J Korean Med Sci 1999; 14: 643-7
ISSN 1011-8934

Diagnostic Criteria for Malignancy in Bile Cytology and Its Usefulness

Fifty three bile specimens from 42 patients were reviewed to assess the diagnostic role of the bile cytology and to define more reliable cytologic indicators of malignancy. Forty three bile specimens came from 34 patients with malignant biliary strictures and 10 bile specimens were from eight patients with benign conditions. There were no false positives. The diagnostic specificity of bile cytology was 100% while diagnostic sensitivity was 55.8%. Overall diagnostic accuracy was 64.2%. We identified four key criteria as cytologic indicators of malignancy among 20 variables by using multiple regression analysis: loss of honeycomb arrangement, hyperchromatism, increased N/C ratio, and coarse chromatin. When bile specimens with three or more of these four criteria are thought to represent malignancy, the sensitivity of diagnosis of malignancy was 65.2%, specificity was 90% and diagnostic accuracy was 69.8%.

Key Words: *Cytology; Bile; Biliary tract neoplasms; Patient selection*

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Received: 7 September 1998
Accepted: 9 July 1999

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INTRODUCTION

In the management of patients with obstructive jaundice, the cytologic examination of the bile is a necessary procedure to evaluate the cause of obstruction. Since the 1960s, bile has been obtained easily from the biliary tree through diagnostic procedures such as percutaneous transhepatic cholangiography (PTC) or endoscopic retrograde cholangiopancreatography (ERCP) (1). In keeping with this, the clinical usefulness and cytologic features of bile cytology have been described in many literatures (2-5). However, it is sometimes difficult to distinguish benign from malignancy because of reactive or degenerative changes in the exfoliated benign biliary epithelial cells. Especially, well-differentiated adenocarcinoma in the biliary system or pancreas causes frequent confusion with their benign counterparts. Although several cytologic features of malignancy in bile cytology has been described in the previous literatures (2, 3, 6), the cytologic hallmarks of malignancy have been rarely reported. In this study, we evaluated the sensitivity, specificity, overall accuracy of bile cytology and compared cytologic features of carcinoma cells with benign epithelial cells in the bile specimens to select diagnostic criteria for malignancy in bile cytology.

MATERIALS AND METHODS

Between 1996 and 1997, 53 bile specimens collected via bile duct aspiration during PTC or ERCP were obtained from 42 patients. All unsatisfactory bile samples for cytologic evaluation were excluded. Of the 53 bile specimens, 43 were obtained from 34 patients with malignant biliary strictures, of which the causes were revealed by operation. They consisted of 17 bile duct carcinomas, 13 pancreatic carcinomas and four ampulla of Vater carcinomas. The histologic types were all adenocarcinomas. The patients, 22 men and 12 women, ranged from 41 to 73 years old (average, 57). The remaining 10 bile specimens were obtained from eight patients with benign conditions. Five patients had biliary obstruction by biliary calculi, which were removed by operation. In one patient with malignant lymphoma, the obstructive jaundice was caused by extrinsic compression by enlarged lymph nodes around biliary tree, which were revealed via radiologic-clinical correlation. The remaining two patients were biopsied. One patient had mucinous duct ectasia and one patient had hyperplastic polyp in the ampulla of Vater. They, six men and two women, ranged from 40 to 82 years old (average, 58).

Following centrifugation of the bile samples, the sed-

iment was smeared on several slides, immediately fixed in 95% ethanol and stained using the hematoxylin and eosin and the Papanicolaou technique. Twenty atypical cytologic features were compared in the carcinoma and benign groups. The loss of honeycomb arrangement denoted the loss of an orderly, sheet-like structure of cells, devoid of nuclear piling up. Variation in nuclear size were designated when cells having a nucleus more than double the size of others were detected in the same cluster. The incidence of each cytologic indicators of malignancy were evaluated for each patient with benign and malignant conditions. Statistical analysis for the selected parameters was performed with the multiple regression analysis.

RESULTS

The diagnostic correlation in benign and malignant conditions is shown in Table 1. In the 10 bile specimens from the benign conditions, the results were all negatives. In the 43 bile specimens collected from patients with malignant conditions, 19 samples were interpretated as benign, one sample as atypia, three samples as suspicious for malignancy and 20 samples as malignancy. For statistical analysis, one atypical and three suspicious smears were included in the malignant group, making a total of 24 positive versus 19 negative specimens.

There were no false positives. The diagnostic sensitivity of bile cytology was 55.8%, specificity 100%, and overall accuracy 64.2%. The incidence of various cytologic features and their diagnostic sensitivity and specificity are shown in Table 2 (Fig. 1-4).

By using multiple regression analysis, four cytologic features were shown to be most significant in relation to malignancy: loss of honeycomb arragement, hyperchromatism, increased N/C ratio and coarse chromatin.

Table 1. Diagnostic correlation of bile cytology in benign and malignant conditions

Cause of obstruction	Cytologic diagnosis				Total
	Benign	Atypia	Suspicious	Malignant	
Benign	10				10
Malignant	19	1	3	20	43

Table 2. Comparison of cytologic features in carcinoma and benign group

Feature	Carcinoma (n=43)	Benign (n=10)	Sensitivity (%)	Specificity (%)
Background				
Necrotic	14	1	32.6	90
Bloody	7	1	16.3	90
Bile pigment	6	3	14.0	70
Presentation				
Papillary structure	13	2	30.2	80
Loss of honeycomb pattern	23	3	53.5	70
Isolated single cells	12	1	27.9	90
Loosely cohesive	9	3	20.9	70
Acinar arrangement	7	1	16.3	90
Nuclear overlapping	12	2	27.9	80
Cell-in-cell arrangement	9	1	20.9	90
Cytoplasm				
Vacuole	9	3	20.9	70
Nuclei				
Irregular shaped	18	2	41.9	80
Variation in size	17	3	39.5	70
Hyperchromatism	22	3	51.2	70
Increased N/C ratio	22	2	51.2	80
Nucleoli				
Prominent	8	2	18.6	80
Multiple	4	0	9.3	100
Chromatin				
Fine	10	8	23.3	20
Clearing and clumping	14	1	32.6	90
Coarse	19	1	44.2	90

Fig. 1. Bile showing sheets of benign ductal cells with honeycomb pattern (Papanicolaou stain, ×400).

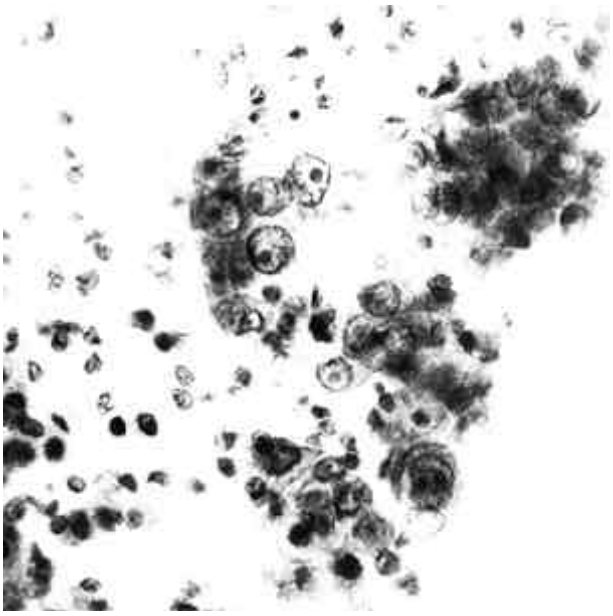

Fig. 2. Bile showing clusters of malignant cells with loss of honeycomb arrangement, enlarged nuclei, increased N/C ratio, prominent nucleoli and cell-in-cell arrangement (H&E stain, ×400).

Loss of honeycomb arrangement were seen in 53.5% of the carcinoma group and 30% of the benign group. Hyperchromatism, increased N/C ratio and coarse chromatin were detected in 51.2%, 51.2%, 44.2% of the carcinoma group and 30%, 20%, 10% of the benign group. Table 3 lists the probabilities of carcinoma combined with the four variables. Twenty eight of 43 specimens in the carcinoma group had three or four cytologic criteria.

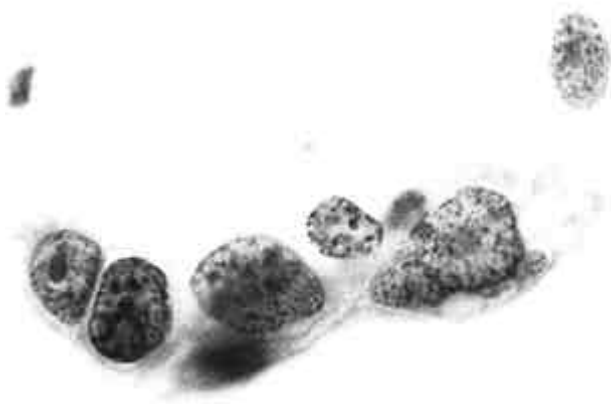

Fig. 3. Bile showing malignant cells with prominent nucleoli and coarse chromatin (Papanicolaou stain, ×1,000).

Fig. 4. Bile showing dyscohesive clusters of pleomorphic malignant cells with increased N/C ratio and hyperchromatic nuclei in the necrotic background (H&E stain, ×400).

By using these criteria, the overall sensitivity of diagnosis of malignancy was 65.2%, the specificity was 90% and diagnostic accuracy was 69.8%.

DISCUSSION

In early days, bile for diagnostic cytology was obtained by duodenal aspiration. Bile collected in this way was

Table 3. Incidence of key criteria in carcinoma and benign groups

No. of criteria	Carcinoma (n=43)	Benign (n=10)	Probability of carcinoma (%)
4	13	0	100
3	15	1	93.8
2	7	1	87.5
1	6	4	60
0	2	4	33.3

Key criteria: Loss of honeycomb arrangement, hyperchromatism, increased N/C ratio, coarse chromatin

almost always contaminated with gastroduodenal and pancreatic juices, which caused degenerative changes in exfoliated epithelial cells, making cytologic interpretation difficult (7-9). As a result, bile cytology did not seem to be practical enough for routine use. With advances in fiberoptics such as PTC and ERCP since 1960, false positive results has been markedly decreased because bile samples were collected directly from the biliary tree (7-9). In our study, there were no false positives. In contrast, there were high false negatives. This means that a negative report is not as useful as a positive diagnosis. A positive diagnosis could avoid an exploratory laparotomy in advanced malignant disease and in high-risk cases (9).

There are several reasons for false negatives: 1) degeneration or autolysis of the cells, in part caused by enzymatic digestion of tumor cells or improper handling; 2) submucosal tumor growth without significant intraductal exfoliation; 3) site of bile collection too proximal to the location of the tumor; 4) fiberoptic stenosis of the pancreatic or bile duct, preventing access to exfoliation; and 5) technical problem or improper collection (3, 10-13). Although there were no false positives in our study, there are still difficulties in diagnosing bile cytology because reactive or degenerative changes in exfoliated benign biliary epithelial cells often appear to be abnormal. Furthermore, in well-differentiated adenocarcinoma of the biliary tract and pancreas, cytologic distinction between benign versus malignancy is extremely difficult.

In previous literatures, a number of cytologic features such as necrotic and/or bloody background, bile pigment, papillary structure, loss of honeycomb arrangement, acinar arrangement, isolated single cells, enlarged nuclei, hyperchromasia, irregular shaped nuclei, increased N/C ratio, cytoplasmic vacuole, cell-in-cell arrangement etc. have been reported to be associated with malignancy (3, 6, 11, 14-16). However, cytologic hallmarks of malignancy has been rarely reported. Nakajima et al. (17) reported six key criteria as useful indicators of malignancy: loss of honeycomb arrangement, enlarged nuclei, loss of polarity, bloody background, flat nuclei and cell-in-cell arrangement. They noted that three or more of these criteria were often observed in carcinoma cases. By using these criteria, the overall sensitivity of diagnosis of malignancy by bile cytology (86.4%) compared favorably with that in other major studies [46.2% (3) and 48.0% (6)] in which bile was collected directly from the biliary tract. This difference may be partially related to the lack of key cytologic criteria for cytodiagnosis.

In this study, we identified four cytologic indicators of malignancy via multiple regression analysis: loss of honeycomb arrangement, hyperchromatism, increased N/C ratio and coarse chromatin. By using these criteria, the overall diagnostic sensitivity of malignancy was 65.2%, specificity was 90% and we concluded that the presence of three or more of four cytologic indicators could indicate biliary malignancy.

In summary, we have illustrated the usefulness of bile cytology in the diagnosis of biliary obstruction through sensitivity, specificity and diagnostic accuracy. The diagnostic specificity of bile cytology was 100%, diagnostic sensitivity was 55.8% and diagnosic accuracy was 64.2%. In addition, we identified four key criteria for separating malignancy from benign biliary stenosis in bile cytology by using multiple regression analysis.

We can conclude that recognition of key criteria in diagnosing bile cytology is very important in differential diagnosis between benign and malignancy cases. When carefully collected and promptly processed, bile cytology can be a valuable adjunct to other diagnostic procedure in the detection of carcinoma causing biliary tract obstruction.

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