

Research Article

The role of brush cytology in diagnosing lesions of biliary tract

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Received: 18 August 2016

Accepted: 10 September 2016

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ABSTRACT

Background: Biliary brushing during endoscopic retrograde cholangiography pancreatography (ERCP) helps in the diagnosis of lesions of biliary tract. We compared the cellular and diagnostic results of various samples taken from the stricture site with the help of brushings taken from stricture site. The aim of this study was to know the age and sex prevalence in subjects suffering from biliary tract lesions and to know the role of ERCP guided brush cytology smears in diagnosing various lesions of extrahepatic biliary tract.

Methods: It is a hospital based study conducted in the Department of Pathology, Andhra Medical College. Brush cytology smears were obtained from twenty patients and cytological examinations was conducted. All the clinical details and investigation reports were collected.

Results: The age group of patients in this study ranged from 30 to 75 years. The numbers of cases analyzed during this period were 20. The mean age of presentation was 55 years. Male: Female ratio was 3:2. Majority of these cases presented with obstructive jaundice. Brush cytology smears from distal bile duct showed malignant cells hence our study revealed distal bile duct is more vulnerable to malignancy. Among all the 20 cases, 12 cases (60%) were reported as cholangiocarcinoma and the remaining 8 cases (40%) were included under benign/reactive. Among benign/reactive lesions, one case showed Micro-filarial infestation with reactive atypia of cells.

Conclusions: The current study was conducted to assess the relative accuracy of brush cytology for diagnosis of malignant biliary strictures.

Keywords: Bile duct, Brush cytology, ERCP, Cholangiocarcinoma

INTRODUCTION

Osnes et al 1975 introduced the technique of ERCP (Endoscopic Retrograde Cholangio Pancreatography), guided brush cytology under direct endoscopic visualization with the help of specially designed brush. ERCP guided brushings is a safe diagnostic procedure for the evaluation of the biliary tract lesions as endoscopic biopsies are difficult to obtain from this area.

There is little morbidity associated with brushing of the bile duct.¹ National cancer institute; United States estimated an incidence of 0.54 cases per 100,000 populations. The Literature concerning cytology of the biliary system is minimal. There are only few articles that describe the cytopathological features of cells from

biliary tract. Carcinomas of extra-hepatic bile ducts are relatively uncommon. Bile duct carcinoma occurs in patients in 60-70years age group and somewhat more common in men than women. More than 90% of patients presented with symptoms of biliary obstruction i.e. jaundice. The sensitivity of brush cytology alone for the diagnosis of all malignant biliary strictures ranges from 33% to 58%.²⁻¹⁰

METHODS

This is a prospective study conducted in the Department of Pathology, Andhra Medical College for a period of 2years from January 2012 to January 2014. A total of twenty cases referred from Gastroenterology department were analyzed.

All the smears were obtained during ERCP. The most common indication for the procedure was obstructive jaundice with stenosis. Other indications are sclerosing cholangitis and vomittings. Among 20 cases a series of 11 consecutive patients presented with obstructive jaundice, with a mean age of 55 years (ranging 30 to 70 years).

Table 1: Age distribution of 20 cases with biliary tract lesions.

Age group (years)	Positive for malignant cells (n=12)	Negative for malignant cells (n=8)
21 - 30	1	3
31 - 40	NIL	2
41 - 50	5	2
51 -60	2	L
61 - 70	4	Nil

Table 2: Sex distribution of 20 cases with biliary tract lesions.

Gender	Positive for malignant cells (n=12)	Negative for malignant cells (n=8)
Male	8	4
Female	4	4

Laboratory values including liver transaminases, alkaline phosphatase, total and direct bilirubin were elevated (Table 3). CT scan and sonography had revealed biliary dilation in all patients. Other information included clinical appearance and course, the site of lesion, final diagnosis and treatment.¹¹ In all cases abnormal appearing biliary mucosa was brushed. Smears, averaging six slides per case, were prepared directly from the endoscopic brush. Two pathologists independently reported the cytology slides.

Table 3: Biochemical parameters of 20 cases with biliary tract lesions.

Investigation	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Serum Bilirubin mg/dl total	19.4	18.6	14.3	19.8	13.8	14.1	20.2	22.3	13.4	14.6
Direct	15	14	9.3	16	9.2	8.4	17.2	18.5	7.8	10.8
SGPT	1500	9700	1100	4500	96	112	4800	123	89	82
Alkaline phosphatase (IU/L)	345	243	223	248	182	166	234	180	174	161
Investigation	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Case 20
Serum Bilirubin mg/dl total	17.4	16.7	18.1	20.6	12.6	19.4	22.3	14.6	19.2	13.4
Direct	13	14	11.1	15	7.2	10.4	12.4	10.5	14.8	9.2
SGPT	1800	5400	1900	6500	89	2345	4500	540	1128	76
Alkaline phosphatase (IU/L)	264	290	242	268	174	231	286	186	312	146

Technique

Brush cytology smears are taken after passing a guide wire through the obstruction. The sheath of the cytology brush can be inserted over the guide wire through the stricture. The guide wire is then removed and the cytology brush inserted through the sheath.

The sheath is then pulled back to allow the brush to emerge into the dilated proximal system. The brush and catheter are then pulled back through the stricture. An X-ray is taken to document the position of the brush through the stricture. Care is taken to ensure that the guide wire tip remains above the obstruction. After the brush is pushed and pulled through the stricture several times the catheter sheath is advanced back into the proximal dilated

system, the brush is removed and the tip prepared for cytology.

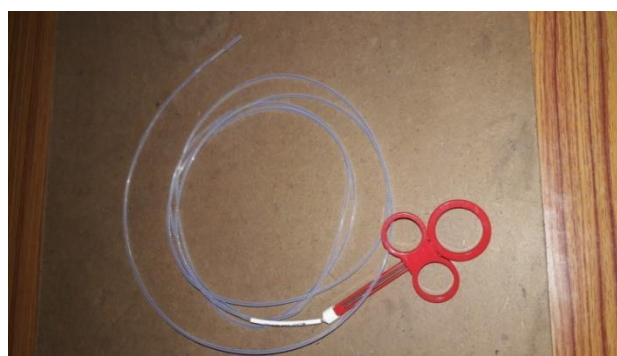


Figure 1: The brush used in the technique.

This set-up ensures access is maintained across the stricture by the guidewire for the ease of subsequent stenting or drainage and also avoids cell loss. When the brush is withdrawn the tip of the brush is cut off and saved in the cytology solution (Figure 1).

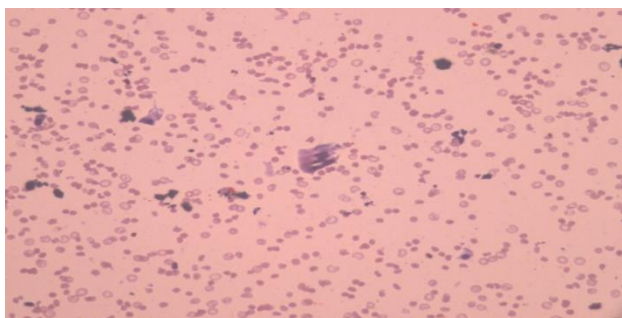


Figure 2: Normal bile duct epithelial cells-ciliated columnar epithelial cells (H&E, 40X).

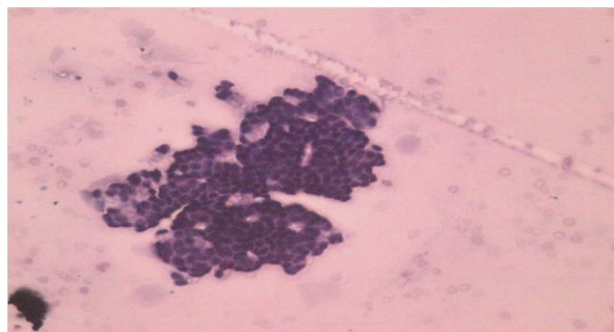


Figure 3: Monolayered sheets of benign ductal epithelial cells (100X, H&E).

The smears are immersed immediately in 50% alcohol fixative and routinely stained with H & E. The smears were correlated with clinical and ERCP features (Table 4).

Table 4: Clinical presentation, ERCP and smear findings in 20 cases with biliary tract lesions.

Investigation	Case 1	Case 2	Case3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Clinical presentation	Jaundice	Jaundice	AD	Jaundice	Vomiting	AD	Jaundice	Jaundice	AD	AD
ERCP findings	CCA	?MBS	?MBS	?MCA	DCCA	?MCC	HCCA	CCA	DCCA	?HCCA
Smear findings	+CCA	+CCA	-VE	+CCA	-VE	-VE	+VE	+CBD	-VE	-VE
Investigation	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16	Case 17	Case 18	Case 19	Case 20
Clinical presentation	Jaundice	Jaundice	Jaundice	Jaundice	AD	Jaundice	Fever	AD	Jaundice	AD
ERCP findings	HCCA	?HCCA	MCCA	?DCCA	?CCA	CCA	?HCCA	?HCCA	DCCA	?HCCA
Smear findings	+CCA	+HCCA	+CCA	+CCA	-VE	+CCA	+CCA	-VE	+CCA	-VE

AD-Abdominal discomfort , CCA-cholangiocarcinoma, MBS-Malignant biliary strictures; MCCA-Middlecholangiocarcinoma, DCCA-Distalcholangiocarcinoma,HCCA-Hilar cholangiocarcinoma; CBD-Carcinoma bile duct.

RESULTS

The smears are adequately cellular with good preservation, were basically categorized into benign/reactive and positive smears (for malignancy). The number of cases analyzed during this period was twenty. The mean age of presentation was 55 years (range 30-75 years) (Table 1).

Male: Female ratio was 3:2 (Table 2). Majority of these cases presented with obstructive jaundice. Cases with serum total bilirubin levels above 18 mg/dl were reported as positive smears for malignant cells (Table 3). Out of 20 cases ERCP showed 3 cases of proximal bile duct stricture, 4 cases of middle part bile duct stricture, 7 cases

of distal bile duct stricture and 6 cases of hilar region stricture.

Out of 7 cases, 5cases of brush cytology smears from distal bile duct showed malignant cells hence our study revealed distal bile duct is more vulnerable to malignancy (Table 5).

Among all the 20cases, 12 cases (60%) were reported as cholangiocarcinoma and the remaining 8 cases (40%) were included under benign lesions. Among the benign lesions one case showed Micro-filarial infestation with reactive atypia of cells. In the present study percentage of correlation between ERCP features and brush cytology smears was 60%.

Cytological features

Benign and reactive smears were composed of cohesive, monolayered sheets of well aligned, benign glandular epithelial cells and tiny tissue fragments (Figure 2). The cells showed 2 dimensional mosaic clusters of tall columnar cells (Figure 3). The individual cells had distinct cytoplasmic borders, abundant fine cytoplasm, round to oval regular nuclei and evenly dispersed fine chromatin. Reactive cells in clusters displayed similar features except for slight variability in nuclear size and inconspicuous nucleoli (Figure 4).

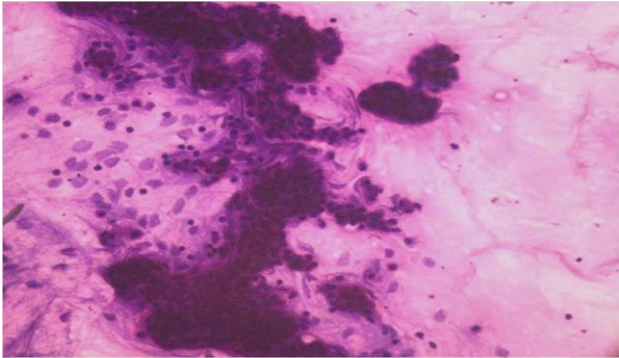


Figure 4: Reactive atypia of benign ductal epithelial cells (100X, H&E).

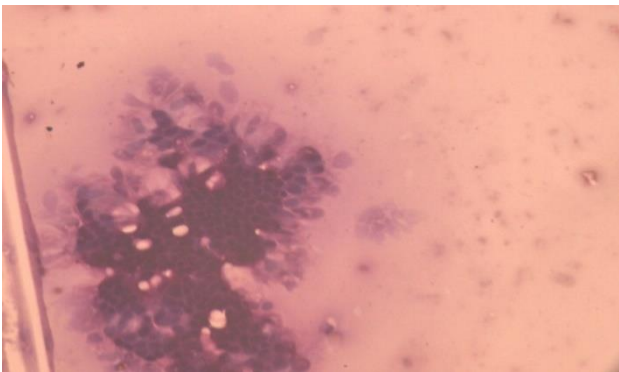


Figure 5: Three dimensional view of malignant cell clusters (100X, H&E).

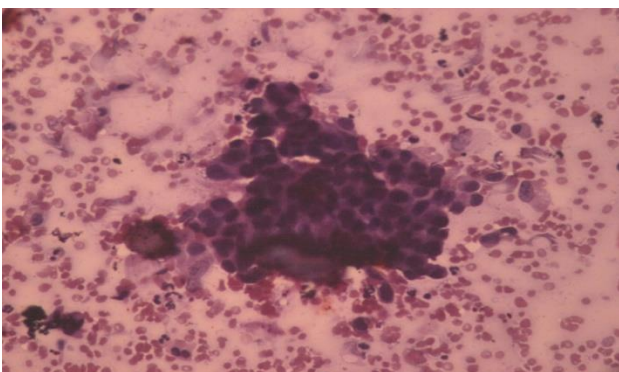


Figure 6: Malignant tumor cells arranged in balls and attempted glandular pattern (400X, H&E).

Smears positive for malignancy consistently displayed tightly cohesive, three dimensional cell clusters that formed balls (Figure 5). The cells in the clusters had enlarged nuclei with scant cytoplasm, irregular nuclear membrane and coarsely clumped chromatin with multiple nucleoli (Figure 6).

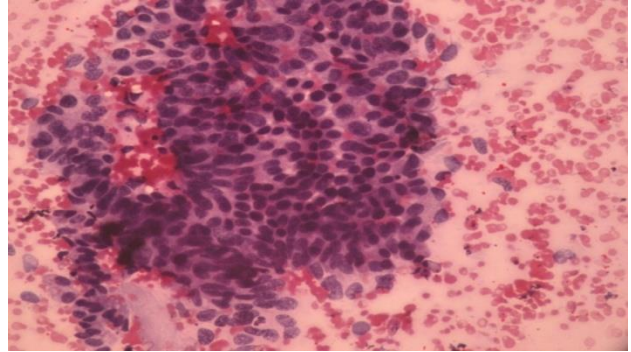


Figure 7: Malignant tumor cell clusters in the haemorrhagic background (400X, H&E).

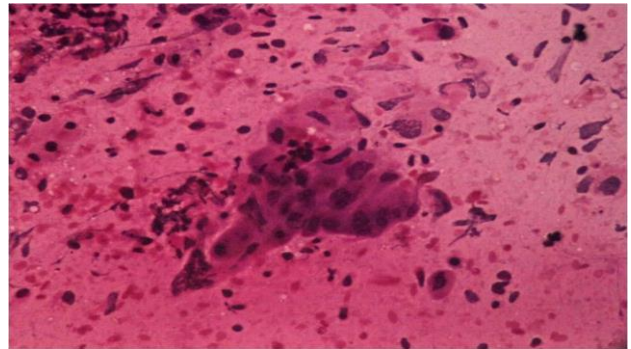


Figure 8: Malignant tumor cells with irregular nucleus arranged in clusters (400X, H&E).

Observations

We studied brush cytology smears in 20 patients with biliary obstruction. ERCP guided brush cytology was obtained from the stricturous segment during endoscopic retrograde cholangio-pancreatography with a mean age of 55 years (range 30-75 years) (Table 1).

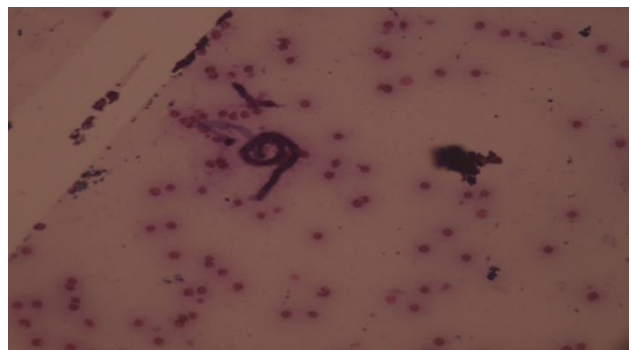


Figure 9: Microfilaria parasite with reactive epithelial cells in bile duct brushings (100X, H&E).

The study comprised of 20 patients out of which 12 (60%) are male patients and 8 (40%) are females. Brush cytology was positive for malignancy in 12 cases (60%) (Table 4). Another 8 cases (40%) are negative for malignancy. Laboratory values including liver transaminases, alkaline phosphatase, total and direct

bilirubin helps in assessment of the stricture's severity and chronicity as well as the etiology. In the present study the smears of patients with cholangiocarcinoma showed high total bilirubin levels ranging between 18-23mg/dl.

Table 5: Smear findings in various parts of bile duct in 20 cases with biliary tract lesions.

Site of CBD stricture	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Proximal	-	+Ve	-Ve	-	-	-	-	+Ve	-	-
Middle	-	-	-	+Ve	-Ve	-Ve	-	-	-	-
Distal	+Ve	-	-	-	-	-	+Ve	-	-Ve	-
Hilar	-	-	-	-	-	-	-	-	-	-Ve
Site Of CBD Stricture	Case11	Case12	Case13	Case14	Case15	Case16	Case17	Case18	Case19	Case20
Proximal	-	-	-	-	-	-	-	-	-	-
Middle	-	-	+Ve	-	-	-	-	-	-	-
Distal	-	-	-	+Ve	-Ve	+Ve	-	-	+Ve	-
Hilar	+Ve	+Ve	-	-	-	-	+Ve	-Ve	-	-Ve

Brush Smears from the distal CBD strictures shows more positivity of cholangiocarcinoma.

Alkaline phosphatase levels are elevated ranging between 180-345 IU/L. Case-2 showed very high level of SGPT (9700 IU/L) where smears showed positive for malignancy. Mild to moderate elevations of alkaline phosphatase, indicates impairment to bile flow due to intra or extrahepatic etiology. Elevations of transaminases imply either a hepatitis process or onset of obstruction. Total bilirubin values are indicative of complete obstruction with healthy liver, bilirubin is said to generally peak under 20 mg/dl, whereas values beyond this imply hepatocellular injury, with or without obstruction. In the present study 11-cases with bile duct strictures presented with obstructive jaundice, the remaining 7 cases presented with abdominal discomfort, 1 case with vomittings and 1 case with fever.

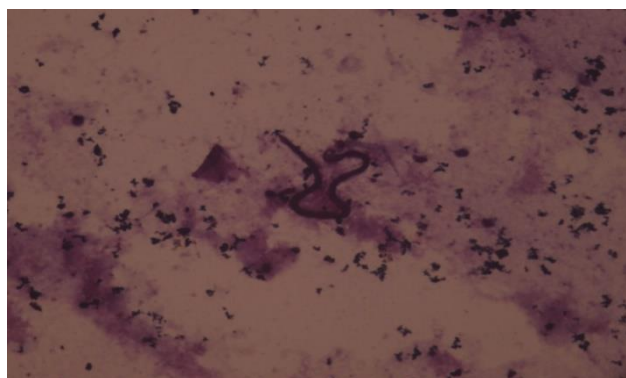


Figure 10: Microfilaria parasite with inflammatory cells in bile duct brushings (100X, H&E).

Out of 20 cases ERCP showed 3 cases of proximal bile duct stricture, 4 cases of middle part bile duct stricture, 7 cases of distal bile duct stricture and 6 cases of hilar region stricture. Out of 7 cases, 5 cases of brush cytology smears from distal bile duct showed malignant cells hence our study revealed distal bile duct is more vulnerable to malignancy (Table 5). Brush cytology was positive for malignant cells in 2 cases (10%) proximal part, 2 cases (10%) in middle part, 5 cases (25%) in distal part and 3 cases (15%) in common bile duct strictures (Table 6).

In other studies brush cytology yielded a sensitivity of 60% in the diagnosis of malignant biliary strictures [12]. In this study one case of micro-filarial infestation was reported in a patient with obstructive jaundice and distal bile duct stricture ,in this case the smears showed mild reactive atypia in the chronic inflammatory cell background (Figures 9 and 10).

In the present study smears from the middle part of bile duct (case 6) showed sheets of benign looking columnar cells in the mucoid background, which were included under benign lesion. The cells are mostly cohesive, with well-defined cell borders and polarity. They have small, centrally placed, round to oval, regular nuclei and abundant cytoplasm. The chromatin is fine and nucleoli inconspicuous (Figure 2 and 3). Smears in Case 3 showed benign looking columnar cells with mild nuclear atypia where the smears are reported as negative for malignant cells and here the site of stricture was proximal part of

bile duct (Figure 4). In the present study significant cytological features are noticed in case-14 and case-16 where the smears are highly cellular and the site of stricture was distal part of bile duct. The key cytological feature noted in positive smears in our series was small, three dimensional, tightly cohesive cell balls with considerable nuclear overlapping; these cells are round to oval shape with moderate nuclear pleomorphism and hyperchromatic nuclei arranged in acinar and papillary pattern (Figure 7) in the haemorrhagic background.

Table 6: Comparison of present study with other studies.

Year	Authors	No. of cases	Cases of carcinoma	Cytological specimens	Sensitivity
1993	Kurzwinski et al ¹⁹	42	Bile duct	Bile only	33%
2002	Hemagovil et al ²⁶	278	Bile duct	Brushing	68%
2003	Sachdev et al ¹³	58	Bile duct	Brushing	48.2%
2012	Present study	20	Bile duct	Brushing	60%

Cholangio carcinomas may arise anywhere between the papilla of Vater and the small branches of the bile ducts within the liver, but they originate most often from the large hilar bile ducts at the bifurcation of the common hepatic duct or from the extra hepatic bile ducts. Intrahepatic cholangiocarcinoma is much less common than hepatocellular carcinoma. Cholangiocarcinoma is known to follow *C. sinensis* infestation, hemochromatosis, and Thorotrast injection and the tumor occasionally arises in patients with chronic ulcerative colitis.

The main indication for biliary cytology is suspected malignancy in a patient with a biliary stricture. Reactive and reparative changes are frequently seen with infectious diseases, and primary sclerosing cholangitis (PSC). Dysplasia may be observed in the biliary tract. Adenocarcinoma in the biliary tract (cholangiocarcinoma) is cytologically similar to those seen in the GI tract. The mucinous variant can be especially difficult to diagnose due to its bland cytologic features. These cells contain abundant mucin and can be sometimes mistaken for histiocytes.

The positive for malignant cells of fluid aspiration cytology at ERCP varies from 19% to 86%.^{7,14,15} Because of small masses and difficulty in localization, the sensitivity of fine-needle aspiration cytology is low, and certain malignant stenosis may not be clearly demonstrated by this invasive method.¹⁶ Most benign strictures are managed conservatively with ductal dilatation and stenting. Malignant strictures may be treated by Whipple resection, bile duct resection, or simple stenting if the patient's disease is unresectable.

Parasitic organisms induce deoxyribonucleic acid (DNA) changes and mutations through the production of

DISCUSSION

Obstructive jaundice, the symptom commonly associated with biliary system, is caused by either a stricture or obstruction. Strictures could be due to ascending cholangitis, primary sclerosing cholangitis, gall stones or neoplasms. Brush cytology plays a very important role in the evaluation of these lesions and in distinguishing benign from malignant strictures or an obstruction.^{12,13}

carcinogens and free radicals and the stimulation of cellular proliferation of the biliary epithelium, which is thought to cause cancer. In the literature there are only a few filariasis cases diagnosed via gastrointestinal cytology.^{17,18} Present study indicated that ERCP targeted biliary tract brush cytology is a useful diagnostic procedure and has several advantages.

Malignant strictures of the extrahepatic bile duct cannot be easily distinguished from benign strictures. Although some sophisticated techniques for the preoperative diagnosis of obstructive jaundice are available, the difficult manipulation and low sensitivity have limited them being used widely in clinical practice.^{14,19} Biliary tract brush cytology, however, has been becoming the method of choice in the evaluation of patients with biliary tract strictures. Cytologically positive smears for malignant cells showed nuclear molding, chromatin clumping, high nuclear/ cytoplasmic (N/C) ratio and less frequently, disordered cell clusters, nucleoli, mitosis and cytoplasmic, vacuoles (Figure 5-8).^{4,20,21} In cases with adequate material on cytology, small three dimensional epithelial clusters with marked atypia suggest the diagnosis of malignancy and complicated surgical biopsies can be avoided as biliary tract is very narrow.

ERCP guided brushings is a safe diagnostic procedure for evaluation of biliary tract lesions. Combined approach of ERCP findings and brush smears would enhance the accuracy in diagnosing the various lesions of biliary tract. There are many new methods for evaluating the pancreatico-biliary stricture such as EUS-guided FNA or spyglass cholangioscopy. These new technologies are not only expensive but also user-dependent devices and they are neither applicable nor present in every ERCP room particularly in developing countries. Easy feasibility, low cost, low complication rate and high specificity make

brush cytology still essential as a baseline investigational procedure.²²

The diagnostic accuracy (sensitivity) of cytologic material obtained by endoscopy from biliary system varies according to each study. In general, the accuracy of positive diagnosis is 34-83% with specificity upto 98%.^{14,17,23} The site of stenosis did not influence the sensitivity and technical success.²⁴⁻²⁶ Present study showed sensitivity of 60% and various authors showed the sensitivity (33% to 83%) of their studies (Table 6).

CONCLUSION

We find that biliary brush cytology, although mainly depending on the skill of endoscopist, as well as the experience of the cytopathologist, is a valuable method for obtaining accurate tissue diagnosis of biliary strictures thus solving eternal diagnostic dilemma: benign or malignant. The current study was conducted to assess the relative accuracy of brush cytology for diagnosis of malignant biliary strictures.

ACKNOWLEDGEMENTS

Authors would like to acknowledge unhesitating and immense help received from Dr P. Murali Krishna, Professor and Head, Gastroenterology, Andhra Medical College, in providing cytology material as needed for preparation of this article.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Sridevi S, Lakshmi AB, Kartheek BVS. The role of brush cytology in diagnosing lesions of biliary tract. *Int J Res Med Sci* 2016;4:4624-31.