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Original Research Article

Ki-67 Expression in Premalignant and Malignant Lesions of Gallbladder in Southern Part of Assam

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Abstract:

Introduction: Gallbladder cancer is the most common malignancies of the biliary tract and among the gastrointestinal malignancies. Gall stones, pyloric and intestinal metaplasia have been found to be associated with gall bladder carcinoma. Ki-67 is a good marker for cell proliferation and its expression is correlated with various lesions of gall bladder.

Aim: The aim of the study is to assess the ki-67 expression in different malignant and premalignant lesions of gall bladder.

Materials and Methods: The retrospective study included 212 cases of gall bladder lesions, out of which 22 cases were malignant and 75 premalignant lesions. Immunohistochemistry was done for ki-67 expression. A percentage of >20% stained cells was considered to be positive regardless of the intensity of staining.

Results: It was observed that out of 212 cases, 179(84%) cases were female and 33(16%) cases are males. The incidence of gall bladder lesions was highest in 41-50 years (36%) and malignant cases were highest in >50 years (55%). In the malignant groups, Ki-67 expression was <20% in 59% cases, 20-30% in 27% cases and >30% in 14% cases. Ki-67 expression was highest in moderately and well differentiated gall bladder carcinomas than poorly differentiated carcinomas.

Conclusion: Ki-67 can be used as a good marker of aggression of various lesions of gall bladder.

Keywords: Ki-67, Gallbladder Carcinoma.

Introduction

The gallbladder is a pear shaped organ present in the inferior surface of right hepatic lobe. The gallbladder wall has three layers namely mucosa, muscularis propria and serosa. Gallbladder cancer is the most common malignancies of the biliary tract and among the gastrointestinal malignancies; gall bladder cancer ranks the 5th [1]. The Indian council of medical research cancer registry has reported incidence rate 4.5% in males and 10.1% in females per 100,000 populations in north India [2]. Due to the biological behaviour of the tumour, diagnostic delay and tumour's extension to the nearby organs at the time of diagnosis, the prognosis is poor and the survival rate is also less [3]. It is one of the most deadliest malignancies and females are mostly affected [4].

There is a strong correlation between gallstones and increased risk for gall bladder cancers. Pyloric and intestinal metaplasia has been found to be associated with gall bladder carcinoma and metaplasia is more commonly seen in the area surrounding the tumour. Dysplasia is also seen in the surrounding of tumour. [5, 6] For the growth and behaviour of many human tumours, cell cycle activity and kinetics act as good indicators. [7-9]. Ki67 is a nucleoprotein. It is a marker for cell proliferation. In G0 and G1 phase of cell cycle, it is not expressed. In G2 and M phase, the highest concentrations of Ki-67 are achieved.

As an indicator of cell proliferation, Ki-67 detection by immunohistochemistry has been accepted.[8] Recent studies established the fact that an increased expression of Ki67 indicates a better survival in few tumour as these tumours have better response to radiotherapy as irradiation destroys preferentially the quickly dividing cells. [10,11,12] There is a good correlation of Ki67 labelling index and the morphologic aggression indicators of hyperplastic, dysplastic and malignant diseases of gallbladder. [13, 14] This study was done with the aim to assess the ki-67 immuno expression in malignant and premalignant lesions of gall bladder.

Materials and Methods

The present study is a retrospective study conducted in the department of Pathology of Silchar Medical College and hospital. We included 212 cases that underwent cholecystectomy for

various reasons like cholecystitis, cholelithiasis, gall bladder carcinoma from 1st November, 2021 to 31st October, 2022. Metastatic carcinoma and any lesions of the biliary tract were excluded from the study.

Gross examination of the specimen was done and respective sections were taken and routine processing and staining with H&E was done. The cases were divided into three groups- group1 (malignant lesions), group 2(premalignant and premalignant like lesions) and group 3 (chronic cholecystitis).

IHC staining was done for ki-67 in all the specimens. For IHC, standard protocol was followed- slides were baked and deparaffinised, rehydration was done with graded alcohol and then antigen retrieval was done. The slides were then washed with buffer and peroxidase blocking was done. Primary antibody was added and then after washing the slides, secondary antibody was added and washed again. After that, DAB chromogen was added and counter stain was done with H&E. all the slides were then examined under light microscope at 400x magnification. Ki-67 labelling index (MIB-1ndex) was calculated. The calculation was done as the percentage of positively stained tumour cell nuclei out of the total tumour cell counted (n=1000). A percentage of >20% stained cells was considered to be positive.

Data was entered into the Microsoft excel data sheet and statistical analysis was done using IBM SPSS version 21 and Chi square test and proportion test was done and p value of less than 0.05 was considered to be statistically significant. Institutional ethical committee approval was obtained (No. SMC 4849).

Results

We conducted the study on total 212 cases, who underwent cholecystectomy for various reasons from 1st November, 2021 to 31st October, 2022. We indented to study the ki-67 expression in the lesions.

We tried to figure out the gender preponderance in gall bladder lesions:

Table 1: Gender distribution according to case number:

sex	All cases	Malignant	Premalignant	Chronic cholecystitis
Male	33(16%)	03(14%)	15(20%)	22(19%)
Female	179(84%)	19(86%)	60(80%)	93(81%)
Total	212	22	75	115

In our study, out of 212 cases, 179(84%) cases were female and 33(16%) cases are males. Out of 22 malignant cases, 19(86%) and 03(14%) were females and males respectively with a ratio around 1:5. We tried to determine the distribution of cases according to age group.

Table 2: Number of cases distributed according to age group:

Age	All cases	Malignant	Premalignant	Chronic cholecystitis
<30 years	34(16%)	00(00%)	08(11%)	26(23%)
30-40years	42(20%)	02(09%)	12(16%)	28(24%)
41-50 years	76(36%)	08(36%)	33(44%)	35(30%)
>50 years	60(28%)	12(55%)	22(29%)	26(23%)
Total	212	22	75	115

From the above table, we found that the incidence of gall bladder lesions as highest in 41-50 years (36%) and malignant cases were highest in >50 years (55%).

Table 3: Group wise distribution of cases:

Sl No.	Groups	No. Of Cases	Percentage
1	Malignant lesions	22	10.4%
2	Pemalignant lesions and premalignant like lesions	75	35.4%
3	Chronic cholecystitis	115	54.2%

Out of the 212 cases, 22(10.4%) cases were malignant lesions of gall bladder, 75 (35.4%) cases were premalignant and premalignant like lesions of gall bladder and 115 (54.2%) cases were chronic cholecystitis.

Table 4: distribution of cases according to diagnostic type in each group:

Type	Number of cases
1. Malignant (22)	
Adeno carcinoma (well differentiated)	08(36%)
Adenocarcinoma (moderately differentiated)	10(45%)
Adenocarcinoma (poorly differentiated)	03(14%)
Adenosquamous carcinoma	01(05%)
2. Premalignant and premalignant like lesions (75)	
Dysplasia	04(05%)
Xanthogranulomatous cholecystitis	27(36%)
Intestinal metaplasia	09(12%)
Pyloric metaplasia	21(28%)
Pyloric and intestinal metaplasia	03(04%)
Cholesterolosis	11(15%)
3. Chronic cholecystitis	115(100%)

From the above table, it was found that tumor in malignant lesions were predominantly moderately differentiated adenocarcinoma (45%) followed by well differentiated adenocarcinoma (36%). In premalignant lesions, xanthogranulomatous cholecystitis (36%) were highest followed by pyloric metaplasia (28%).

Table 5: Ki-67 expression in different groups:

SL. NO.	Groups	<20%	20-30%	>30%
1.	Malignant (22)	13(59%)	06(27%)	03(14%)
2.	Pre malignant (75)	75(100%)	00(00%)	00(00%)
3.	Chronic cholecystitis (115)	115(100%)	00(00%)	00(00%)

From the above table, it was found that in malignant groups, Ki-67 expression was <20% in 59% cases, 20-30% in 27% cases and >30% in 14% cases. In premalignant group and chronic cholecystitis cases, <20% expression was seen in 100% cases. Statistically the difference was found to be significant (p value <0.05)

Table 6: Ki-67 expression in gall bladder adenocarcinoma cases:

Group	Total	No. of cases with positive Ki-67 expression	Percentage (%)	P-value(<0.05 significant)
Well differentiated adenocarcinoma	08	03	38%	0.35
Moderately differentiated adenocarcinoma	10	04	40%	0.38
Poorly differentiated adenocarcinoma	03	01	33%	0.45

From the above table, it was found that 3 (38%) out of 8 cases of well differentiated adenocarcinoma, 4 (40%) out of 10 cases of moderately differentiated adenocarcinoma and 1 (33%) out of 3 cases of poorly differentiated adenocarcinoma had ki-67 expression >20%. There was no statistically significant difference between ki-67 expression and various gall bladder adenocarcinomas.

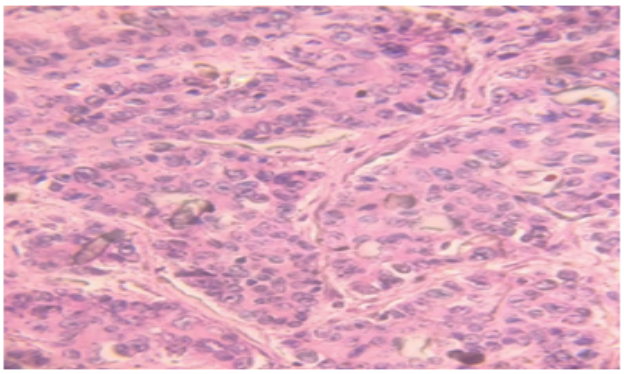

Figure 7: Well differentiated adenocarcinoma of gall bladder (40x, H and E)

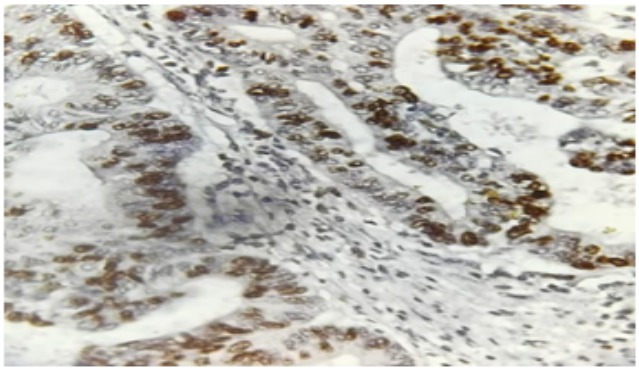

Figure 8: Ki-67 expression in well differentiated adenocarcinoma of gall bladder (40x)

Discussion

The present study entitled "Ki-67 Expression in Premalignant and Malignant lesions of Gallbladder in Southern part of Assam" was carried out at the department of pathology of Silchar medical College and hospital and we included 212 cases of cholecystectomy specimens from 1st November, 2021 to 31st October, 2022. The present study showed there was a female preponderance (84%) in gall bladder lesions with male: female ratio 1:5. Our results were in consistent with the study by Ajay kr. Singh et al, Kumar R. et al. [15, 16]

In our study, the maximum incidence of gall bladder lesions was in the age group of 41-50 years (36%) and >50 years age group patients had maximum incidence of gall bladder carcinoma. In a study by Nahar et al, it was found that maximum incidence of gall bladder carcinoma was in age group of 51-60 years.[17] Ajay kr. Singh et al found that majority of patients in malignant and premalignant groups were aged >40 years.[16] Ki-67 is a nuclear and nucleolar protein which is expressed in G, S, G2, M phases of cell cycle except G0 and G1 phase. It can be used as a good marker for cell cycling [18, 19].

In our study, ki-67 expression was <20% in 59% cases of malignant, 20-30% in 27% and >30% expression was seen in 14% cases of malignant lesions whereas premalignant lesions and chronic cholecystitis have less than 20% ki-67 expression. Our study is in concordance with study of Ajay kr. Singh et al. Kumar R. et al [15, 16] found there was no expression of ki-67 in cases of chronic cholecystitis. Lee CS also had similar findings.[20]

In a study by Stancu et al, expression of ki-67 was present in all cases of chronic cholecystitis and carcinoma.[21] Ki-67 antigen is low in premalignant and chronic cholecystitis cases when compared to gall bladder carcinoma and it implies that epithelial hyperplasia with increased cellular proliferative activity plays an important role in carcinogenesis.[20,22,23]

In our study, positive ki-67 expression was more in moderately differentiated adenocarcinoma (40%) and well differentiated adenocarcinoma (38%) than poorly differentiated adenocarcinoma (33%). Kumar R. et al [16] had similar findings.

Luis A et al [24] in their study found that out of 41 cases of gall bladder carcinoma, 69% of moderately differentiated carcinoma, 55.5% of well differentiated carcinoma and 50% of poorly differentiated carcinoma had positive ki-67 expression. Doval DC et al [25] found that ki-67 Li was significantly higher in poorly differentiated tumor. Xuan YH et al [26] found that ki-67 in carcinoma patients was more prevalent in advanced stage, older patient and the staining intensity was higher in advanced stage and poorly differentiated carcinoma.

Conclusion

From our study, we can conclude that gall bladder carcinoma was higher in females of age more than 50 years. Ki-67 expression was higher in malignant group of lesions and premalignant lesions did not show positive Ki-67 expression of more than 20%. Ki-67 expression was more in moderately and well differentiated gall bladder carcinomas than poorly differentiated carcinomas. So, Ki-67 can be used as a good marker of aggression of various lesions of gall bladder.

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