

Role of Intraoperative Imprint Cytology in Diagnosis of Suspected Gallbladder Carcinoma

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Abstract

Background: The present study was conducted to assess whether touch imprint cytology can help in rapid diagnosis of Gallbladder carcinoma and thus facilitate individualized treatment. Rapid and early diagnosis is essential to gain the benefits of initiating the treatment early. Cytological examination provides useful information for guiding therapeutic strategy. These cytological findings will subsequently be confirmed by histopathological findings.

Methods: A prospective investigation was performed on 45 cases of suspected gall bladder carcinoma on the basis of clinical and radiological examination. Imprint smears were made intra-operatively of fresh samples from various representative areas, and stained with Leishman Giemsa for air-dried smears and Papanicolaou for alcohol-fixed smears. A rapid opinion regarding the benign or malignant nature of the lesion and the type of tumour was given.

Results: The overall sensitivity was 92%, specificity 100%, positive predictive value 100%,. Characteristic cytological patterns were noted in various lesions.

Conclusions: Imprint cytology can be used as an adjunct to histopathology for rapid and early diagnosis in the operation theatre, thus helping better management of patients.

Keywords: Intraoperative diagnosis - touch imprint cytology - Gallbladder carcinoma.

I. Introduction

Carcinoma of gall bladder is the 5th most common tumor of G.I tract. It is the most frequent malignant tumor of the biliary tract ⁽¹⁾. Approximately 70% of tumors are unresectable and radical surgery has not proved to be of value. Secondary spread occurs in liver, to the adjacent cystic, common bile duct and peripancreatic lymph node and diffuse carcinomatosis is the usual final outcome. 5 years survival is less than 10%. Study of progression and development of gall bladder carcinoma have been shown passage through stage of atypical hyperplasia and carcinoma in situ. No easy way of detecting these early lesions has been discovered ⁽²⁾.

Incidence rate of gallbladder carcinoma is 0.2% to 3% of all performed cholecystectomies. These rates may increase to 5%–7% in patients over 65 years old. Highest incidence is seen in Chileans, American Indian & in Northern part of India-9.1 % ⁽³⁾. Patients may be asymptomatic or if symptoms are present, usually indistinguishable from benign gall bladder disease like chronic cholecystitis (pain, anorexia, and elevated alkaline phosphatase), and manifest only after the tumor has inflicted substantial damage ^(4, 5). Macroscopically, gallbladder adenocarcinoma typically produces a scirrhous, gray-white, firm mass because of the prominence of desmoplasia ⁽⁵⁾. When diffuse, it may be difficult to distinguish from cholecystitis; hence microscopic examination of every resected gallbladder is very important. Diffuse infiltrative types may create a picture reminiscent of linitis-plastica, and may spread linearly along the extra hepatic bile ducts as well.

Owing to the risks of bile extravasations, particularly in the presence of bile duct stenosis, gall bladder puncture with bile aspiration is not done preoperatively. So cytological diagnosis is therefore restricted to immediate preoperative or intraoperative procedure. Cyodiagnosis is a single technique and on a single sample has a lower sensitivity in the detection of malignancy here. But this restriction does not negate the value of cytology in this situation as cytological methods may be the only way of establishing the diagnosis ⁽⁶⁾. With these considerations, imprint cytology of the gallbladder mucosa is very useful method. Touch imprint cytology has shown almost equivalent sensitivity as compared to frozen section ^(7, 8).

So aim of this study is to correlate cytological feature of gall bladder lesion with histopathological diagnosis and to evaluate diagnostic accuracy of imprint cytology in the diagnosis of gall bladder carcinoma.

II. Materials And Methods

During Study period, a total of 45 cases of gall bladder tumor which were diagnosed as malignancy by clinical and radiological examination, subsequently undergo resection of tumor. Among these 45 cases only 39 cases were confirmed as malignant lesion by histopathological examination and 6 cases were diagnosed as inflammatory lesion.

The tissue is received in Operation Theater, than macroscopically examination of the tumor was done for site, size, appearance, consistency, margins, presence or absence of any necrosis, presence or absence of any hemorrhage is done along with examination of surrounding normal tissue if present.

After cutting the tumor with a clean scalpel in proper site, imprint smears were taken by gentle touching. Then the smears was air dried and stained by standard method(MGG) and cytological diagnosis was made in the department of pathology, I.P.G.M.E&R. and was informed to the surgeon in best of the earliest time(25 to 30 minutes).

The specimen was then transferred into 10% neutral buffered formalin and kept overnight for fixation. Representative tissue sections submitted on next morning for histopathological study. Tissues were then processed and paraffin blocks were prepared. The tissue sections were put on albumin coated slides and preserved for different stains such as haematoxylin eosin [H & E] stain.

At first all the sections were stained by H & E stain to identify the nature of tumor. Examination of these slides done under light microscope and histopathological diagnosis is made.

III. Results & Analysis

In this study total 45 cases were diagnosed as malignant lesion of gall bladder by clinical and radiological examination. In five of our cases the appearances of xanthogranulomatous foci resembled carcinoma for the surgeon grossly ^(Fig 1), diagnosed as benign lesion by imprint cytology ^(Fig 2). Macroscopically, gallbladder adenocarcinoma of diffuse variant poses a diagnostic problem as it typically produces a scirrhous, gray-white, firm mass which may be difficult to distinguish from chronic cholecystitis ^(Fig 3), even the mucosa were healthy in some cases ^(Fig 4), hence the importance of imprint cytological examination of every resected gallbladder lesion.

We take imprint smear intraoperatively in O.T. and inform the surgeon about benign nature of 5 GB lesion and findings of malignant cells ^(Fig 5) in touch smear in 37 cases of gall bladder lesion. These definite diagnoses of malignancy help the surgeon to modify the operation ^(Fig 6). Only in two cases cytological findings were inconclusive.

On final histopathological examination, there were 37 cases of invasive gall bladder carcinoma (61%) ^(Fig 7) and 2 case of gall bladder carcinoma in situ (3%). Out of 37 cases of invasive gall bladder carcinoma 33 cases were of adenocarcinoma, 3 cases of adenosquamous carcinoma and one case of undifferentiated carcinoma of epitheloid variety. Two cases of carcinoma in situ of gall bladder were inconclusive in imprint cytology. Among 37 cases only one case was misdiagnosed in touch imprint cytology as the cells lack marked cytological atypia or pleomorphism. But histological examination diagnoses the case as well differentiated adenocarcinoma and not involving the serosa. With considering immunoreactivity, especially in gall bladder tumor, it was noted that gall bladder carcinoma show CK7 positivity in 90% cases ^(Fig 8).

Correlation of cytological diagnosis with histopathological findings in gallbladder lesion:

HISTOPATHOLOGICAL DIAGNOSIS	TOTAL CASE NO	CORRELATION WITH CYTOLOGICAL DIAGNOSIS	FALSE POSITIVE	FALSE NEGATIVE
Invasive papillary adeno ca	33	32/33	0	1
Adenosquamous ca	3	3/3	0	0
Undifferentiated Ca,epitheloid Variety	1	1/1	0	0
Ca in situ	2	0/2	0	2
Xanthogranulomatous Cholecystitis	5	5/5	0	0
Acute exaggeration Of chronic cholecystitis	1	1/1	0	0

Specificity: 100%

Sensitivity: 92%

Positive predictive value: 100%

Negative predictive value: 67%

IV. Statistical analysis

Statistical analysis was done with Cohen's kappa coefficient using SSPS software. Cohen's Kappa coefficient is a statistical measure of inter rater agreement for qualitative items. It is generally thought to be a more

robust measure than simple percent agreement calculation since 'k' takes into account the agreement occurring by chance. Cohen's k measure the agreement between two raters which classify 'N' items into 'C' mutually exclusive categories. The first mention of kappa like statistics is attributed to Galton (1892). The equation for 'K' is

$$K = \frac{\text{Pr (a)} - \text{Pr (e)}}{1 - \text{Pr (e)}}$$

Where Pr (a) is the relative observed agreement among raters and Pr (e) is the hypothetical probability of chance agreement, using the observed data to calculate the probabilities of each observer randomly saying each category. If the raters are in complete agreement than k=1. if there is no agreement among the raters (other than what would be expected by chance), the k ≤ 0. Cohen's kappa measures agreement between two rater only. The measurements of observer agreement for categorical data in biometrics vol: 33 gave the following table for interpretation of k values.

'k' interpretation:

- <0 No agreement
- 0 – 20 Slight agreement
- 20-40 Fair agreement
- 41-50 Moderate agreement
- 50-60 substantial agreement
- >60 Strong agreement.

Comparison of imprint cytological diagnosis with histopathological diagnosis of gall bladder lesion in the present study:

Input of DATA:				Level of Confidence:			
		Disease					
		Yes	No	Total			
Test	+	36	0	36	<input type="radio"/> 90 % <input checked="" type="radio"/> 95 % <input type="radio"/> 97.5 % <input type="radio"/> 99 % <input type="radio"/> 99.5 %		
	-	3	6	9			
Total		39	6	45			
RESULTS:							
	%	Lower Lim	Upper Lim				
Sensitivity	92.308	83.945	100.000				
Specificity	100.000	100.000	100.000				
True Prevalence	86.667	76.734	96.599				
Apparent Prevalence	80.000	68.313	91.687				
Predictive Value +	100.000	100.000	100.000				
Predictive Value -	66.667	35.868	97.465				

Interpretation:

In the present study, gall bladder carcinoma was the most common diagnosis and imprint cytology shows sensitivity of 92%, specificity -100%, positive predictive value was 100% and negative predictive value 66.6%. So from present study it can be inferred that for diagnosis of gallbladder carcinoma imprint cytology technique is very much applicable.

V. Discussion

A total of 45 patients were enrolled according to the inclusion criteria in the present study and all cases were suspicious of gall bladder carcinoma by clinical and radiological examination but 6 cases were diagnosed as benign lesion in both cytological and histopathological finding. Out of six cases, 5 cases were of xanthogranulomatous cholecystitis and one case was diagnosed as acute exacerbation of chronic cholecystitis. Out of 39 cases of gall bladder tumor 29 patients were female and only 10 cases were male. Here the ratio is almost 3:1. Lohe et al in their series found the male: female ratio of 3:1⁽⁹⁾. So GB carcinoma has a female preponderance. Fine needle aspiration cytology as a preoperative investigation is contraindicated in gall bladder malignancy. Because, there is chance of spillage of bile and increase incidence of tumor implantation in needle tract. Pre operative diagnosis is not possible as most of the gall bladder malignancies are diffuse type and do not produce any polypoid growth, so ultra sonogram or CT scan show thick gall bladder wall only. So it is very difficult to differentiate from benign lesion like chronic cholecystitis or xanthogranulomatous cholecystitis. In cases of gall bladder lesion which were suggestive of gall bladder malignancy according to clinical and radiological findings, were diagnosed as benign lesion in imprint smear examination under microscopy. These

findings were confirmed by histopathological examination. 5 cases were reported as xanthogranulomatous cholecystitis and there were one case of acute exacerbation of chronic cholecystitis.

In the present study, for diagnosis of gall bladder carcinoma (which was the most common diagnosis in our study) imprint cytology technique shows the sensitivity 92% specificity 100% positive predictive value 100% negative predictive value 67%.

But out of 39 gall bladder malignancy 36 were diagnosed correctly by imprint cytology and six cases which were suggestive of malignant lesion by clinical and radiological investigation, but by intra operative imprint cytology were diagnosed as benign and prevent unnecessary radical surgery. So the diagnosis of early gallbladder carcinoma is almost impossible before surgery; in many cases the tumors are grossly in apparent. The usefulness of imprint cytology in detecting early carcinoma of the gallbladder mucosa was assessed. It is very important to take imprint smear samples from as much area as possible. Carcinoma in situ is not diagnosed by imprint smear as in cytology structural loss occurs. We diagnose case by cellular morphology. So it is a limitation of imprint cytology.

Policlín have studied significance of imprint cytology of the gallbladder mucosa and its use in diagnosing macroscopically in apparent carcinoma. Eight macroscopically in apparent carcinomas were diagnosed, and nine grossly evident carcinomas were confirmed by imprint cytology in 120 cholecystectomies. The sensitivity of cytological diagnosis of macroscopically in apparent carcinoma was 80%, specificity and predictive value were 100%, and efficiency was 97.7%. If overt carcinomas are included, both the sensitivity and efficiency increase, to 89.6% and 98%, respectively. For the diagnosis of dysplasia the sensitivity and predictive value was 84%, specificity 97.6% and efficiency 95.8%. Because of the simplicity and rapidity of imprint cytology of gallbladder mucosa, coupled with its high sensitivity and reliability, it is recommended for the detection of in apparent carcinoma during cholecystectomy in patients at high risk of cancer⁽¹⁰⁾. Both Touch Imprint Cytology and Frozen Sections are dependable intraoperative consultation diagnostic modalities. Touch Imprint Cytology provided better cellular morphology and fewer artifacts⁽¹¹⁾. On the other hand Frozen Sections provided more tissue architectural details but frequently hampered by freezing artifacts. Touch Imprint Cytology alone may provide a correct diagnosis in vast majority of cases with minimal expense and without the need of sophisticated cryostat machine thus making it quite suitable for many hospitals where cryostat machines are not available.

Ahmareen Khalid et al have studied the efficacy of Touch Imprint Cytology in the diagnosis of various pathological processes was evaluated and compared with Frozen Section in intraoperative consultation. We examined 60 cases from various sites of the body that were referred for intraoperative consultation. All cases were analyzed by Touch Imprint Cytology followed by Frozen section. We found diagnostic accuracy of both procedures comparable. The diagnostic accuracy in distinguishing benign from malignant lesions by combined procedures was 100%. There were no false positive or false negative cases. For diagnosing specific subtypes of malignancy, the diagnostic accuracy of each method alone was 96.6% with a sensitivity of 86% and specificity of 100% and the combined sensitivity 90%⁽¹²⁾.

In conclusion, this study on gallbladder tumors is an attempt to define the significance of imprint cytology for characterizing the nature of tumor, whether it is benign or malignant and to evaluate the strength of correlation between imprint cytology and histopathology.

Data suggest that imprint cytology is an invaluable tool for rapid intra operative diagnosis of gallbladder tumor and aids in formulating future therapeutic strategies.

Imprint cytology technique is very much cost effective, simple and quick method of diagnosis. It is reliable in terms of accuracy, sensitivity, specificity and positive predictive value. It does not affect the utility of the specimen for histopathology. Imprint cytology is the only method which gives intra operative diagnosis within 25 minutes. Both frozen section and touch imprint cytology provide accurate diagnoses. The accuracy is quite high in both, particularly in differentiating benign from malignant lesions. Imprint cytology reveals crisper cytological details and it has further advantage of being inexpensive, simple, and quicker than frozen section. The major limitation of present study is the sample size. The size taken does not give enough power to detect a difference between individual groups of tumor. Therefore the present study may be reviewed as a component of a large multicentric study to reach a definite conclusion. The skills and experience of the cytopathologist is pivotal to improve the sensitivity of the diagnostic method. In addition application of immunocytochemistry may help to increase the sensitivity.

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Fig 1: Plaque like thickening of the gallbladder in Xantho granulomatous cholecystitis

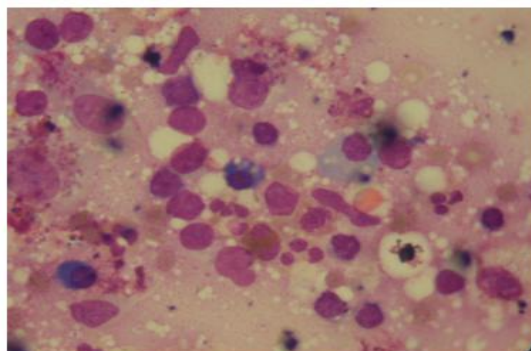


Fig 2: Xantho granulomatous Cholecystitis showing lympho Plasmacytic infiltration and Foamy macrophages



Fig 3: Thick walled gall bladder Resembling chronic cholecystitis.



Fig 4: Healthy non bile stained mucosa in thick walled gallbladder.

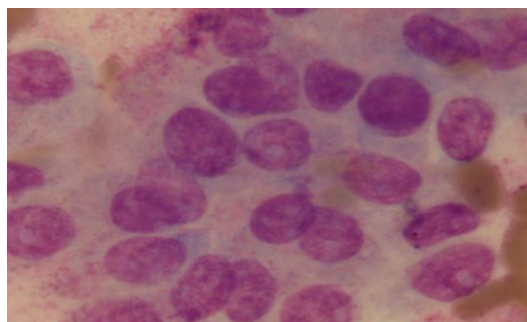


Fig 5: Adenocarcinoma ,pleomorphic cells with loss of cohesion,acini formation in gall bladder.



Fig 6: Gall bladder along with adjacent liver bed and regional Lymph nodes.

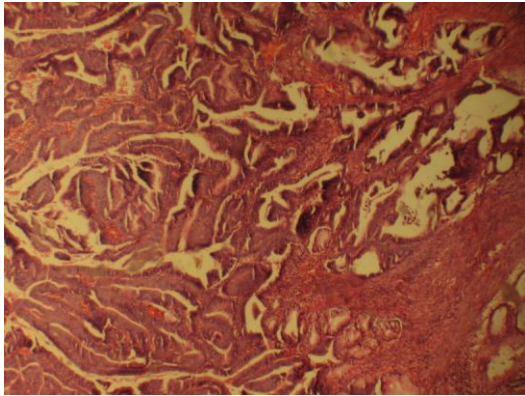


Fig 7: Adenocarcinoma of bladder subtle, infiltrating pattern.

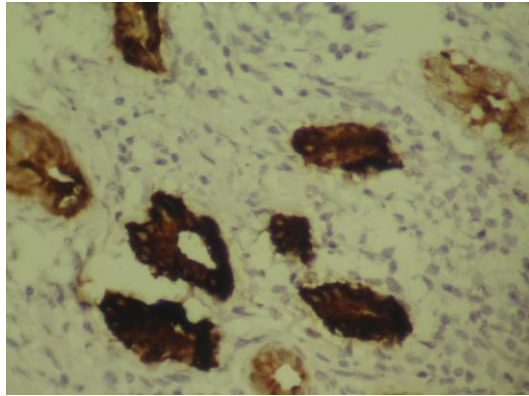


Fig 8: ck 7 positivity in another case Of Gall bladder carcinoma