A Comparison of various Endoscopic Cytological Techniques in the Diagnosis of Periampullary Carcinomas

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ABSTRACT

Background/objective: Cytology is a valuable adjunct to histology in the diagnosis of periampullary carcinoma (PCA). Aim of this study was to perform a comparative evaluation of various endoscopic cytological techniques in addition to forceps biopsy (FB) in the diagnosis of PCA.

Methods: A total of 120 specimens were obtained from twenty cases of suspected PCAs', using five different endoscopic cytological techniques [Brushing before biopsy (BB), Brushing after biopsy (BA), Fine needle aspiration cytology (FNAC), Suction cytology (SC) and Bile cytology (BC)], in addition to FB. All samples were obtained in a single sitting after performing endoscopic sphincterotomy.

Results: All patients turned out to be positive for malignancy. The diagnostic accuracy of BB, BA, FNAC, SC, BC and FB for diagnosis of PCA was 50%, 65%, 60%, 35%, 35% and 95% respectively. When any cytological technique was combined with FB, 100% diagnostic accuracy was obtained.

Conclusion: The diagnostic accuracy of various cytological techniques for the diagnosis of PCA varies from 35-65%. BB offers the maximum diagnostic accuracy of 65%, and when combined with FB, offers very high (up to 100%) chances of diagnosing suspected PCA (J Dig Endosc 201;2(3):172-77)

Keywords: Periampullary carcinoma - Cytological techniques - Endoscopy

Introduction

Periampullary carcinomas (PCAs’) include all neoplasms arising at or within 1 cm of the ampulla of Vater. It includes cancer of the ampulla, cancers arising from the head of pancreas, second part of duodenum or the distal segment of the common bile duct. The diagnosis of PCAs’ has important implications for the management and prognosis of patients. Currently, none of the serological or biochemical markers can be relied upon to make or exclude the diagnosis of PCA. In addition, non-invasive imaging can miss small focal lesions, though larger unresectable lesions are easily seen. The ultimate diagnosis of malignancy is based on positive histopathological or cytological criteria[1].

Endoscopic retrograde cholangiopancreatography (ERCP) is indicated when obstruction at the level of ampulla is suspected on the basis of dilated bile duct seen at non-invasive imaging. The objective of early ERCP in symptom-atic patient is to identify malignancy, when present. This can be facilitated by various histological and cytological techniques. Cytology is a valuable adjunct to forceps biopsy (FB), the combined yield of two being superior to the yield of either individual technique[1]. The various endoscopic cytological techniques available are brushing before biopsy (BB), brushing after biopsy (BA), fine needle aspiration cytology (FNAC), suction cytology (SC) and bile cytology (BC). However, all of these techniques cannot be performed...
routinely in all patients in a clinical setting, and the best technique to be used as an adjunct to FB needs to be established. To the best of our knowledge, no single study has compared all these cytological techniques with FB in a given set of patients. Therefore, this study was planned to compare the utility of various cytological techniques in the diagnosis of PCAs’.

Material and Methods

Patients

Twenty consecutive cases with obstructive jaundice due to suspected PCAs’ presenting to medical or surgical OPD or admitted in wards of Dayanand Medical College & Hospital, Ludhiana, India were enrolled. Written informed consent was taken and study was approved by ethics committee.

ERCP and preparation for the procedure

Patients were kept fasting overnight. Premedication [injection midazolam (2-5 mg) and injection hyoscine (20-40mg) i.v.] and antibiotic prophylaxis were given before ERCP. Water-soluble contrast medium (Conray 76%), diluted with normal saline (60:40) was used for visualization of biliary tree. After visualizing the periampullary area, sphincterotomy was performed and 6 samples were taken from each patient in a single setting in the following order: BB, FB, BA, FNAC, SC and BC. Thus, cumulative of 120 samples were taken.

Methods used for sampling

Brushing for cytological evaluation was obtained before and after FB. Lesions were brushed with disposable nylon brush and combo cath brush. Each brushing was smeared on 3-4 clean glass slides. FB was performed using endoscopic biopsy forceps 24U. FNAC was done by using aspiration biopsy catheter assembly based on modified injection sclerotherapy equipment. It consisted of two polyvinyl chloride catheters with internal diameter of 1.0 mm (Tube A) and 1.7mm (Tube B). After visualizing a lesion endoscopically, catheter assembly was passed through the biopsy channel of the ERCP scope. The inner tubing was slid forward so that approximately 6 mm of the needle projected out of the sheath. With in-and-out movements of the catheter assembly, the needle tip was pushed into the lesion, while suction was applied with the syringe. The assembly was withdrawn after retracting tube A into tube B. The aspirated material was flushed onto glass slides. Samples for SC were taken using a suction catheter made of polyvinyl chloride. After taking aspiration BC samples, the suction catheter was passed through the biopsy channel of the endoscope and its tip was abutted against the target area from which the biopsies were taken. Suction was then applied for 15-30 seconds, using 20 ml syringe connected to the hub of the catheter. The tip of suction catheter was moved to cover as much area of the lesion as possible. After discontinuing suction, catheter was withdrawn and the aspirated material flushed on to glass slides.

Preparation and staining of the smears

Aspirates from all the above techniques were smeared on 3 to 4 clean glass slides soon after removal from the endoscope, dried and stained with May-Graunwald-Giemsa stain and hematoxylin eosin stain (H&E) for microscopic examination. The biopsy samples were processed in the standard manner. The smears were examined and findings recorded as:

a. Positive cytology: presence of frank/unequivocal malignant cells
b. Suspicious cytology: atypical cells, suspicious but not confirmatory, for malignancy
c. Negative cytology: unequivocally negative, or atypical cells consistent with an inflammatory or reparative process, or cases in which a diagnosis could not be made because of inadequate material

These smears were further categorized into squamous cell carcinoma and adenocarcinoma depending upon the cytological features wherever possible. The results of the histopathology were compared to the various cytological reports. The diagnostic accuracy (effectiveness) for each technique was calculated. Diagnostic accuracy was calculated as a proportion of correctly classified subjects (True Positive + True Negative) among all subjects (True Positive + True Negative + False Positive + False Negative).

Results

A total of 120 cytological/histopathological specimens from 20 patients with suspected PCAs’ were obtained during ERCP using different techniques. The median age of patients was 60 years (range 45-86), and the male: female ratio was 13:7. All 20 cases turned out to be positive for malignancy. The endoscopic appearances of the lesions and the results of various cytological techniques and histology with their diagnostic accuracy for malignancy are shown in Table 1. The diagnostic accuracy of different techniques in various combinations is shown in Table 2, and the correlation between endoscopic appearance of tumor and results of cytological techniques is shown in Table 3.

Of the different cytological techniques, BA had the highest diagnostic accuracy (65%), and both SC and BC had the lowest diagnostic accuracy (35% each) for diagnosing PCAs’. The design of the study provided for reporting benign lesions but none of the cases studied turned out to be benign or negative. Because of the accidental lack of true negative, false negative and false positive cases in this study, specificity and sensitivity could not be calculated.

FB was positive for malignancy in 19 out of 20 cases. In
one case, the biopsy specimen was inadequate. In this patient, the biopsy was not repeated, as this case was positive for malignancy by all other cytological techniques. The diagnostic accuracy of FB alone was 95%, and when used in combination with any of the cytological techniques was 100%.

BB was positive in 10 out of 20 cases. Two negative cases had scanty material. Brushing was not repeated in them as histology was positive in these cases. The diagnostic accuracy of BB was 50%. BA was positive for malignancy in 13 out of 20 cases (Figure 1). All the negative cases had very scanty material. In these cases, ERCP was not repeated as all of these were positive on histology. The diagnostic accuracy of BA was 65%. When the two brushing techniques were taken together, the overall accuracy was 80%. On combining any of the brush techniques with FB, a 100% diagnostic accuracy was achieved.

FNAC was positive for malignancy in 12 out of 20 cases (Figure 2). In three negative cases, the material was inadequate. However, repeat FNAC was not done in them. Diagnostic accuracy of FNAC alone was 60%, and when combined with BB and BA, the accuracy was increased to 65% and 85% respectively. FNAC, in combination with FB, yielded a 100% diagnostic accuracy.

SC was positive in 7 out of 20 cases. The diagnostic accuracy of SC alone was 35% only. When combined with BB or BA, SC yielded a diagnostic accuracy of 60% and 70% respectively. In combination with FB, the diagnostic accuracy increased to 100%.

BC was positive in seven cases (Figure 3), with a diagnostic accuracy of 35%. All cases positive by SC were also positive by BC. BC, when combined with BA or FB, yielded a diagnostic accuracy of 85% and 100% respectively.

Discussion

ERCP is an important tool for the evaluation, diagnosis and management of lesions of the periampullary region. Though relatively simple, tissue sampling techniques at ERCP have generally returned disappointing results with low sensitivity. The most commonly used technique is BC, which has a sensitivity in the range of 30–69%[2-3]. The diagnostic yield increases with an increase in the number of tissue sampling techniques combined in any particular
patient, with a sensitivity ranging from 52%–77%, depending on the clinical interpretation of reported cytological atypia[4]. This prospective study assessed the usefulness of 5 cytological modalities, that is BB, BA, FNAC, SC and BC along with FB in the diagnosis of PCAs’.

FB during ERCP is a safe, simple and convenient technique, and is commonly used as an initial method for diagnosis of suspected PCAs’. In a study by Kubota et al, of the 43 patients in whom FB was done from stricture, it was successful in 41 patients (95.3%)[5]. All specimens were adequate, no complications were seen and no false positive results were seen. In our study, results of FB were positive in 95% patients and false negative in 5% due to scanty specimen.

Biliary brushing of suspected lesions has produced encouraging results. Osnes et al was first to report about cytological sampling from the biliary tree using a brush[6]. It has been demonstrated that direct brushing of strictures is superior to simple aspiration of bile; the latter confirms the diagnosis of malignant stricture in only 10% to 32% of cases[7]. Studies using biliary brushing have reported highly variable results with sensitivity rates ranging from 30% to 69%[6]. In a study by Foutch et al using brush cytology for diagnosis of malignant biliary obstruction in 39 patients reported a sensitivity of 54%, specificity of 100% and an overall accuracy of 72%[8]. Ferrari et al used brush cytology for diagnosis of 55 bile duct and 19 pancreatic duct strictures and reported a sensitivity of 56.2%, specificity of 100% and an accuracy of 70%[9]. Stewart et al reported that brush cytology identified 147 of 246 (59.8%) neoplasms in a series of 406 consecutive patients evaluated over a 6.5 year period[10]. Singh et al studied 30 patients with biliary obstruction caused by carcinoma gallbladder (n=16), carcinoma head of pancreas (n=10), or cholangiocarcinoma(n=4). Brush cytology was positive for malignancy in 8 cases (26.7%)[11]. In our study, the diagnostic accuracy of BB was 50% and of BA was 65%. When both were combined, the diagnostic accuracy rose to 80%. Although studies comparing FB with brush cytology favor the former technique, particularly for distal strictures, the largest comparative study by Schoeffl et al found that yields were similar for proximal biliary strictures, and brush cytology was superior to FB for intrahepatic lesions[12-13]. The combination of biopsy and brushing resulted in an overall sensitivity rate of 70% compared with 65% for FB alone and 47% for brush cytology alone. In our study, both BB and BA, when combined with FB, showed 100% diagnostic accuracy.

FNAC is an effective means of diagnosing many malignant tumors. The main attraction of endobiliary FNAC is in its potential in diagnosing lesions that produce extrinsic compression of biliary tree for which standard diagnostic methods have failed. Cope et al obtained a diagnosis in 80% of pancreatic cancers and 60% of bile duct strictures using a 21-22G 4mm Chiba needle[14]. No complications were observed. In the present study, a similar diagnostic accuracy of 60% was obtained with FNAC.

SC has been performed on epithelial cells exfoliated into biliary tract and aspirated from the duodenum. Harell et
al reported an overall sensitivity of 47% using specimens from 15 patients with neoplastic strictures[15]. They stressed on the need for aspirating large volumes of bile and sending bile promptly to cytopathologist, as cell morphology may deteriorate after an hour. Muro et al collected 10cc of bile cytology samples in 100 patients[16]. They concluded that sensitivity of cytology sampling could be maximized by transversing the strictured bile duct and keeping the site of sampling closer to the area of stricture. Mohandas et al studied BC in 64 patients with malignant biliary strictures[17]. In control group, bile was aspirated without dilating the biliary stricture, while in the other group, bile was aspirated after dilating bile stricture. BC was positive for malignancy in 26.6% and 63.3% patients in the control and dilated group respectively. Cytology was positive in 73% of gall bladder cancers, 62.5% of bile duct cancers, 40% of pancreatic cancers and 60% of metastasized cancers after dilation. Suggested reasons for high false negative rate on exfoliative cytology of PCAs include the enzymatic digestion of cells, pancreatic duct stenosis and intramural or extramural tumor growth. Another study reported a sensitivity of 55.8% using BC in the diagnosis of biliary obstruction[18]. Uchida et al showed that repeated cytological samplings using bile obtained via an endoscopic nasobiliary drainag tube for diagnosis of malignant biliary stricture increased sensitivity to 72.4%[19]. In our series, the diagnostic accuracy of SC and BC taken alone was 35%, which is quite low as compared to that of other cytological techniques. The reasons for these could be that large amount of bile could not be aspirated and repeat bile aspiration was not done.

Several studies compared bile aspiration to brush cytology and/or FB. These studies showed that BC alone was inferior in sensitivity to brushing (26% vs. 69%) and to FB (32% vs. 88%)[20-21]. Chang et al reported in a series of 18 patients that a combination of FB, BC and BR did not show superior results to FB alone (sensitivity 64%)[22]. Similar results were obtained by Pugliese et al. who achieved only a small gain in sensitivity when combining both the techniques (61% for BC plus FB versus 53% for each alone)[23]. In our study, BC and SC had lower diagnostic accuracy (35%) than BB, BA or FNAC (50%, 65%, 60% respectively). However, when any of these techniques were combined with FB, 100% diagnostic accuracy was obtained.

Stent cytology is another endoscopic technique which has been applied by many workers. Foutch et al obtained a cumulative of 78 specimens from 30 patients with bile duct strictures[24]. Overall sensitivity was highest for stent (36%) and brush cytology (33%), compared with results obtained from BC (6%). Overall, 47% of patients with cancer could be diagnosed by one or more cytological techniques. Desa et al studied 117 patients with extrahepatic biliary stricture using FNAC, brushings or BC[25]. A final diagnosis of cholangiocarcinoma was made in 88 patients, and 29 patients had benign strictures. Intraoperative cytology was more sensitive than non-operative examination (80% vs 42%). Overall sensitivity of FNAC (67%) was greater than that of brush (50%) and exfoliative cytology (30%). No complications were encountered.

The limitation of our study is that the number of patients was small. However, we obtained samples by 6 different methods in each patient and a total of 120 specimens were studied. To the best of our knowledge, this is the only study comparing 6 different sampling techniques for the diagnosis of PCA.

**Conclusion**

The positive yield of various cytological techniques alone for the diagnosis of PCAs’ varies from 35-65%. BA alone offers the maximum yield of 65%, and when combined with FB, offers a very high (up to 100%) probability of identifying malignancy in peripancreatic region. All cytology techniques are safe and can be performed simultaneously with an ERCP scope.

**References**

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