



RESEARCH ARTICLE

CARCINOMA OF THE GALL BLADDER: A RETROSPECTIVE STUDY IN A TERTIARY CARE HOSPITAL OF DELHI WITH IHC PROFILE

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ABSTRACT

Background: Gall Bladder Cancer (GBC) is a malignancy of aggressive nature with ethnic and geographical patterns. It is most commonly reported from Chile (16-27/100,000), Japan. In India, its incidence is from 1.01 per 100000 to 10.1 per 100000 in males & females respectively.

Aim and Objectives: The objectives of the study were to analyze the demographics, incidence, histopathological findings with immunoprofiling in gall bladder carcinomas.

Material and Methods: A retrospective analysis of 1208 cholecystectomy specimens were received for histopathology from 2011 till 2015 in a tertiary care hospital Delhi and analyzed.

Results: A total number of 45 cases of carcinoma of gall bladder were diagnosed which included 43 cases of Adenocarcinoma, 2 cases of Adenosquamous carcinoma. Most gall bladder adenocarcinomas were of pancreatobiliary-type, showing variable degrees of differentiation. Female: male ratio of 3.6: 1 was observed, with mean age being 51 years.

Conclusions: Gall bladder carcinoma is rare, constituting 3-5% of all the gastrointestinal tumours. Gall stones are considered as one of the most common factors for initiating the malignant process. The patients are in advanced stages at initial presentation. Early diagnosis is difficult usually as they are common with benign disease of gall bladder. Further, correlation with radiographic and clinical findings is also important to avoid misdiagnosis.

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INTRODUCTION

Gall Bladder cancer (GBC) is a highly malignant tumour. It has varied geographical and racial distribution. The incidence of this cancer is more in countries such as Chile (16-27/100,000), Japan (7/100,000), Poland (14/100,000), Israel (5/100,000) and southern Pakistan (11/100,000). The incidence of carcinoma gall bladder in India ranges from 1.01 per 100000 for males to 10.1 per 100000 for females (Indian Council of Medical Research (ICMR) Annual Report of population-based cancer registries of the National cancer Registry Programme, 1996). In India, it is most common in northern and north-eastern states of Uttar Pradesh, Bihar, Orissa, West Bengal and Assam. Delhi and Bhopal have recorded the highest incidence rates i.e. 6.6 and 5.2 for females and 1.9 and 2.2 for males per 100,000 respectively.

It is higher in Northern India than South India (National Cancer Registry Programme, 2010). The incidence of GBC increases after the age of 45 years and is maximum at the age of 65 years (National Cancer Registry Programme, 2010). Gall stones have been found to be associated with GBC in 86% cases. Other normalities detected are porcelain gall bladder (10-20%) and abnormal choledocho-pancreatic duct junction. Larger gallstones >3 cm are more predisposing to cancer. Recently, Helicobacter infection has also been found to be linked with GBC (Albores-Saavedra, 2011 and Fukuda, 2002). According to site of origin, fundus has been the commonest location with frequent invasion of liver, lymph node and other adjacent organs. GBC can be divided into are pancreatobiliary-type adenocarcinomas, showing variable degrees of differentiation. The remaining epithelial cell types occurring in the gallbladder include adenosquamous carcinoma, squamous cell carcinoma, small cell carcinoma, and undifferentiated carcinoma. Histological classification is often difficult (Pandey, 2001 and Saul, 2009). The objectives of the study

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were to analyze the demographics, incidence, histopathological findings with immunoprofiling in gall bladder carcinomas.

MATERIAL AND METHODS

A retrospective study was carried out from 2011 to 2015 in Department of Pathology in a tertiary hospital, Delhi, in 1208 cholecystectomy specimens submitted for cholelithiasis and the clinically suspected cases of gall bladder tumors and correlated with the biochemistry findings. We included all the diagnosed cases of Gall bladder carcinomas and excluded all those cases which were clinically suspected as carcinoma but were not histopathologically confirmed and cases for which specimen or paraffin blocks were not available. All specimens had minimum three sections from different anatomical regions of the gall bladder or processed entirely. Histopathological sections were stained with hematoxylin and eosin stain, special mucin stains such as PAS & Alcian blue. Immunohistochemical markers such as Ki-67, P53, CK-7, CK-5, CK-20, CEA, EMA and CA19-9 were performed in cases of malignancy. Also our emphasis was to know the following

- Thrust was laid on intraoperative examination of gall bladder for selection of samples to be sent for histopathology which helps to decrease the expenditure on resources required for histopathological examination.

RESULTS

A total 45 cases of carcinoma of gall bladder were diagnosed which included 43 cases of adenocarcinoma and 2 cases of adenosquamous carcinoma. Most gall bladder adenocarcinomas were of pancreatobiliary-type, with variable degrees of differentiation. Carcinoma insitu changes were noted in 7 other cases. Female:male ratio of 3.6:1(BC-1) was observed with mean age being 51 and median 45 years. 38 cases showed presence of gallstones. 33 cases showed growth arising from the fundus, 3 cases from body, 1 involving neck and the rest were diffusely involving the wall (Piechart, Fig.1). Out of 45 cases, only 6 cases were suspected as malignant either radiologically or as preoperative findings which showed concordance.

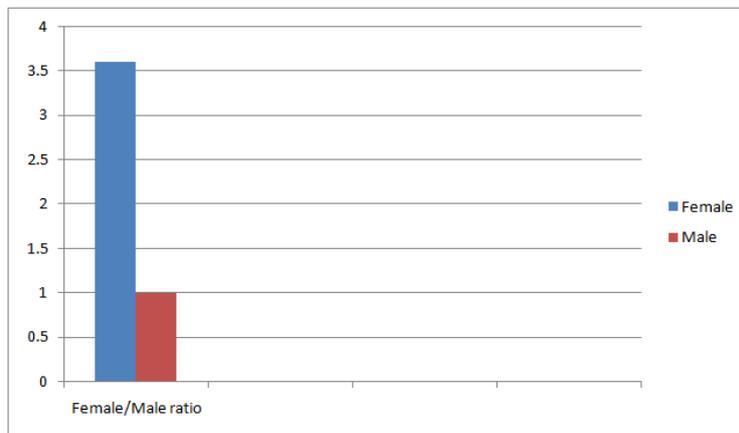


Figure 1. Female/Male Ratio

Table 1. Histological Diagnosis with Grading

Histological Type	Number of cases	Percentage
1. Adenocarcinoma	43	96
Well differentiated	29	67.4
Moderately differentiated	10	23.2
Poorly differentiated	4	9.3
2. Adenosquamous carcinoma	2	4
Total	45	100

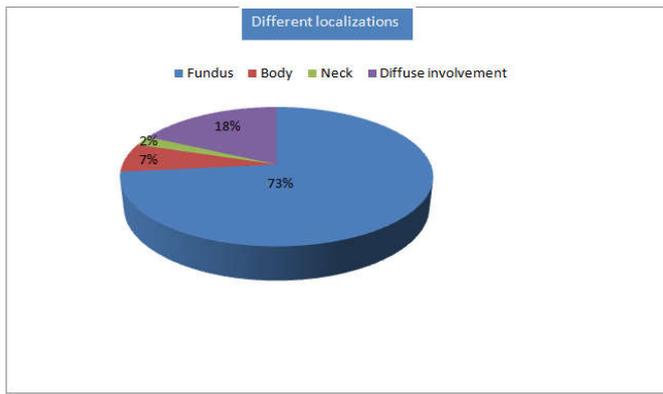
Advances in knowledge

- To know the incidence of GBC in North India, with most common pattern.
- Study of various patterns of GBC.
- As seen in our study the number of clinically suspected cases and incidental carcinomas of Gall bladder are high and we endorse the histopathological examination of all excised GB specimens
- To study IHC for GBC and its use in GBC.

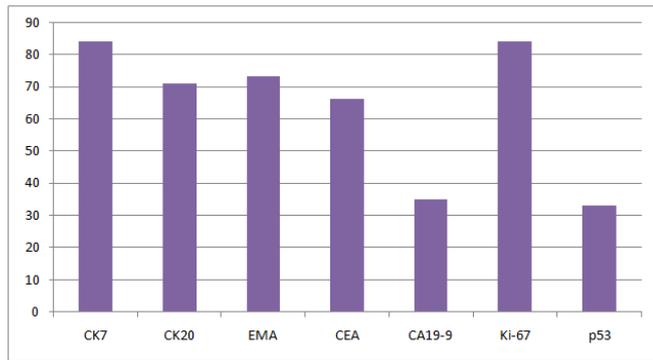
Application in patient care

- Factors such as age & sex with dietary factors which are important for early detection of GBC.

Out of the remaining 39 cases, in 32 cases, lesions were detected on gross examination whereas only in 7 cases, malignancy was detected microscopically. Of the 43 cases of adenocarcinoma, 2 cases of adenocarcinoma with focal micropapillary configuration with pyloric and intestinal metaplasia (Fig.6) and 1 case of Adenocarcinoma with neuroendocrine differentiation. A case showed Adenocarcinoma of GB with involvement of the duodenal mucosa (Fig.4) whereas another case showed involvement of CBD and Pancreas (Fig.5). Five cases showed polypoidal configuration, 4 cases associated with insitu changes. In terms of grading, 29 cases were of well differentiated adenocarcinomas (Fig.2), 10 cases of moderately differentiated adenocarcinomas and 4 cases of poorly differentiated carcinomas (Table 1).



Piechart1



Barchart-2- IHC study. Percentage of Carcinoma GB cases showing positivity for different markers (n-45)

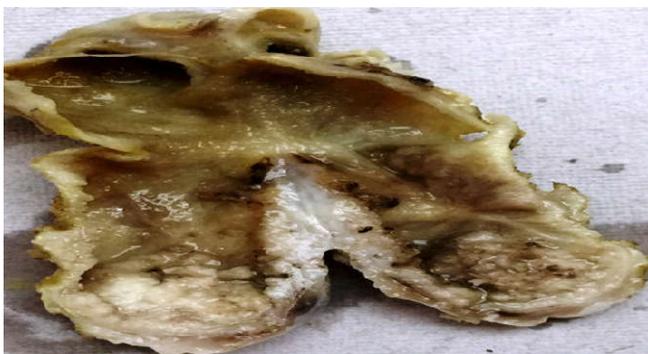


Fig. 1. Gross findings showing Papillary grey white growth

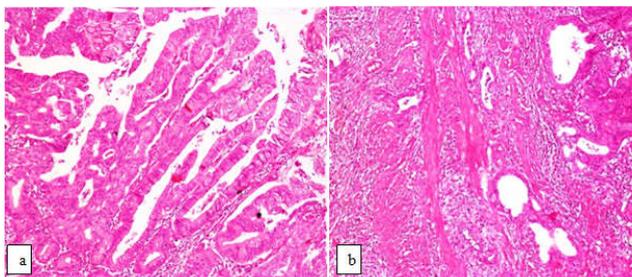


Fig. 2a,b Well differentiated AC with villoglandular pattern and invasion into muscle layer (100X,H&E)

Adenosquamous carcinoma was seen in 2 cases (Fig.3). Mucin stains, PAS showed positivity in 22 cases and Alcian blue in 17 cases of malignant cases. CK-7 positivity was seen in 38 cases, CK-20 in 32 cases out of which, 20 cases were also positive for CK-7. CK-5 positivity of squamous cell component in Adenosquamous carcinoma was also noted (Fig.9).CEA was positive in 30 cases whereas EMA in 33 cases.

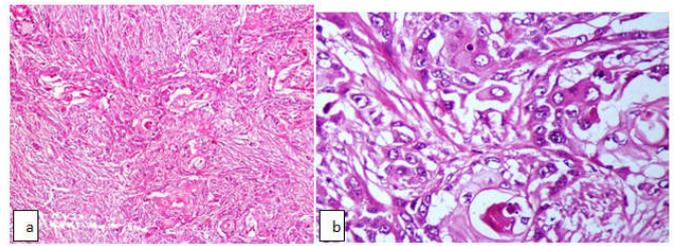


Fig 3 a,b- Adeno squamous carcinoma (100X,400XH&E)

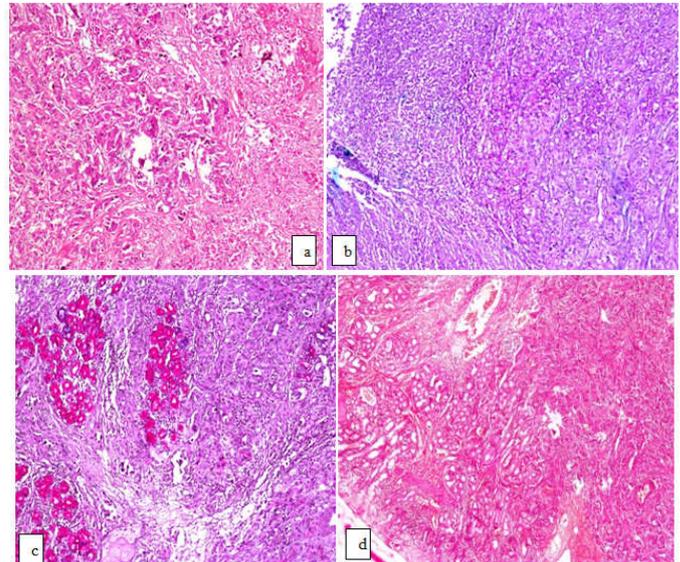


Fig. 4a,b,c,d Moderately differentiated AC with invasion into duodenum(100X,100X,100X,H&E, PAS with AB-Positive, PAS with AB-Negative, H&E

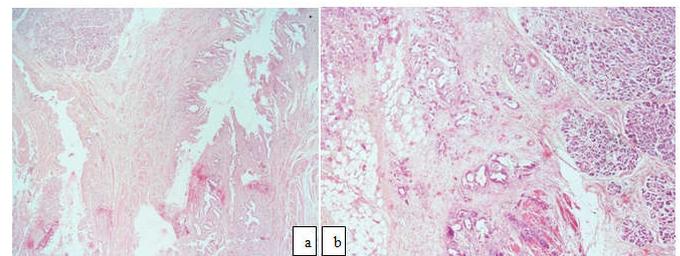


Fig.5a,b. Involvement of CBD and pancreas (40x,100x,H&E)



Fig. 6a,b,c- AC with micropapillary configuration, intestinal metaplasia and in-situ changes. (100X,100X,100X,H&E)

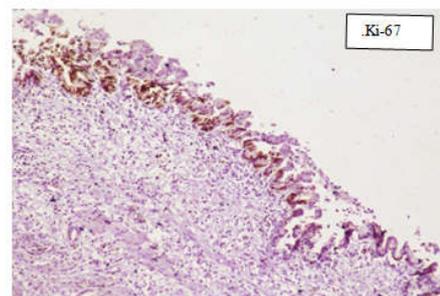


Fig.7-Ki-67 positivity (40X)

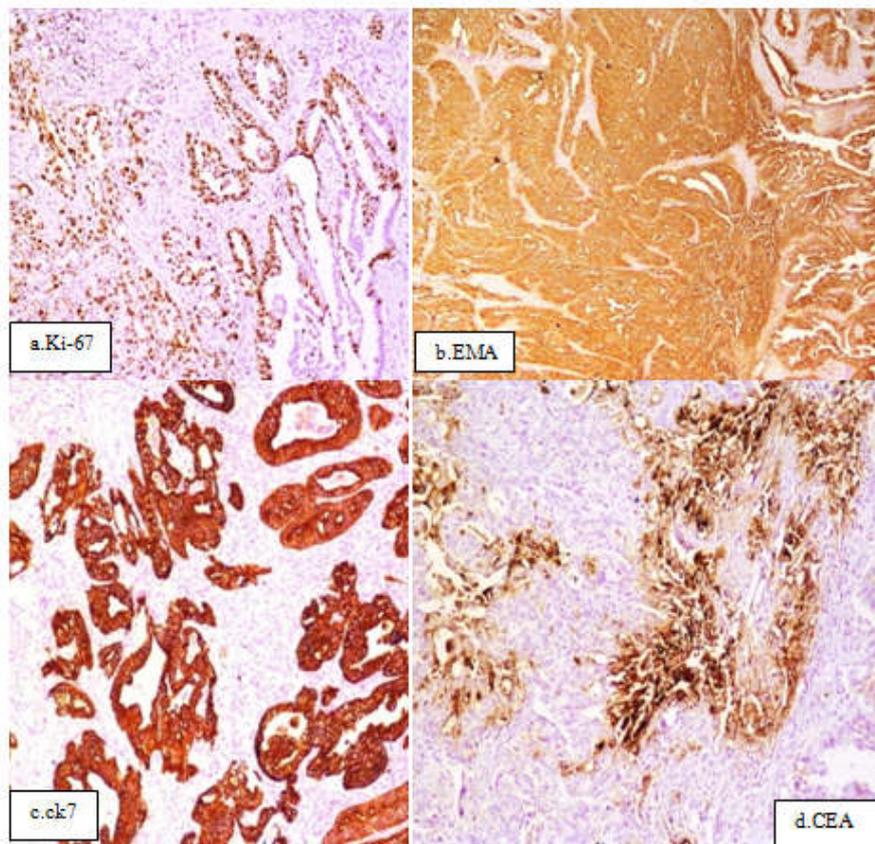


Fig 8 a,b,c,d- Ki-67, EMA, CK-7, CEA positivity in Adenocarcinoma(40X,40X,100X,40X)

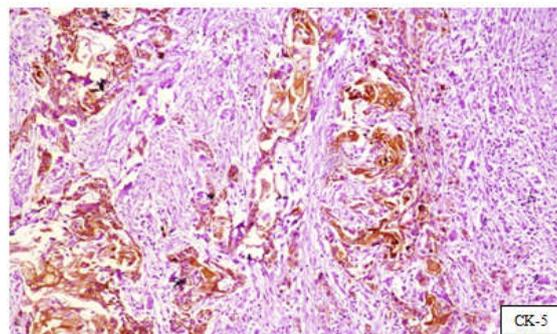


Fig.9. CK-5 positivity of squamous cell component in Adenosquamous carcinoma (40X)

CA19-9 was positive in 16 cases. Ki-67 scoring was high in 38 cases whereas p53 in 15 cases (BC-2, Fig.7, 8). The majority of the cases presented at stage –II (35 cases).

DISCUSSION

Gallbladder cancer is the commonest cause of death among the biliary malignancies (Khan, 2010). It is usually detected at an advanced stage due to its non-specific symptoms (Le, 2011). Gallbladder cancer is among the top five common cancers in females in Delhi (Tyagi, 2008), whereas in the endemic zone of eastern India, it is the third most common malignancy of the gastrointestinal tract (Shukla, 1985). Based on the Adyar Cancer Registry report, the prevalence figure of GBC was 0.52 % amongst men and 0.66 % amongst women (Shanta, 1990). In our study, 45 patients were followed up for over a period of 4 years. The incidence seen in our study observed was 3.7%. The age group of the patients was 25-80 years with a maximum incidence in the sixth decade of life, similar to study by Khan *et al.* (Khan, 2010).

The geographical variations describe the cultural, dietary or genetic differences in the Indian population. The reported female to male ratio worldwide is 4:1 and we found a ratio of 3.6:1. The disease was found to be more common in the northern states of India. The various risk factors included age, sex, gall stones, diet and chronic inflammation, all of which could not be established in this retrospective study, except for a high incidence of gall stones (85%) and chronic inflammation (Shukla, 1985). Gall stones are the most common factor in carcinogenesis. Physical trauma which was produced by the stones might have resulted in epithelial dysplasia and ultimately in the progression to carcinoma. In our study, 85% of the patients had associated gall stones. Similar results were noted by Khan *et al.*, in 96.15% of the cases as well as from MD Anderson Hospital in which 88% incidence of gallstones (Khan, 2010 and Perpetuo, 1978). Gall stones have been present in 70% of cases in Indian population (Pandey, 2001). These studies favour possible causal association between gallstones and GBC. The present series show the mean age of the patients to be 51 years, with a wide range of 28-82 years as also noted by in other studies (Shukla, 1985; Pandey, 2001 and

Shanta, 1990). Results from our study showed that gallbladder cancer is predominantly a disease of elderly females; with an overall Female to male ratio of 3.6:1. These results were consistent with the results of other studies (Shukla, 1985 and Pandey, 2001) where it was reported to be 1:3 and 1:2.5 respectively. Adenocarcinomas are the most frequent histological subtype of the malignant gallbladder neoplasms, representing approximately 90-95% of all cases. In contrast, squamous cell or 'epidermoid' carcinomas and adenosquamous carcinomas are rare (Roa, 2011). In our study Adenocarcinoma, not otherwise specified, constituted the most common (96%) histological type followed by Adenosquamous (4%). Histopathology revealed comparable findings in other study done by Hamdani *et al.* who reported 114 cases of Adeno carcinoma and 1 case of Adenosquamous carcinoma GB (Hamdani, 2012). Seven cases of incidental GBC which had negative clinical, radiological and intraoperative macroscopic findings for any growth (1.5%) were observed in the current study, five cases had polypoidal mass (1.1%), these cases were report as adenocarcinomas.

Our results were comparable with a study from Malaysia where 9 cases of incidental GBC were reported, whereas Lobo *et al.* reported 4 cases of incidental carcinomas as unexpected histopathological finding in the resected specimens of elective cholecystectomy which were performed for benign gall bladder diseases (Khoo, 2008; Lobo *et al.*, 2012). PAS and Alcian blue positivity was observed in 48.8% and 37.7% malignant cases which was similar to study done by Gupta *et al.* in 57% and 36% cases. The immunoprofile of GBC is similar to that of bile duct carcinoma (intrahepatic and extrahepatic) and pancreatic carcinoma. Cytokeratin 7 (CK7) is almost always positive, Cytokeratin 20 (CK20) can be positive, more often in extrahepatic bile duct carcinoma than intrahepatic cholangiocarcinoma.

In addition, carcinoembryonic antigen-monoclonal (CEA-M), carbohydrate antigen (CA19-9), B72.3, MUC1, and MUC5AC are also positively expressed in bile ducts and GBC but can be focal (Pritchard, 2009). CK-7 positivity was seen in 84% cases whereas CK-20 in 44% cases which was similar to study done by Duval *et al.* in 82% cases for CK-7 and 27% cases for CK-20 (Duval, 2000). Despite the advances in medical imaging it is still difficult to diagnose gall bladder cancer pre-operatively. According to study done by Gonzalez *et al.*, he emphasized on diagnosing GB carcinomas based on patient's clinical details and intraoperative macroscopic findings instead of sending all the specimens for histopathology as it can help decrease human and material resources needed for the traditional method for detecting gallbladder carcinoma (Romero-González, 2012).

Conclusion

As seen in our study with concordance to other studies the incidence of GB carcinomas is quite high in Delhi and North India. Carcinoma of gall bladder is rare and accounts for only 3-5% of all the gastrointestinal tumours. Stones are considered as one of the most common factors for initiating the malignant process. Carcinoma of the gallbladder has a poor prognosis since it usually presents at a very advanced stage. Detection of carcinoma at an earlier stage is very difficult because the symptoms most of the time mimic benign gallbladder diseases. As seen in our study the number of clinically suspected cases and incidental carcinomas of Gall bladder are high.

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