Histopathological Spectrum of Metaplasia, Dysplasia and Malignancy in Gall Bladder and Association with Gall Stones.

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Abstract: *Cholecystectomy is the most common abdominal surgery & in majority of the cases, is performed due to gall-stones that seems to cause premalignant changes like dysplasia.The objectives of this study was to determine the incidence of metaplasia, dysplasia and malignancy in cholecystectomy specimens and explored its association with gall stones. All total 900 cholecystectomy specimens were collected for the period of 2 years done in the institute. Clinical features were reviewed & prevalence of gall stone was noted with reference to presence or absence of dysplasia or malignancy. A total of 50 cases were found to have their dysplasia done or dysplasia with malignancy or malignancy without dysplasia 36 cases showed isolated dysplasia & 14 cases carcinoma, out of which 12 had dysplasia in adjacent mucosa. Out of 30 specimens showing metaplastic changes, including one rare case of squamous metaplasia ,19 cases were associated with dysplasia and 10 cases with carcinoma. Age range was 12 to 80 years for dysplasia and 38 to 84 for carcinoma. Gall stones were found in 88.8% (i.e., 32/36) cases showing dysplasia and 78.5% (i.e.,11/14) of cases showing carcinoma. The incidence of dysplastic change in the adjacent mucosa in cases of GB carcinoma was 85.7 % (i,e, 12/14). The lesions were predominantly found in females.* ***Conclusion:*** *These observations thus reveal a relationship between gall-stones and premalignant and malignant conditions of gall bladder, though carcinoma may appear de novo.*

INTRODUCTION

Gallstones are the most common biliary pathology making cholecystectomy the most common abdominal surgery throughout the world. Gallbladders with carcinoma usually also contain calculi (80-90% of cases.)[1]. A high incidence of carcinoma of gall bladder is observed in subjects with high incidence of stones or who have been harbouring stones for a longer duration[2].

Two schools of thought have emerged as to the pathogenesis of the malignant lesions of the gall bladder due to gall stone disease: (a) Epithelial hyperplasia > through dysplasia to > neaplasia. (b) Epithelial metaplasia leading to dysplasia, carcinoma-in-situ and invasive cancer[3]. Current literature favors the second hypothesis. Presumably, gall bladder containing stones develop cancer as a result of constant irritation, trauma and chronic inflammation.

It has been observed that subcellular, biochemical, histochemical and molecular alterations in epithelial cells of the gall bladder may precede the development of malignancy[4]. Therefore, the study of dysplastic changes and malignancy associated with gallstone disease is important for better understanding of gall stone disease. The present work has been designed with the aims of finding out: (1) An association between mucosal metaplasia, dysplasia and carcinoma. (2) Correlation between carcinoma and gallstone.

MATERIALS AND METHODS

900 surgical specimens of gall bladder were included in the study. Clinical features were noted in each case including the presence or absence of stones. The gall bladders were promptly fixed in 10% formalin following excision to avoid autolytic changes in mucosa. If stones were present they were washed and estimated in terms of number, size and type of stones. Sections were taken from any suspicious areas of mural thickening or mucosal ulceration. Small masses or polyps were also submitted for histopathologic examination. A total of three full-thickness tissue sections including fundus, body and neck of gall bladder were taken from each specimen. Subsersal Cystic lymph node, if, present was also taken.

After fixation, the tissue were processed with routine methods and 5 mm thick sections were cut and stained with hematoxylin and eosin for studying histopathological changes. When dysplasia was found additional multiple sections were taken for microscopic examination.

RESULTS

Total number of cases included in the study for analysis was fifty. Isolated dysplasia was present in 36 cases and gall bladder carcinoma (GB CA) in 14 cases.

Gallstones were present in forty-three out of fifty cases among which thirty-two cases i.e. 88.8% were associated with *dysplasia* and eleven cases i.e., 78.5% were associated with *dysplasia* and *carcinoma*.

The age range of isolated dysplasia (not associated with carcinoma) was 12-80 years, the mean age being 47 years & male to female ratio being 1:1.2.

The age range in cases of ***gall bladder*** carcinoma was 38 to 84 years, the mean age being about 57.3 years & male: female being 1:1.8.There was difference of 10 years in mean age in patients showing dysplasia & carcinoma.

Of the total 14 cases of ***gall bladder*** carcinoma, 12 cases revealed *dysplasia* in the adjacent mucosa. In one case, tumour presented as a gall bladder mass and the superficial or adjacent mucosa was not available for study. In another case, no dysplasia was seen in adjacent mucosa.

The *metaplastic changes* were seen in continuity with dysplastic epithelium as well as focal separate lesions. These changes were more frequently seen in areas showing dysplasia. It was seen that stones were present in all (100%) gall bladders with antral/intestinal metaplastic changes (Table1).

Table 1: *Incidence of Gall Stones in dysplasia and carcinoma*

	Total cases	Stone present	Stone absent
Dysplasia	36	32(88.8%)	4(11.1%)
Carcinoma	14	11(78.5%)	3(21.4%)

Metaplasia/dysplasia, in the adjacent gall bladder mucosa, 12 out of 14 cases showed metaplastic changes (antral or intestinal or both) i.e. 78.5%. 11 out of 14 (85.7%) cases shows dysplastic changes. Intestinal metaplasia was present in 6(42.8%) and antral/peudopyloric metaplasia in 4(28.5%) cases One case showed squamous metaplasia (71%) and in one cases, both antral as well as intestinal metaplasia was present (table 2). identified by the presence of goblet cells in the epithelium (fig-1). Antral metaplasia was identified as glands lined by cuboidal to columner epithelium with large quantities of clear mucin having morphological resemblance to Brunner's glands (fig-

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1). Squamous metaplasia was identified by the presence of typical squamous epithelium differention in gall bladder mucosa. Cholesterolosis was identified by presence of cholesterol laden foamy macrophages in the lamina propria of gall bladder mucosa (Fig. 1,2,3)

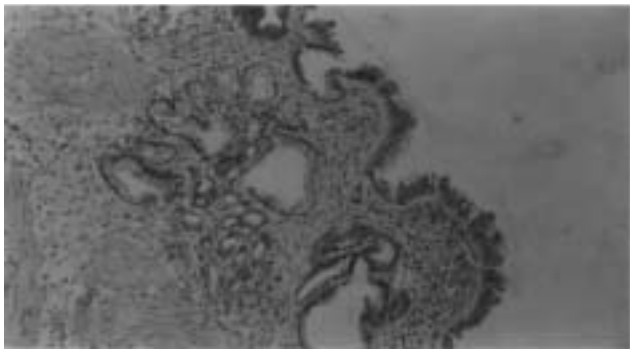

Figure 1: *Gallbladder wall showing normal mucosa in continuity with intestinal metaplasia and dysplasia. Antral type glands are seen in lamina propria some of which are showing dysplastic changes. (H&EX100)*

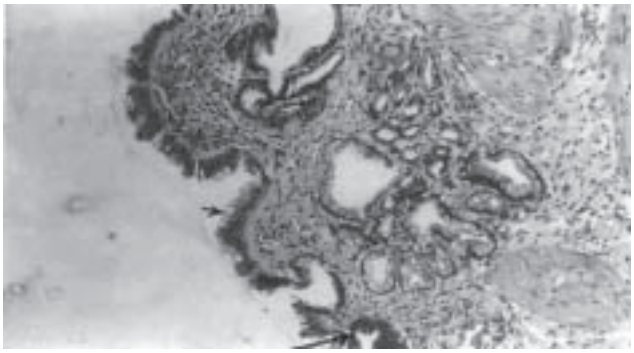

Figure 2: *Picture of gallbladder wall showing mucosa in transition from normal to low grade dysplasia as shown in inset. (H&EX100), inset (H&EX400)*

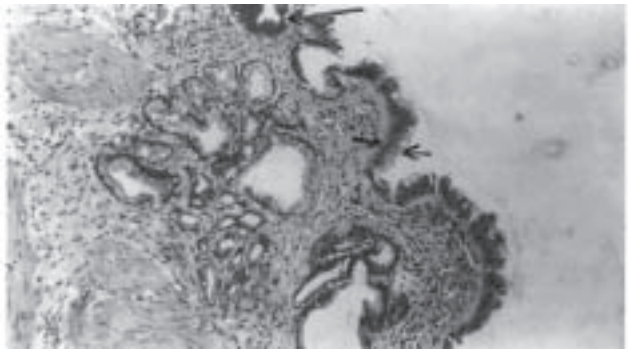

Figure 3: *Picture of gallbladder wall transition from normal (small arrow) to high grade dysplasia. (Big arrow). Adysplastic gland is sesn in lamia propria. (marked by star). (H&EX100)*

Table 2: Histopathological features in Adjacent Mucosa in Cases of Isolated Dysplasia and their Incidence with Gall Stones

		Stones present	Stones absent
Acute cholecystitis	7(19.4%)	5(71%)	2
Chronic cholecystitis	29(30.5%)	27(93%)	2
Cholesterolosis	5(13.8%)	5(100%)	0
Antral metaplasia	10(27.7%)	10(100%)	0
Intestinal metaplasia	9(25%)	9(100%)	0

Table3: *Histopathological Changes in Adjacent Mucosa in Cases of Gall Bladder Carcinoma*

	Total	With stone	Without stone
Dysplasia	12	11(91.6%)	1
Antral metaplasia	4	4(100%)	0
Intestinal metaplasia	6	5(83%)	1
Squamous metaplasia	1	0	1(7.14%)
Cholesterosis	1	1(7.14%)	0

Table 4: *Histological Classification of Gall Bladder Carcinoma found in this Study*

Histological type	No. of cases	Percentage
Well differentiated adenocarcinoma	**1**	**7.1%**
Papillary adenocarcinoma	**2**	**14.2%**
Mucin secreting adenocarcinoma	**2**	**14.2%**
Moderately differentiated adenocarcinoma	**5**	**35.7%**
Poorly differentiated adenocarcinoma	**3**	**21.4%**
Adenosquamous carcinoma	**1**	**7.1%**
Total	**14**	

DISCUSSION

It is well known that gallstone is an important risk factor for the development of carcinoma gall bladder but causal relationship is still unproven. Finding of gallstone in over 75% of cases of GB CA in this study do suggest a possible link. Although this does not prove gall stone to be a causative factor, but such high association points towards an important etiological risk factor. Martinez et al[5] have also illustrated that low and high grade dysplasia, tubular adenomas, carcinoma in situ and invasive carcinoma were more frequent when cholilithiasis was present($p<.05$) than in cases without lithiasis[5]. The results of the study done by Vitteta et al[6] showed that primary carcinoma of gall bladder was always associated with single or multiple cholesterol gallstones that were impacting on gall bladder wall. In present study gallstones were present in over 78.5% of cases of gall bladder carcinoma. Piehler JM, Crichlow RW et al[7] have also found the incidence of 65-90% of gallstones in the patients with gall bladder carcinoma that is in concordance with present study. No patients with gall bladder carcinoma had brown or black pigment stonesand in present study too, none of the patients had pigment stones.

The pathogenesis, by which the gallstones lead to gall bladder carcinoma is a matter of debate, but it has been suggested that the frequency of various epithelial changes that is ulceration, mucosal hyperplasia, metaplasia and dysplasia is more common in calculous cholecystitis than in normal gall bladders. It is due to this constant chronic irritation and inflammation leading to such epithelial changes that gall stone may be involved in the pathogenesis of gall bladder cancer. In the gall bladders that showed dysplasia, such changes were seen more frequently in the fundus and body region rather than neck.

The incidence of metaplastic changes in dysplastic gall bladders in our study was 57%. In 27% of cases antral metaplasia was seen and in 25% of cases intestinal metaplasia was also present. Gallstones were present in 91% of gall bladders showing dysplasia

and in all cases (100%) of cholelithasis showing both antral and intestinal metaplasia.

Yamigawa H[8] have found gallstones in 86.9% of cases with dysplasia, results; very similar to present study. They have found intestinal metaplasia and pseudopyloric gland metaplasia in 80.4% and 100% of patient with gallstones.

Gupta SC et al[9] have also found high prevalence of gall stones in gall bladders with metaplastic, dysplastic and neoplastic changes than those gall bladders which showed no epithelial changes.

Sanjay Mukhopadhyaya et al[10] showed significant association between antral and intestinal type metaplasia (p<.001) & between intestinal metaplasia and dysplasia(p<.001,chi square test) suggesting, a progression from antral type metaplasia to dysplasia.[10]

Gupta SC et al[7] have also found high prevalence of gall stones in gall bladders with metaplastic, dysplastic and neoplastic changes than those gall bladders, which showed no epithelial changes.

In gall bladder carcinomas, gallstones were found in 78.5 percent of cases. 3 cases i.e. 21 percent were not associated with gallstone. Eleven cases i.e. (78.5%) showed metaplastic changes. Out of which stone was present in all but two cases. Among these two cases one case showed squamous metaplasia and another case showed intestinal metaplasia. The cause of metaplastic changes in these two cases is not clear.

The intestinal metaplasia was focal and interrupted and more frequently associated with dysplastic changes. In few cases goblet cells representing intestinal metaplasia were also seen arising within the area of antral metaplasia.

Liatio M[4] studied 71 cholecystectomy specimens and found dysplastic changes in 24 gall bladders. Metaplasia was noted in 20 cases and in 14 cases was associated within the dysplastic portion.

In present study dysplasia was seen in 12 out of 14 (85.7%) cases of gall bladder carcinoma in the adjacent or superficial.

Such high percentage suggests definite close association between dysplasia and carcinoma Albores Saavedra et al[11] and Dowling J.P.Kelly[3].

Metaplastic changes were seen in 11 out of 14 cases of gall bladder carcinoma. Intestinal metaplasia was seen more frequently than antral metaplasia. One case showed both intestinal as well as antral metaplasia. These metaplastic changes were seen adjacent to dysplastic portions as well as in the areas of frank malignany further strengthening the hypothesis that dysplasia may occur in simultaneously metaplastic epithelium. One case showed squamous metaplasia in superficial mucosa most of which was ulcerated and showed a poorly differentiated aderosquasous cell carcinoma infiltrating throughout the wall the lanes of gall bladder. This case was not associated with gallstone.

Although cholesterolosis is not considered a premalignant condition, but we have found one case of GB CA showing features of cholesterolosis focally. This was a well differentiated papillary adenocarcinoma. It could be primary event or it may so happen that adenocarcinoma arose first and then tumour epithelium absorbed cholesterol from bile & foamy cells were produced secondarily. Cholesterolosis is usually associated with benign papillary hyperplasia.

Akiyama T et al[12] have reported two cases of carcinoma in situ of gall bladder associated with cholesterolosis. One was mucinous carcinoma on subsequent sections & other was papillary adenocarcinoma.

The male and female sex ratio in cases of dysplasia was 1:2.2 and in cases of carcinomas was 1:1.8 in the present study, hence the frequency of GB cancer is more common in females. [4,5,6,14]

The study shows a difference of about 10 years in the cases of dysplasia and gall bladder carcinoma suggesting that a period of about 10 years is needed for dysplasia to convert into carcinoma. Roa et al[13] have also studied preneoplastic lesions and gall bladder cancer. They statistically analyzed the time required for change of dysplasia to carcinoma of gall bladder using age as main parameter and concluded that the period required to progress from dysplasia to carcinoma would be around 15 years observing a continuum in the progression of disease.

CONCLUSION

The results of this study showed presence of gallstones in 88.8% of cases with dysplasia and 78.5% cases of adenocarcinoma of gall bladder and thereby support a good association with gallstone and the incidence of dysplastic and malignant changes seen in choleaystectomy specimens of gall bladders. The high incidence (85.7%) of severe dysplastic changes in the adjacent mucosa in cases of GB carcinoma strongly suggest that it could be a *premalignant* lesion. However, the absence of dysplastic change in adjacent mucosa in one case means that GB carcinoma can also arise directly without the preceding change of dysplasia. Mean age of patients with dysplasia was 10 years younger than patients with carcinoma. The disease prevalence shows a female preponderance in the study.

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