Original Article

DOI: 10.7860/JCDR/2018/38090.12395

Pathology Section

Role of Endoscopic Retrograde Cholangiopancreatography Guided Brush Cytology in Evaluation of Malignant Biliary Tract Strictures: Experience of a Tertiary Care Teaching Centre in Northern India

PREETI AGARWAL[1], SHIVANJALI RAGHUVANSHI[2], SUCHETA YADAV[3], SHALINI BHALLA[4], RAJ MEHROTRA[5], SAMEER GUPTA[6], VISHAL GUPTA[7], MADHU MATI GOEL[8]

ABSTRACT

Introduction: Endoscopic Retrograde Cholangio-Pancreatography (ERCP) is a gold standard procedure for evaluation of biliary tract lesions. There is a need to obtain a timely diagnosis without subjecting patient who harbours benign disease to major surgery. Strictures due to malignancy are one of the common reasons for obstructive jaundice in our set up. ERCP procedure is performed in such cases to relieve the block by stenting or papillotomy. Biliary strictures that are suspected to be malignant but lack a tissue diagnosis, termed indeterminate strictures, often pose a diagnostic dilemma. Pathological confirmation is desirable for deciding further management protocol. Brush cytology proves to be safe and sensitive procedure in diagnosis of biliary tract lesions.

Aim: This study aims at evaluating the cytomorphological features of intra/extrahepatic malignant lesions directed by ERCP guided brush cytology and to establish the usefulness of biliary brushings in reaching a diagnosis in cases of malignant strictures presenting with obstructive jaundice.

Materials and Methods: The study was a prospective study of one year time period. Cytological material was obtained from 40 cases of malignant strictures presenting with obstructive jaundice, who underwent ERCP for evaluation and treatment. Five cases of benign stricture were assessed to define benign baseline cytomorphology. The smears were prepared and analysed for standard cytological features.

Results: Out of 40 malignant cases, smears from eight were classified as benign, five as reactive, five as NOS-suspicious, and 22 as malignant. Benign and reactive smears were considered as negative (13/40), suspicious and malignant smears (27/40) were considered as positive. On follow up 62.5% of the cases (25/40) were diagnosed as Carcinoma Gall bladder, 20% (8/40) as cholangiocarcinoma, 12.5% (5/40) as periampullary carcinoma and 5% (2/40) as pancreatic ductal adenocarcinoma. Detection rate by cytology was 100% in pancreatic ductal adenocarcinoma, 87.5% in cholangiocarcinoma, periampullary carcinoma (80%) and 56% in carcinoma gall bladder involving common bile duct. Overall sensitivity of brush cytology was 67.5%. Cytomorphological features of increased cellularity, loss of polarity, nuclear hyperchromasia, irregular nuclear outline and nucleolar prominence were found consistent with malignancy. Additional features seen in malignant diagnosis were nuclear variability, background atypical cells and multinucleate cells.

Conclusion: The present study support that ERCP guided brush cytology is a sensitive method in evaluating cases of malignant biliary tract strictures.

Keywords: Cytomorphological features, Endobiliary brush cytology, Gall bladder carcinoma, Obstructive jaundice, Papillotome

INTRODUCTION

Obstructive jaundice is a common presentation seen in biliary lesions encountered in clinical practice in India. It may be due to intrahepatic and extrahepatic causes. Extrahepatic cholestasis, characterized by dilated bile ducts, is often caused by a bile duct stricture or stone or malignancy [1]. Most of the literature published from Northern India clearly shows that majority of patients presenting in large set-ups have malignant lesions as cause of obstructive jaundice [2]. Reported incidence of malignant lesions ranges from 63.3% to 75% in comparison to benign cases [2-4]. Most of these cases undergo either diagnostic or therapeutic ERCP. Direct visualisation by ERCP still remains the gold standard in evaluation of the extrahepatic bile duct.

Biopsy is usually difficult and a very small tissue piece is submitted for histological evaluation which might not be representative of the diseased area in case of malignant strictures. However, properly performed biliary brush cytology can give better output in these cases. The advantages offered by biliary brush cytology over biopsy are that more area is sampled and a flexible brush can be negotiated in stricture area beyond the scope. However, skill of the endoscopist and the time taken to make smears plays a major role in the application of biliary brushing in interpretation of biliary strictures.

Present study emphasises the role of doing brush cytology at the time of ERCP procedure which is inadvertently performed in most of the cases of malignant biliary stricture, so that patient is not subjected to any subsequent procedure for diagnosis and subtyping of tumour. In addition, the sensitivity of the procedure can be increased, if strict cytological criteria are followed and also correct procedure of smearing is followed.

MATERIALS AND METHODS

Study sample: This was a prospective study done over a period of one year, we analysed 40 patients reporting to Department of Surgical Gastroenterology of King George Medical University,

Lucknow, Uttar Pradesh, India. Patients who presented with signs and symptoms of obstructive jaundice (due to biliary strictures) with clinical suspicion of malignancy were included in the study. Diagnosis of malignancy was confirmed clinically and radiologically or by clinical course of the disease or histopathology or fine needle aspiration cytology. In this study, some of the patients showed rapid increase in size of mass on radiology and showed clinical signs of malignancy. All patients with benign causes of obstructive jaundice or any other associated malignancy were excluded. Five cases of benign causes of biliary stricture were taken as controls to define baseline benign cytomorphology in the smears and for standardization of the technique. Smear staining and analysis of data was done in Department of Pathology. Ethical committee clearance was taken.

Methods: Patients were assessed clinically and demographic data of the patients were recorded. Clinical and biochemical parameters were properly recorded for each case (including detailed clinical history and Liver function test). Radiological investigations including ultrasound, computed tomography and cholangiogram findings were also noted. ERCP procedure was performed using a standard video duodenoscope. After visualisation of ampulla, endoscopic sphincterectomy was performed using a papillotome introduced over a guide wire. Under radiographic guidance using a contrast fluid, bile duct strictures were localised. Strictures were brushed with rapid to and fro movements using a double lumen brush. The procedure was performed by experienced gastro-surgeon under strict aseptic protocol and after well informed written consent from the patient. Adequate brush cytology sample could be obtained in all the study subjects. Cytosmears were prepared by experienced pathologist in the endoscopy procedure room.

Cellular material adherent to the brush was directly transferred to the glass slide and five smears were prepared and stained by rapid Papanicolau (2 smears), Haematoxylin and eosin (2 smears) and Giemsa stain (1 smear). Detailed cytological evaluation was done and recorded. Statistical analysis was done by SPSS 23 version software.

Cytomorphological evaluation: Cytological smears were assessed for described cytological characteristics in detail. Morphological features assessed included: 1) cellularity; 2) background; 3) pattern of cell distribution; 4) polarity; 5) nuclear size; 6) nuclear to cytoplasmic ratio; 7) nuclear variation >(x2 or x4); 8) nuclear shape; 9) nuclear outline; 10) chromatin; 11) mitosis; 12) amount of cytoplasm; 13) background atypical cells; and 14) multinucleate cell. Other cytological features like nuclear grooving, convolutions, notches and moulding and necrosis were also studied. The nuclear size was comparable to size of RBC and nuclear variation was compared to a benign glandular cell from benign smears. Nuclear cytoplasmic ratio was reported as increased when it was greater than 2:1 or when the nucleus constituted more than 50% of entire cell volume. The smears were seen by two independent pathologist and final consensus was taken by a third pathologist in case of discrepancy. The smears were classified as benign, reactive, malignant and Not Otherwise Specified (NOS)-suspicious. For purpose of analysis NOS-suspicious smears were considered to be positive and reactive as negative. The patients were followed up clinically and radiologically. Diagnostic sensitivity of the brush cytology procedure was assessed along with correlation with the demographic and cytological characteristic. Sensitivity of the procedure was calculated by the formula; sensitivity=true positive cases/(true positive+false negative) x100.

RESULTS

Total 40 malignant cases underwent transpapillary brush cytology during the study period. The mean age of the patients was 54 years. Males were 13 and females were 27(M:F ratio=1: 2.1). Rest were confirmed by clinical follow-up and radiology. Carcinoma gall bladder was the most common malignancy seen in our cases (62.5%; 25/40) followed by cholangiocarcinoma (20%; 8/40), periampullary carcinoma (12.5%; 5/40) and pancreatic carcinoma (5%; 2/40) respectively. In 22 out of 40 cases histological confirmation for malignancy was available. In 18/22 cases of carcinoma gall bladder USG guided aspiration cytology was also available and was positive for malignant cells.

Amongst the biochemical investigations, the mean serum bilirubin level was 11.32 mg/dL and mean alkaline phosphatise level was 1595 IU/L. These levels were consistent with laboratory parameters of obstructive jaundice. Patents did not have any procedure related complications while undergoing the procedure.

Biliary brush cytology: Ninety five percent of the cases sampled by biliary brush (38/40) had adequate cellular material for interpretation in the first brushings, only 5% (2/40) were inadequate. The procedure was repeated as there was strong radiological and clinical suspicion of malignancy and adequate samples were obtained in them. The cytological smears were classified as benign, reactive, NOS-suspicious and malignant. One case was sampled thrice during course of the disease in which first two smears revealed reactive cytology and last smear was malignant. The final diagnosis was considered. The procedure was repeated as the patient was clinically deteriorating and the tumour was increasing in size radiologically. Out of 40 malignant cases, the cytological smears of eight were classified as benign, five as reactive, five as NOS-suspicious, and 22 as malignant. For purpose of analysis NOS-suspicious smears were considered to be positive and reactive as negative. Diagnostic sensitivity of brush cytology for detection of all malignancies was 67.5% (27/40); 56% cases (14/25) of carcinoma gall bladder, 87.5% cases of cholangiocarcinoma (7/8), 80% cases of periampullary carcinoma (4/5) and 100% of pancreatic ductal adenocarcinoma (2/2) were diagnosed by brush cytology [Table/Fig-1].

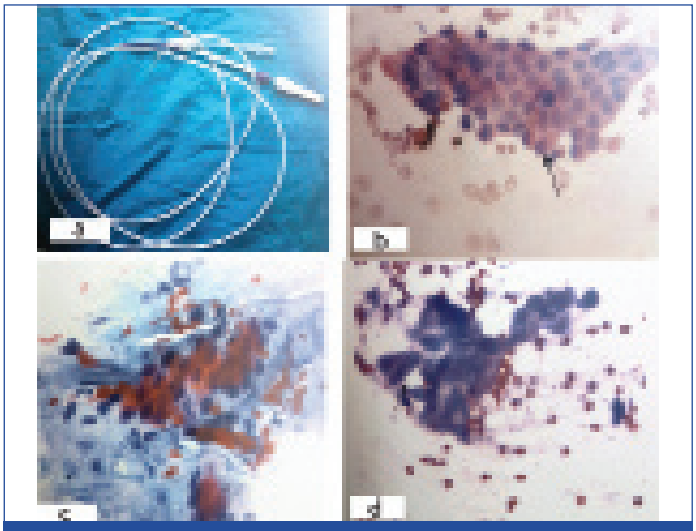
Cellular details of all the smears are summarized in [Table/Fig-2]. The benign smears were low in cellularity with cells disposed in monolayer sheets of regular round epithelial cells, nuclear to cytoplasmic ratio was normal and cytoplasm was adequate. No atypical cells, giant cells, mitosis or necrosis was seen. Reactive smears had moderate to high cellularity with haemorrhage and acute inflammation in the background. Cells were disposed in sheets and clusters with mild pleomorphism, polarity was maintained and nuclear crowding was absent [Table/Fig-3]. The malignant smears had moderate to high cellularity with haemorrhage and acute inflammation in background. Cells were disposed in sheets and clusters with pseudopapillae and acini formation. Polarity was lost with increased nuclear cytoplasmic ratio. The tumour cells were round to oval, some were spindled. Nuclear size was >1.5 RBC. Reactive cell clusters were seen in five of the malignant smears. Necrosis was present in 18/22 malignant smears. Smears from five cases displayed cytomorphology intermediate between reactive and malignant smears. They were classified as NOS -suspicious. The smears were cellular with cell disposed in sheets and clusters. Nuclear clumping was present but the nuclear membrane was regular, nuclear variability intermediate (between x2-3) with nucleolar prominence in one case. No mitosis, multinucleate cells were seen in the background. 4/22 malignant smears had significant inflammation in the background [Table/Fig-4].

Clinical diagnosis	Number of cases	Detected	Not detected	Sensitivity (%)
Carcinoma gall bladder	25	14	11	56
Cholangiocarcinoma	8	7	1	87.5
Periampullary carcinoma	5	4	1	80
Pancreatic carcinoma	2	2	0	100
Total	40	27	13	67.5

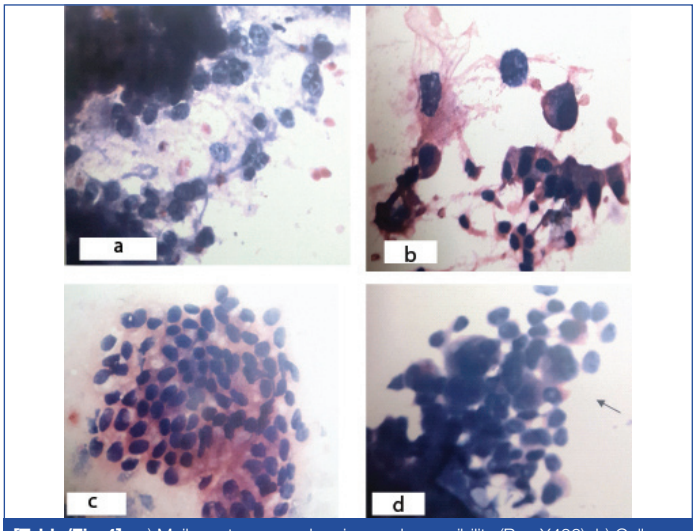
[Table/Fig-1]: Sensitivity of biliary brush cytology.

	Benign smear (n=8)	n=8	Reactive smear (n=5)	n=5	Malignant (n=22)	n=22	NOS-suspicious (n=5)	n=5
Cytologic features	**Category**							
Cellularity	Low	8	Moderate High	3 2	Moderate High	10 12	Moderate High	3 2
Background	Clear with slight hemorrhage	8	Hemorrhage Acute inflammation	5 3	Hemorrhage Acute inflammation	18 8	Hemorrhage Acute inflammation	4 1
Cell disposed in	Monolayer sheets(1-3) Benign columnar cells<2 Cluster of <5 cells	8 8 8	Sheets(>5) clusters	5 5	Sheets(>5) Clusters Pseudopapillae Acini formation	15 17 8 7	Sheets(>5) Clusters Pseudopapillae Acini formation	2 3 1 1
Polarity	Maintained	8	Maintained	4	Lost	19	Maintained	3
Nuclear size	>1.5 RBC size	0	>1.5 RBC size	0	>1.5 size of RBC	17	>1.5 size of RBC	4
Nuclear to cytoplasmic ratio	Normal	8	Normal	4	Increased	20	Increased	3
Shape	Oval to round	8	Oval to round	5	Oval to round Angulated Grooving Moulding	4 10 5 4	Oval to round to elongated	4
Margins	Regular	8	Regular	5	Irregular	21	Irregular	3
Variation	Absent	8	<(x2)	5	>(x2) >(x4)	10 12	Between (x2-3)	5
Chromatin	Fine	8	Fine	5	Hyperchromatic	12	Clumped	4
Nucleoli	Absent	8	Absent	5	Present	21	Present occasionally	4
Cytoplasm	Pale eosinophilic	8	Pale eosinophilic	5	Moderate Scant	9 13	Moderate	5
Background atypical cells	Absent	8	Absent	5	Present	8	Present	2
Giant cells	Absent	8	Absent	5	Present	7	Absent	5
Mitosis	Absent	8	Absent	5	Present	4	Present	5
Necrosis	Absent	8	Absent	5	Present	18	Absent	5

[Table/Fig-2]: Detailed cytological feature evaluation in each cytological category.



[Table/Fig-3]: a) Double lumen 8 freanch brush which increased yield in study. b) Benign smear showing a benign sheet (arrow), (H&E X 400). c) Smear showing nuclear variability (<X2), nuclear membrane is regular (Pap X 100). d) Smear showing nuclear grooving arrow, (Pap X 400).



[Table/Fig-4]: a) Mailgnant smears showing nuclear varibility (Pap X400). b) Cells showing increased nucleo cytoplasmic ratio (Pap X400). c) Atypical cells forming sheets (HE X400). d) Smear showing multinucleate cells (Pap X400).

DISCUSSION

Early diagnosis of malignant biliary strictures still remains a challenge. Most of the patients present late when palliative treatment like stenting is the mainstay of treatment. Usually the malignancy is locally advanced with or without distant metastases and curative resection cannot be done in such patients. Even then an accurate tissue diagnosis is needed in both resectable and non-resectable tumours to plan chemotherapy [5].

Biliary and pancreatic duct lesions are not always readily accessible to biopsy, and cytological techniques have become the initial diagnostic modality in many cases [6,7].

Amongst the diagnostic procedures, percutaneous radiologically guided fine needle aspiration is a very accurate technique in pancreatobiliary malignancies but for this there must be a distinct mass lesion for adequate sampling. Other methods like bile, pancreatic and duodenal fluid aspirates have low sensitivity and specificity because of poor cellular preservation [8]. Brush cytology performed during ERCP has now become the preferred initial method of diagnosis in many patients with pancreatico–biliary strictures. The technique allows sampling from most sites within the pancreatic and biliary duct systems. Well prepared and sampled specimens usually have well preserved cellular samples suitable for cytological analysis [9,10].

In the present study, malignancy was common in older age group. Furthermore, women were affected more frequently than men. Similar observations were seen by other authors [11,12].

Modest diagnostic sensitivity is recorded in most studies to date

(33-78%; mean 42%) and negative predictive value, despite high test specificity (90-100%). A comparison between various studies with respect to sensitivity, specificity, negative predictive value and positive predictive value is summarised in [Table/Fig-5] [13-22].

In present study the procedure had a moderate diagnostic sensitivity of 67.5%. The procedure was performed by a well-trained gastro-surgeon and protocol based evaluation of smears was done. The reasons for low sensitivity in most of the studies may be due to

Author	Year	Total No. of patients	No. of patients with cancer	No. of cases detected by Brush cytology	Sen-sitivity (%)	Speci-ficity (%)	PPV (%)	NPV (%)
Lee G J et al., [13]	1995	149	106	40	37	100	100	39
Ponchon T et al., [14]	1995	204	127	45	35	97	96	44
Pugliese V et al., [15]	1995	94	64	35	54	100	100	50
Glasbrenner B et al., [16]	1999	78	57	32	56	90	94	43
Mansfield JC et al., [17]	1997	43	41	17	42	100	100	8
Jailwala J et al., [18]	2000	133	104	31	30	100	100	28
Macken E et al., [19]	2000	106	62	35	57	100	100	62
Stewart CJR et al., [20]	2001	406	246	147	60	98	98	61
Fogel EL et al., [21]	2006	102	94	28	30	-	-	-
Shieh FK et al., [22]	2014	32	32	25	78	-	-	-
Present study	2018	40	40	27	67.5	-	-	-

[Table/Fig-5]: Summary of studies comparing diagnostic efficacy of Endscopic retrograde Cholangiopancreatography guided brush cytology.

sampling and interpretative differences. Sampling errors might occur when tumours at these sites show a predominantly submucosal spread, with limited or absent surface epithelial abnormality [23,24]. Strictures might also be caused by external compression like by lymph node metastasis. The site of the tumour might also affect the sensitivity. Interpretative errors are more likely to occur in well differentiated carcinomas, in which the cytological abnormality may be minimal [25].

Several studies have shown that diagnostic accuracy is greatest for ampullary neoplasms, intermediate for cholangiocarcinoma, and lowest for pancreatic carcinoma, particularly for tumours in the pancreatic tail [26]. Although in this study, detection rate was 80% in ampullary carcinoma cases, 87.5% in cholangiocarcinoma, 100% in pancreatic ductal adenocarcinoma and 56% in carcinoma gall bladder causing obstruction.

An interesting finding found in present study was that an increase in detection rate was found during later half of study. Possible explanations are the use of double lumen brush instead of single lumen later in the study and increase in surgeon's expertise in taking the sample. In addition, more of forward movement of the brush was done to increase the cellular yield. Also, removing the brush and catheter together improved cancer detection compared with pulling the brush through the catheter sheath.

As per present study cytological features which were found more consistent with malignancy were high cellularity, loss of polarity, hyperchromatic nucleus with clumped chromatin, nuclear crowding, irregular nuclear membrane and nucleolar prominence. Nuclear variability, background atypical cells and multinucleate cells were also seen in smears which were reported as positive.

Cytological features like inflammation had a similar frequency in benign and malignant bile duct brushings, making it less useful in the accurate identification of malignant brushings. However, it should be noted that acute inflammation should not be ignored in all cases, particularly when associated with other concerning malignant characteristics. Five of our malignant smears had inflammation in the background and few clusters were atypical.

In an effort to improve the diagnostic accuracy of cytologic examination, several investigators have proposed specific diagnostic criteria for malignancy.

Avadhani V et al., had seven reviewers for bile duct brushing specimens who examined strength of several well established cytological characteristic in predicting malignancy [27]. They studied change in chromatin pattern, nuclear irregularity, pleomorphism, 2- cell population and three dimensional clusters were helpful in accurately identifying malignancy in these specimens and were present in more than 50% cases. Okonkwo AM et al., studied strict morphologic criteria: major (nuclear contour, chromatin pattern) and minor (polarity, cell types, nuclear size, nuclear grooves, nucleoli, mitosis, nuclear/cytoplasmic ratio). Irregularities in nuclear membrane and abnormal chromatin pattern were the most consistently useful features correlating with malignancy, which are similar to the criteria we have studied. Their study was based on conventional smears which is also similar to this study [28].

However, Renshaw AA et al., observed that overall assessment of malignancy based on degree of atypia was more reproducible than any set of criteria and also resulted in higher sensitivity for malignancy with small decrease in specificity [29].

Regarding inflammation in the background of malignant smears, it has been demonstrated that pancreatic ductal adenocarcinomas, particularly its micropapillary and undifferentiated subtypes, may demonstrate marked intra-epithelial infiltration in pancreatic ductal adenocarcinoma which may involve the bile ducts resulting in 'positive' bile duct brushings [30].

Lack of obvious features of carcinoma should be prompt the surgeon to do repeat sampling to prompt a conclusive diagnosis of malignancy. Two cases in our study had repeated aspirations as they had strong clinical and radiological suspicion of malignancy.

Present study did not have any procedure related complications. In a series of 223 consecutive biliary strictures sampled by brush cytology, Ponchon T et al., reported one retroperitoneal bile duct perforation related to the brushing. The patient was treated with placement of a biliary stent and remained asymptomatic. These brushings were obtained without the use of a guide wire; when brushing is performed over a guide wire, the likelihood of this adverse event is low [14].

Considering current diagnostic aids to brush cytology, immunohistochemistry is another useful adjuvant study in bile duct brushing assessment which is been recently studied. In a recent study, over 50% of biliary cancers showed a maspin +/IMP3+/S100P+/pVHL – staining profile, and 20% showed a maspin+/IMP3 – /S100P+/pVHL– profile [31]. However immunohistochemistry lacks specificity. A newer pancreato-biliary tract specific FISH probes have been identified that targets 1q21, 7p12, 8q24, and 9p21, with a sensitivity of 65% and specificity of 93% [32]. However, FISH poses technical and financial challenges.

The search for improvements in diagnostic accuracy of bile duct brushings has led some to suggest triple testing (brush cytology, fluorescence in situ hybridization and forceps biopsy) which has 82% sensitivity, 100% specificity, 100% positive predictive value, and 87% negative predictive value as compared to brushing alone [33]. However, these studies had sufficient specimen cellularity for additional testing, which is often not the case in clinical practice and also most centres lack facilities and molecular tests are not cost effective.

LIMITATION

Furthermore, current study also has few limitations, adequate number of benign cases was not included in the study, therefore, specificity could not be calculated. Biochemical investigations from benign cases could not be compared with the malignant cases and any cut-off value to differentiate between benign and malignant cases could not be assessed.

CONCLUSION

To conclude, the results of the present study clearly show that biliary brushing is good and cost effective if these guidelines are properly followed: 1) smears are appropriately prepared by a double lumen brush with more of forward movement rather backwards during sample collection; 2) strict cytological criteria for interpretation and reporting is followed. The limitations of the technique must be recognised, brush cytology is useful as the initial investigation of patients with suspected pancreatico–biliary neoplasia and the present study fulfils the need to study individual morphological characteristics and prove their diagnostic utility.

Future recommendations are for development of strategies to improve lesion targeting and better retrieval of cytology specimens with higher quality and also studies with larger number of cases along with multi-reviewer analysis adds to calculation of diagnostic efficacy of the procedure.

ACKNOWLEDGEMENTS

There is no funding involved in this work. We want to thank our technical team members for their support.

REFERENCES

[1] Karvonen J, Kairisto V, Grönroos JM. Stone or stricture as a cause of extrahepatic cholestasis - Do liver function tests predict the diagnosis? Clin Chem Lab Med. 2006;44:1453-56.
[2] Gupta AK, Singh A, Goel S, Tank R. Profile and pattern of obstructive jaundice cases from a tertiary care teaching hospital of Uttar Pradesh. International Surgery Journal. 2017;4(2):743-46.
[3] Sharma MP, Ahuja V. Aetiological spectrum of obstructive jaundice and diagnostic ability of ultrasonography clinician's perspective. Tropical Gastroenterology. 1999;20:67-69.
[4] Madhu MP, Agarwal V, Soni A, Pokharna RK, Nijhawan S, Sharma G, et al. Etiological spectrum of extra hepatic biliary obstructive (EHBO) at a tertiary care centre in Northern India. Tropical Gastroenterology. 2015;36(2):142-43.
[5] Thuluvath PJ, Rai R, Venbrux AC, Yeo CJ. Cholangiocarcinoma: a review. Gastroenterologist. 1997;5(4):306-15.
[6] Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. Acta Cytologica. 1995;39:11-18.
[7] Logrono R, Wong JY. Reporting the presence of significant epithelial atypia in pancreaticobiliary brush cytology specimens lacking evidence of obvious carcinoma: impact on performance measures. Acta Cytol. 2004;48: 613-621.
[8] Leach SD, Rose JA, Lowy AM, et al. Significance of peritoneal cytology in patients with potentially resectable adenocarcinoma of the pancreatic head. Surgery. 1995;118:472-78.
[9] Foutch PG. Diagnosis of cancer by cytological methods performed during ERCP. Gastrointest Endosc. 1994;40:249-52.
[10] Kurzawinski T, Deery A, Davidson BR. Diagnostic value of cytology for biliary stricture. Br J Surg. 1993;80:414-21.
[11] Gupta M, Pai RR, Dileep D, Gopal S, Shenoy S. Role of biliary tract cytology in the evaluation of extrahepatic cholestatic jaundice. Journal of Cytology. 2013;30(3):162-68.
[12] Mahmoudi N, Enns R, Amar J, AlAli J, Lam E, Telford J. Biliary brush cytology: Factors associated with positive yields on biliary brush cytology. World J Gastroenterol. 2008;14:569-73.
[13] Lee JG, Leung JW, Baillie J, Layfield LJ, Cotton PB. Benign, dysplastic, or malignant making sense of endoscopic bile duct brush cytology: results in 149 consecutive patients. Am J Gastroenterol. 1995;90:722-26.
[14] Ponchon T, Gagnon F, Berger F. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. Gastrointest Endosc. 1995;42:565-72.
[15] Pugliese V, Conio M, Nicolò G. Endoscopic retrograde forceps biopsy and brush cytology of biliary strictures: a prospective study. Gastrointest Endosc. 1995;42:520-26.
[16] Glasbrenner B, Ardan M, Boeck W. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. Endoscopy. 1999;31:712-17.
[17] Mansfield JC, Griffin SM, Wadebra V. A prospective evaluation of cytology from biliary strictures. Gut. 1997;49:671-77.
[18] Jailwala J, Fogel EL, Sherman S. Triple tissue sampling at ERCP in malignant biliary obstruction. Gastrointest Endosc. 2000;51:383-90.
[19] Macken E, Drijkoningen M, Van Aken E. Brush cytology of ductal strictures during ERCP. Acta Gastroenterol Belg. 2000;63:254-59.
[20] Stewart CJR, Mills PR, Garter R. Brush cytology in the assessment of pancreatico-biliary strictures: a review of 406 cases. J Clin Pathol. 2001;54:449-55.
[21] Fogel EL, deBellis M, McHenry L. Effectiveness of a new long cytology brush in the evaluation of malignant biliary obstruction: a prospective study. Gastrointest Endosc. 2006;63:71-77.
[22] Shieh FK, Luong-Player A, Khara HS, Liu H, Lin F, Sellenberger MJ, et al. Improved endoscopic retrograde cholangiopancreatography brush increases diagnostic yield of malignant biliary strictures. World J Gastrointest Endosc. 2014;6:312-17.
[23] Adamsen S, Olsen M, Jendresen MB, Holck S, Helthoj A. Endobiliary brush biopsy: Intra- and interobserver variation in cytological evaluation of brushings from bile duct strictures. Scand J Gastroenterol. 2006;41:597-603.
[24] Draganov PV, Chauhan S, Wagh MS, Gupte AR, Lin T, Hou W, Forsmark CE. Diagnostic accuracy of conventional and cholangioscopy-guided sampling of indeterminate biliary lesions at the time of ERCP: a prospective, long-term follow-up study. Gastrointest Endosc. 2012;75:347-53.
[25] De Peralta-Venturina MN, Wong DK, Purslow MJ, Kini SR. Biliary tract cytology in specimensobtained by direct cholangiographic procedures: a study of 74 cases. Diagn Cytopathol. 1996;14:334-48.
[26] Foutch PG, Kerr DM, Harlan JR, Kummet TD. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. Am J Gastroenterol. 1991;86:577-80.
[27] Avadhani V, Hacihasanoglu E, Memis B, Pehlivanoglu B, Hanley KZ, Krishnamurti U, et al. Cytologic predictors of malignancy in bile duct brushings: a multi-reviewer analysis of 60 cases. Modern Pathology. 2017;30:1273-86.
[28] Okonkwo AM, De Frias DVS, Gunn R, Diaz L, Schindler S, Lal A, et al. Reclassification of "Atypical" Diagnoses in endoscopic retrograde cholangiopancreaticography-guided biliary brushings. Acta Cytologica. 2003;47:435-42.
[29] Renshaw AA, Madge R, Jiroutek M, Granter SR. Bile duct brushing cytology: Statistical analysis of proposed diagnostic criteria. Am J Clin Pathol. 1998;110:635-40.
[30] Reid MD, Basturk O, Thirabanjasak D, Hruban RH, Klimsatra DS, Bagci P, et al. Tumor infiltrating neutrophils in pancreatic neoplasia. Mod Pathol. 2011;24:1612-19.
[31] Chen L, Huang K, Himmelfarb EA, Zhai J, Lai JP, Lin F, et al. Diagnostic value of maspin in distinguishing adenocarcinoma from benign biliary epithelium on endoscopic bile duct biopsy. Hum Pathol. 2015;46:1647-54.
[32] Barr Fritcher EG, Voss JS, Brankley SM, Campion MB, Jenkins SM, Keeney ME, et al. An optimized set of fluorescence in situ hybridization probes for detection of pancreatobiliary tract cancer in cytology brush samples. Gastroenterology. 2015;149:1813-24.
[33] Nanda A, Brown JM, Berger SH, Lewis MM, Barr Fritcher EG, Gores GJ, et al. Triple modality testing by endoscopic retrograde cholangiopancreatography for the diagnosis of cholangiocarcinoma. Ther Adv Gastroenterol. 2015;8:56-65.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India.
2. Assistant Professor, Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India.
3. Junior Resident, Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India.
4. Assistant Professor, Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India.
5. Professor, Department of Pathology, Mayo Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.
6. Associate Professor, Department of Surgical Oncology, King George Medical University, Lucknow, Uttar Pradesh, India.
7. Additional Professor, Department of Surgical Gastroenterology, King George Medical University, Lucknow, Uttar Pradesh, India.
8. Professor, Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shivanjali Raghuvanshi,
Assistant Professor, Department of Pathology, King George Medical University, Chowk, Lucknow-226010, Uttar Pradesh, India.
E-mail: joyousshivi@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 02, 2018**
Date of Peer Review: **Aug 22, 2018**
Date of Acceptance: **Oct 20, 2018**
Date of Publishing: **Dec 01, 2018**