreatitis+pseudocyst in one patient, and mucinous cystic neoplasm in one patient.

The results of the patients with malignant cystic lesions were compared to the results of patients with benign cystic lesion considering the biochemical, immunohistochemical results, and the findings observed in paraffin-embedded tissue sections.

The data were entered into the SPSS 13 package program. The Mann-Whitney U-test was used to compare patients with malignant lesions versus patients with benign lesions. The chi-square test was used to evaluate the relationship between the categorical variables. In statistical analyses, the level of statistical significance was set at 5%, and SPSS (version 13.0) was used in statistical analyses.

3. Results

The mean age of the patients was 50.61 years (range: 29 and 83 years). Of the patients, ten (76.9%) were females, and three (23.1%) were males (Table 1). When the patients were categorized according the final pathological results, five patients (38.5%) had malignant lesions and eight patients (61.5%) had benign lesions. Of five patients in whom the examination of paraffin-embedded tissue sections revealed malignancy, three were females and two were males (Table 2).

Table 1. Distribution of Demographic Features

	Min-Max	Mean ± SD
Age (year)	29-83	50.61±14.78
Gender	(n)	(%)
Female	10	76.9%
Male	3	23.1%
Total	13	100%

Table 2. PAS/Mucin staining patterns in malignant and benign lesions

			PAS/Mucin		Total
			+	-	
Pathology	M	Number	3	1	4
	B	Number	1	6	7
Total		Number	4	7	11

M: Malignant B:Benign +:Positive Staining -: Negative Staining Chi-square=4.055; p=0.044 for PAS; p=0.201 for Mucin.

Among the patients with pathological diagnosis of malignant PCL, blood CEA was minimally elevated in one patient, and CA 19-9 was elevated in three patients (Tables 3 and 4). CA 19-9 levels were significantly different between patients with malignant versus benign lesions.

The examination of the aspirated cyst fluid revealed normal CEA levels regardless of the nature of the lesion (malignant or benign). CA 19-9 levels in the aspirated fluid were higher than normal in three patients with malignant lesions and two patients with benign lesions. This group of patients with benign lesions was comprised of those who underwent surgery due to a high index of suspicion on radiological examination and in whom the examination of paraffin-embedded tissue sections revealed MCN and CNT (Tables 5 and 6).

In conclusion, the comparison of patients with malignant and benign lesions revealed no significant difference in terms of CEA levels in the aspirated fluid; however, there was a significant difference in terms of CA 19-9 levels. Due to insufficient fluid aspiration, PAS and mucin staining patterns could not be evaluated in two patients, one of which had a malignant lesion according to the final pathological result and the other had a benign lesion. The immunohistochemical examination in the remaining 11 patients revealed positive PAS and mucin staining in two out of five patients with malignant lesions and in one patient with MCN; PAS-positive staining was reported in one patient with a malignant lesion, and negative PAS and mucin staining in one patient with malignant lesion. In one patient diagnosed with a malignant lesion, an immunohistochemical examination could not be performed due to insufficient aspiration fluid. PAS staining was significantly different between benign and malignant groups; however, there was no significant difference in terms of mucin staining. Seven patients diagnosed with a benign lesion showed negative PAS and mucin staining, and an examination could not be performed due to insufficient aspiration fluid.

Table 3. Blood CEA levels in patients with malignant and benign lesions

			Blood CEA		Total
			N	P	
Pathology	M	Number	4	1	5
	B	Number	8	0	8
Total		Number	12	1	13

M: Malignant B: Benign N: Normal P: Pathological; p=0.03.

Table 4. Blood CA 19-9 levels in patients with malignant and benign lesions

			Blood CA 19-9		Total
			N	P	
Pathology	M	Number	2	3	5
	B	Number	8	0	8
Total		Number	10	3	13

M: Malignant B: Benign N: Normal P: Pathological; p=0.045.

Table 5. CEA levels in the aspirated fluid of malignant and benign lesions

			CEA in the Aspirated Fluid		Total
			N	P	
Pathology	M	Number	4	0	4
	B	Number	7	0	7
Total		Number	11	0	11

M: Malignant B: Benign N: Normal P: Pathological; p=0.383.

Table 6. CA 19-9 levels in the aspirated fluid in patients with malignant and benign lesions

			CA 19-9 in the Aspirated Fluid		Total
			N	P	
Pathology	M	Number	1	3	4
	B	Number	5	2	7
Total		Number	6	5	11

M:Malignant B:Benign N:Normal P: Pathological; p=0.017.

4. Discussion

PCNs account for less than 10% of all pancreatic neoplasms; however, the advancement of modern imaging methods has increased the detection rates for these lesions, which accounted for 30% of the resected lesions, particularly in the last decade (9). The rate of misdiagnosing PCNs as pseudocysts was reported to be up to 37-50% in the past and around 10% in recent years (10).

The management of patients with PCNs is controversial due to the unknown biological behavior of the lesion. Several treatment and follow-up algorithms have been suggested in consideration of the patient's age, gender, medical condition, clinical and physical findings, and location and size of the lesion, particularly premalignant or malignant radiological features of the cysts (11,12). However, no single algorithm has proven to be successful in differentiating benign from malignant lesions in patients with PCN (13).

Most patients with PCN are asymptomatic. The patients may exhibit clinical symptoms such as abdominal pain, jaundice, and weight loss depending on the location and size of the lesion (9). CT is regarded as the preferred choice of method in the diagnosis of PCN, and it was reported to have an accuracy rate between 20% and 90% in differentiating characteristic features of pancreatic cystic lesions (14). Magnetic resonance (MR) imaging or magnetic resonance cholangiopancreatography (MRCP) are more frequently used in lesions measuring less than 2 cm, and the method is useful in demonstrating the association of the tumor with the duct of Wirsung using a noninvasive technique. Endoscopic retrograde cholangiopancreatography (ERCP) is the most sensitive method in demonstrating the relationship between the cystic lesion and pancreatic channel, but this method should not be preferred initially (15).

Endoscopic US (EUS) has been used in the management of PCNs for both diagnostic and therapeutic purposes since 1980s. The accuracy rate for EUS is reported to range between 40% and 96%. Although CA 19-9, CEA, CA-125, and CA72-4 levels are analyzed in the cyst fluid obtained with the guidance of endosonography, particularly CA 19-9 and CEA levels are considered to have a diagnostic value (12,16).

The biochemical analysis of the aspirated cyst fluids revealed CA 19-9 levels above normal ranges in three patients, and blood CA 19-9 levels were within normal ranges in all patients. The examination of CEA levels revealed above normal values in only one patient with a locally-advanced malignant lesion, which is different from the literature. CEA levels in the blood and aspirated cyst fluid were within normal ranges in the other patients.

As mentioned in the studies on the cytopathological examination of the pancreatic cystic lesions in the literature, cuboid cells predominate in serous cysts and columnar cells predominate in MCN and intraductal papillary mucinous neoplasms (IPMN). Furthermore, marked atypia is not observed in serous cysts, but the lesion shows positive glycogen staining. On the other hand, MCNs and IPMNs show positive staining for mucin (7,8,17). In the present study, two patients with malignant lesions and one patient with MCN showed positive PAS and mucin staining, and fluid aspirate from one patient with malignant lesion showed positive PAS staining. The comparative evaluation of mucin and PAS staining did not reveal a significant association with pathological diagnoses; however, the researchers consider that this result was associated with an insufficient number of patients.

The treatment protocols recommend spleen-sparing distal pancreatectomy in PCNs localized in the pancreatic body and tail, segmental pancreatectomy in small lesions localized in the neck, and pancreaticoduodenectomy in lesions localized in the head of the pancreas and uncinate process. Laparoscopic resection can be safely performed in lesions with benign appearance or tumor size less than 5cm in diameter. The centers experienced in laparoscopic pancreatectomy report complication rates ranging between 15 and 20% (18,19). Spleen-sparing distal pancreatectomy can be performed to avoid infection risk and hepatological complications in patients with small lesions and low risk of invasive cancer. Enucleation is not recommended due to high complication rates, primarily the formation of pancreatic fistula and a high risk of tumor recurrence in the surgical margins (20,21). In the current series, five patients underwent surgery based on tumor localization, size, and clinical data in accordance with the literature. Two patients with tumors localized in the head of the pancreas underwent pancreaticoduodenectomy, one patient with a lesion in the tail underwent spleen-sparing laparoscopic distal pancreatectomy, and two patients with lesions localized in the tail and corpus underwent open distal pancreatectomy plus splenectomy.

5. Conclusion

In conclusion, the present study confirmed that CA 19-9 measurement in the blood and aspirated cyst fluid are important diagnostic parameters that can be used in differentiating malignant from benign cystic lesions of the pancreas. Three patients with malignant lesion showed positive PAS staining and one patient with MCN showed positive mucin staining. However, the present study had a small number of patients, and therefore, the researchers consider that further prospective studies with a larger number of patients are required.

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