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• CLINICAL RESEARCH •

p53 protein expression and CA19.9 values in differential cytological diagnosis of pancreatic cancer complicated with chronic pancreatitis and chronic pancreatitis

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Abstract

AIM: To evaluate p53 protein overexpression and to measure serum CA19.9 concentrations in cytological diagnosis of patients with suspected pancreatic cancer.

METHODS: 24 patients with suspected pancreatic cancer due to chronic pancreatitis, had a pancreatic mass determined by imaging methods. The serum CA19.9 concentration was measured by solid phase radioimmunoassay. On laparotomy, puncture biopsy was performed, and specimens were divided into two parts for cytological diagnosis and detection of p53 protein.

RESULTS: Cytology offered a sensitivity of 0.63, a specificity of 1.00, and an accuracy of 0.63. p53 protein analysis offered a sensitivity of 0.44, a specificity of 1.00, and an accuracy of 0.73. CA19.9 offered a sensitivity of 0.44, a specificity of 0.80, and an accuracy of 0.67. The combined cytology and p53 protein analysis showed a sensitivity of 0.78, a specificity of 1.00, and an accuracy of 0.92. Cytology and CA19.9 showed a sensitivity of 0.67, a specificity of 0.80, an accuracy of 0.67. combined cytology and p53 protein analysis and CA19.9 showed a sensitivity of 0.78, a specificity of 0.80, and an accuracy of 0.79.

CONCLUSION: Superior to any single test, the combined approach is helpful for the differential diagnosis of pancreatic cancer complicated with chronic pancreatitis.The combined cytology and p53 protein analysis offers the best diagnostic efficacy.

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INTRODUCTION

The differential diagnosis of pancreatic cancer (PC) complicated with and chronic pancreatitis (CP) is difficult because of their common clinical symptoms and overlapping findings. Increased pancreatic adenocarcinoma risk in chronic pancreatitis patients has renewed the interest in early tumour diagnosis and in the differentiation of neoplastic and chronic inflammatory ductal changes[1, 2]. If a pancreatic mass is discovered by the imaging method, cytological examination is required to make a conclusive diagnosis. Owing to a large number of cases without a conclusive diagnosis[3], it would be worthy to diagnose it with other methods. Genomic alterations in p53 tumour-suppressor gene and overexpression of p53 protein are frequently found in pancreatic cancer, but rarely in chronic pancreatitis. An elevated serum CA19.9 concentrations is found in a high proportion of patients with pancreatic cancer and this is considered to be the standard serum marker for adenocarcinoma of the pancreas. In the current study, we retrospectively evaluated overexpression of p53 protein, serum CA19.9, and cytology in the diagnosis of pancreatic cancer.

MATERIALS AND METHODS

Materials

24 patients,19 men and 5 women, with a mean age 58.6 years, (range 41-69 years,) with jaundice, weight loss, and abdominal pain, underwent pancreatectomy in our hospital between January 1995 and December 2001 because of high suspicion of pancreatic cancer arising from chronic pancreatitis. Preoperatively, they were found to have a mass in the pancreas by CT and ERCP. 19 masses were located in pancreatic head, two in the body, and two in the tail, 1 with three foci.

Methods

CA19.9 determination The serum samples were stored at -20 ℃ for CA19.9 by solid phase radioimmunoassay. A value of 37 U/ml was the upper limit of normal.

Cytological examination Puncture biopsy of the pancreas was performed during laparotomy, and the specimen was divided into two parts: one part was used for making fresh smears, after papanicolaou staining. The presence of malignant cells and suspicious cells were examined under microscope (Figures 1-2). The other part was employed for immunohistochemical analysis.

Immunohistochemical analysis The sample was fixed in buffered formaldehyde and paraffin-embeded. Histological sections (5 μm) were prepared, mounted on poly-L-lysine-coated slides and dried for 12 to 24 h at 37 ℃. Immunohistochemistry was performed with the avidin-biotin complex (ABC) kit[4] using the monoclonal antibody which recognizes both mutant and wild-type p53.

The result was graded as either negative or positive. The specimen was considered to positive when >5 % of the tissure component was unequivocally immunoreactive in the appropriate cellular compartment (Figure 3).

Histological examination The resected specimens were fixed in 10 % formaldehyde and sliced into 5 mm sections and stained with hematoxylin-eosin. The presence or absence of cancer was microscopically determined.

Statistical analysis

Statistical analysis was performed with STATISTICA for Windows. Differences between the results of two groups were tested with one-sided *t* test. A *P* value <0.05 was considered statistically significant.

RESULTS

Histological diagnosis

9 patients were diagnosed as pancreatic cancer complicated with chronic pancreatitis, including carcinoma of the pancreas head in 6 cases; carcinoma of the body and/or tail of pancreas in 2 cases, multifocal cancer with three foci in 1 case. The other 15 patients were confirmed to be chronic pancreatitis, including 13 cases with inflammatory mass in the head of pancreas, and two cases with adenoma in the body and/or tail of pancreas.

To compare with histological diagnosis, cytology offered a conclusive diagnosis in 5 of 24 cases. The cytological report was not conclusive in 19 cases (9 cases with suspicious cells and 8 cases with insufficient material, and two cases without non-malignant cell) (Figures 1,2).

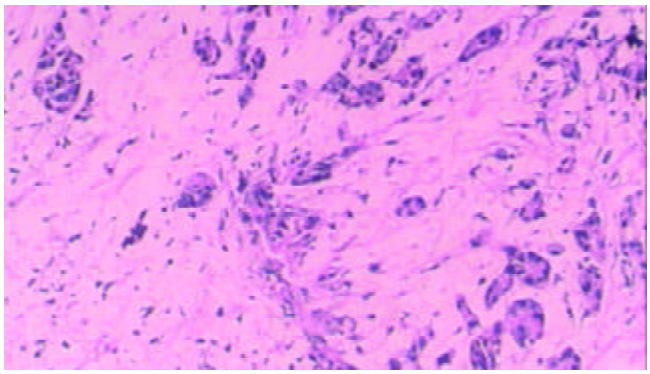

Figure 1 A little glandublar tissue and a large amount of fibrotic tissue.

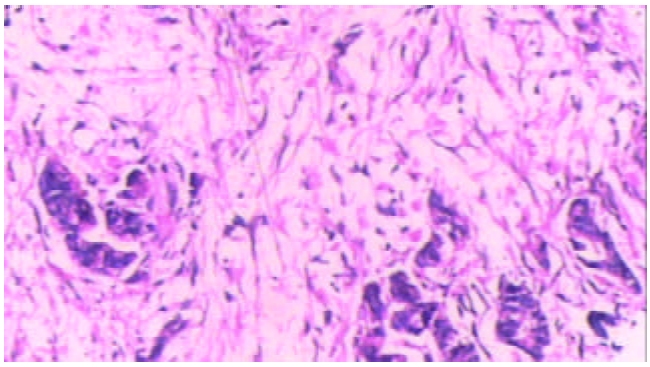

Figure 2 A few cancerous cells scattered in fibrotic gland tissue. (Papanicolaou staining, original magnification ×200).

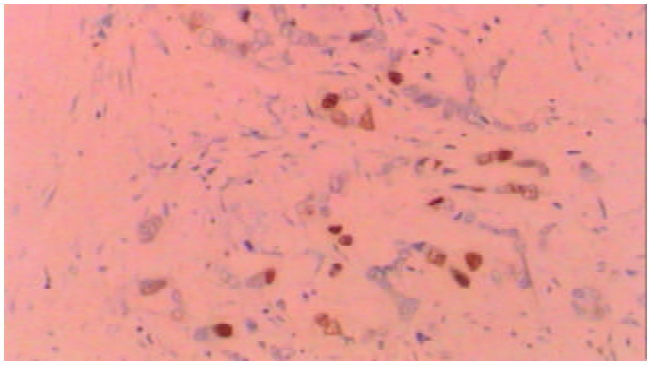

Figure 3 Yellow-brownly stained nuclei of p53 positive cells (ABC, original magnification ×400).

The presence of malignant cells in puncture samples was detected in 5 of 9 pancreatic cancer patients confirmed by histological examination. According to cytological diagnosis. The incidence of p53 protein overexpression and CA19.9 values ovre shown in Table 1.

Table 1 Patients with p53 protein overexpression and high serum CA19.9 with cytologically diagnosed pancreatic disease (*n*=24)

Histological diagnosis	Cytology				
	Patient *n*(%)	Malignant cells	Suspicious cells	Insufficient material	Non-malignant cells
Malignant diseases					
PC	9(37.5) p53(+)	2/5	1/1	1/1	0/2
	CA19.9≥37 U/ml	3/5	1/1	0/1	0/2
Benign disease					
IMH	13(54.2) p53(+)	0	0/5	0/4	0/4
	CA19.9≥37 U/ml	0	2/5	1/4	0/4
Adenoma	2(8.3)p53(+)	0	0/2	0	0
	CA19.9≥37 U/ml	0	0/2	0	0

PC: pancreatic cancer; IMH: inflammatory mass of the head of pancreas.

Table 2 Assay effectiveness in patients with pancreatic diseases (*n*=24)

Assay	Sensitivity TP/(TP+FN)	Specificity TN/(FP+TN)	Predictive value of positive test TP/(TP+FP)	Efficiency (TP+TN)/Total
Cytology alone	5/(5+3)=0.63	10/(0+10)=1.00	5/(5+0)=1.00	(5+10)/24=0.63
CA19.9>37 U/ml	4/(4+5)=0.44	12/(3+12)=0.80	4/(4+3)=0.57	(4+12)/24=0.67
P53(+)	4/(4+5)=0.44	15/(0+15)=1.00	4/(4+0)=1.00	(4+15)/24=0.79
Cytology+CA19.9	6/(6+3)=0.67	12/(12+3)=0.80	6/(6+3)=0.67	(6+12)/24=0.67
Cytology+P53	7/(7+2)=0.78	15/(15+0)=1.00	7/(7+0)=1.00	(7+15)/24=0.92
Cytology+P53+CA19.9	7/(7+2)=0.78	12(3+12)=0.80	7(7+3)=0.70	(7+12)/24=0.79

TP: true-positive; FN: false-negative; FP: false-positive; TN: true-negative.

p53 protein overexpression (shown by immunohistochemical staining) was positive in 4 of 9 patients with pancreatic cancer (Figure 3), and negative in the remaining 20 specimens. The combination of p53 protein overexpression and cytology offered a78 % sensitivity and a 100 % specificity.

Using the cut-off value of 37 U/ml as the normal upper limit, CA19.9 measurement identified 4 of 9 pancreatic carcinomas (range=41-480 U/ml). Additionally, high concentrations were also detected in 3 cases of chronic pancreatitis with a mass (range=210-1 200 U/ml). CA19.9 positivity in the chronic pancreatitis patient was related to common bile duct obstruction. The combined CA 19.9 and cytological assay showed a sensitivity of 67 % and a specificity of 80 %.

Combination of cytological analysis, P53 protein overexpression and CA19.9

Since all positive cytologies carried a final diagnosis of carcinoma, the main diagnostic contribution of p53 and CA19. 9 in the pancreatic cancer group was at the time when the cytology was suspicious or not contributory. Either test contributed to the final diagnosis of pancreatic adenocarcinoma in 4 out of 9 cases: in one case both were positive, in another case p53 protein showed overexpression but CA19.9 value was lower than 37 U/ml. None of the patients with chronic pancreatitis was positive for both markers. The sensitivity of the combined approach was 78 % with a specificity of 80 %. Both the sensitivity and efficiency of the combined approach (cytology, CA19.9, and p53 protein overexpression) were significantly higher than that of either test alone (P<0.01). Both the sensitivity and efficiency of combination of cytology and p53 were higher than that of combination of cytology, p53 and CA19.9 (P<0.05). Finally both the sensitivity and efficiency of the combined approach of cytology, p53 and CA19.9 were better than that of combination of cytology and CA19.9 (P<0.05) (Table 2).

DISCUSSION

Much effort has been devoted to achieve an optimum standard for conventional imaging procedures to increase the sensitivity and specificity of these diagnostic tools. Among which CT, ERCP are the mainstay for pancreatic cancer detection[5,6]. However, these morphologically oriented procedures, have an unsolved drawback ie, the inability to differentiate tumour tissue from pancreatic masses caused by chronic pancreatitis. Therefore, this has limited their application in patients with a suspected resectable mass for the decision of surgical exploration. FNA biopsy of the primary tumor has a significantly false negative rate due to the inflammatory response around the tumor, which accounts for the lower sensitivity[3]. According to a 1986 review, the average sensitivity was approximately 80 %[7]. A more recent, large, single-institution series reported a sensitivity of 72.5 %[8]. FNA-cytological distinction between chronic pancreatitis and pancreatic cancer is occasionally difficult because chronic pancreatitis can induce morphological changes similar to those seen in well differentiated adenocarcinoma[9]. This explains the equivocal results found in two kinds of pancreatic diseases, which can not be discriminated by clinical and imaging tests. In addition, the accuracy of cytology examination also depends on the quality and number of cells. The reported sensitivities of ERCP aspiration cytology, brush cytology, FNA biopsies, and forceps biopsies were in the range of 22 % to 71 %, and the use of multimethod samplings increased the sensitivity to 50-78 %[10]. Even with endoscopic ultrasonography-guided FNA biopsy, there were also false negative results[11]. Sometimes the accuracy of cytology might be damaged by poor staining or inadequate fixation[12]. The distinction between chronic pancreatitis and well-differentiated adenocarcinoma is difficult and it remains to find whether artificial intelligence algorithms can prove themselves useful in the cytologically differential diagnosis of carcinoma and chronic pancreatitis[9].

The greatest usefulness of carbohydrate antigen CA19.9 is its performance in detecting pancreatic cancer using a cutoff upper limit of 37 U/ml[13-16]. However, it can be confusing to interpret elevated concentrations of CA19.9, because the elevated concentration of CA19.9 used for the diagnosis of pancreatic cancer can also be seen in benign conditions such as pancreatitis[17-19], and conversely CA19.9 may be low in malignant conditions[20]. As shown in Table 1 and Table 2, the application of CA19-9 for differentiating cancer from chronic pancreatitis was disappointing in our study because of its low sensitivity and specificity, which were only 44 % and 80 %, similar to those reported by Okaga *et al*[21]. This may be explained by the elevation of CA19.9 in benign inflammatory conditions as well as in malignant diseases. According to Ker *et al*[22] CA19.9 is synthesized by normal biliary ductal cells. In benign biliary obstruction, epithelial cells will proliferate, and as a result, more CA19.9 may be secreted and leaks out into the bloodstream. The other mechanism concerning the false serum elevation of tumour markers in chronic pancreatitis has been shown to be a disturbed antigen polarity[23].

p53 gene abnormalities are considered to play an important role in the carcinogenesis of pancreatic cancer present in almost half of pancreatic cancer, but uncommon in chronic pancreatitis[24-26].

Studies on p53 abnormalities generally look for overexpression or persistence of the p53 protein, or for mutations in the genic sequence. Mutations of p53 gene lead to an accumulation of p53 protein reaching detectable concentrations by immunohistochemistry. In contrast, production of the wild type p53 gene is undetectable because of its short half-life. Thus there seems to be a good correlation between the overexpression of p53 protein and p53 gene mutations[27]. The p53 protein concentrations are correlated with percentage of p53 gene alterations[28,29]. In 40-60 % of pancreatic carcinomas, mutations of p53 gene and the increased accumulation of p53 protein have been shown by both direct sequencing and immunohistochemistry[30,31]. Both methods require tissue specimens. In comparison with cytology the, biggest advantage of gene analysis is no need to search for integrity or large number of cells. This method is sensitive enough to detect 3-30 mutant copies in the presence of 300 000 normal copies of the gene (which would be the equivalent to 0.01 ng of mutant DNA in 1 mcg of total DNA)[32]. In our series we found p53 protein overexpression in suspicious cells and insufficient materials.

In the current study, we analyzed the relative and combined contributions of the detection of p53 protein and CA19.9 concentration to the cytological diagnosis of pancreatic cancer when there was a clinical suspicion of pancreatic cancer corroborated by imaging diagnostic technique. In FNA samples, overexpressions were detected in 44 % of carcinoma. Fortunately no false-positives were detected even in the subset of chronic pancreatitis patients with a mass. Using a cut-off upper limit of 37 U/ml, the high CA19.9 concentrations were not strongly suggestive of pancreatic cancer. Although CA19. 9 is superior to any single markers elevated, it is not suitable for determining the nature of a pancreatic mass in patients with chronic pancreatitis. Similar results were also reported by other authors[33].

The combination of cytology and p53 offered the best diagnostic procedure. In four out of 9 patients with PC, the p53 protein overexpression contributed much to supporting the cytological diagnosis. Therefore, p53 protein overexpressions in FNAs are specific for pancreatic cancer, but CA19.9 values are not. Interestingly, none of the patients with chronic pancreatitis was positive for both markers,

suggesting that the combination of cytology and p53 might be useful in distinguishing PC and CP.

In conlusion, p53 protein analysis enhanced the diagnostic sensitivity of cytological evaluation in chronic pancreatitis patients with clinical suspicion of pancreatic cancer, especially in those with inconclusive cytological results such as the presence of suspicious cells or insufficient cellular material. In this case, p53 protein overexpression analysis offered a highly specific test although it was rarely employed as a clinical decision-making process.The previous clinical evidence also indicated the diagnostic benefit provided by p53 and other molecular marker analysis[34, 35], with an accumulation of more patients in such a study, there will be growing facilities for differentiating PC from CP.

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