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Ki-67 Expression in Premalignant and Malignant lesions of Gallbladder

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Abstract
Introduction: *Cell proliferation is an important pre-requisite for the development of a neoplasm. Ki-67 is one of the most important cell proliferation markers. Its expression is correlated with the aggression of various histopathologic changes in the epithelium of gallbladder.*
Aim: *The aim of this study to assess the expression of Ki-67 in various pathological lesions of gallbladder. In the study immunohistochemical expression of Ki-67 was evaluated.*
Material and Methods: *This case control study included 154 patients who underwent cholecystectomy for various reasons like carcinoma and cholelithiasis. Out of 154 patients of gall bladder which included 85 cases of malignant lesion (adenocarcinoma) and 47 cases of premalignant and premalignant like lesions (including dysplasia, metaplasia, and xanthogranulomatous cholecystitis) along with 22 cases of chronic cholecystitis as control.*
Results: *The result showed Ki-67 Li in malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range 10-25% and remaining 7 (8.2%) had Ki-67 Li expression >25%. The Ki-67 Li expression was higher for patients aged >40 years, females, with well/ moderately differentiated adenocarcinoma and tumor diameter <2 cm.*
Conclusion: *The conclusion can be made that Ki-67 can be used as a marker of aggression of gall bladder histopatholgic lesions.*
Keywords: *Gall bladder, Malignancy, Ki-67.*

Introduction

Biliary tract diseases are common medical problems which affecting a significant populations worldwide and majority of the patients represented with cholelithiasis or cholecystitis [1]. Gall stones predispose to various pathologic lesions in the gallbladder, most importantly is inflammation and cancer[2]. In the glallbladder

epithelium adjacent to invasive carcinoma, dysplasia is noted in 40 - 60% of cases and in about 1% of all elective cholecystectomies done because of gallstones, an occult GB cancer was detected. Carcinoma of gallbladder is an aggressive malignancy and usually presenting at an advanced stage. It ranks fifth among the gastrointestinal malignancy[3]. Its incidence steadily increases with age and women are affected two to six time more than men [4]. The Indian council of medical research cancer registry has reported incidence rate 4.5% in males and 10.1% in females per 100,000 population in north india[5] Histopathologicaly there are 90% of cases of adenocarcinoma and the rest are squamous cell carcinoma and others. Antigen Ki-67 is a protein that corresponds to a nuclear non-histone protein expressed by cells in the proliferative phases G1, G2, M, and S which was described in 1983 [6,7]. In general, there is a good correlation between Ki-67 staining and mitotic count. Most studies have been performed on frozen sections; however, the anti-ki-67,MIB-1 antibody also is applicable on routinely fixed, paraffin embedded tissue after microwave pre-treatment [8].

The score of Ki-67 means the percentage of total number of tumor cells with nuclear staining [9]. Ki-67 is important for cell proliferation and has a relation to ribosomal RNA transcription. It is well characterized at the molecular level, Ki-67 protein is extensively used as a marker for cell proliferation [10]. There is a good correlation of Ki-67 labeling index and the morphologic aggression indicators of hyperplastic, dysplastic and malignant diseases of the gallbladder in addition to its prognostic significance [9,10]. The aim of this study is to assess the proliferative activity of the gallbladder epithelium utilizing Ki-67 score in neoplastic and non-neoplastic diseases of gall bladder.

Materials and Methods

This case control study included 154 patients who underwent cholecystectomy for various reasons like carcinoma and cholelithiasis from two year period iejuly 2014 to july 2016. Total 154 patients of gall bladder which included 85 cases of malignant lesion (adenocarcinoma) and 47 cases of premalignant and premalignant like lesions (including dysplasia, metaplasia, and xanthogr-anulomatous cholecystitis) along with 22 cases of chronic cholecystitis as control.

All the specimens were immediately fixed in 10% formalin and the histological examination of all Hematoxyline and Eosin-stained slides included evaluation of mucosa for signs of inflammation, ulceration, hyperplasia, metaplasia, dysplasia and malignant change.

Immunohistochemical evaluation for the expression of Ki-67 was done by the use of Dako (trade mark) Flex monoclonal antihuman Ki-67 and evaluated under light microscopic observation at x 400 magnification. In the immunostaining analysis, positive brown nuclei were detected and discriminated from negative blue hematoxyline nuclei.

Ki-67 labelling index (MIB-1 index) was calculated as the percentage of positively stained tumour cell nuclei out of the total tumour cells counted (n=1000). A percentage of stained cells was considered positive regardless of the intensity of staining.

The statistical analysis was done using SPSS (statistical package for social sciences) version 15.0 statistical analysis software. The results of ki-67 were expressed in mean, range and Chi square test and level of significance ie $P<0.05$ was considered significance.

Results

The present study was carried out with an aim to evaluate the Ki-67/Li expression in carcinoma and premalignant lesion of gallbladder and to carry out a clinicopathological correlation of the same. For this purpose a case-control study was carried out in which a total of 154 subjects were enrolled. Group-wise distribution of subjects enrolled in the study has been shown in Table 1 below:

Table 1: Group wise Distribution of Subjects enrolled in the study

SN	Group	Description	No. of cases	Percentage
1.	Malignant	Cases with malignant lesions of gall bladder	85	55.2
2.	Premalignant	Cases with premalignant and premalignant like lesions of gall bladder	47	30.5
3.	Controls	Specimen obtained from patients undergoing cholecystectomy	22	14.3

Out of 154 subjects enrolled in the study, a total of 85 (55.8%) were cases of malignant lesions of gallbladder and 47 (30.5%) specimen with premalignant and premalignant like lesions of gall bladder while remaining 22 (14.3%) chronic cholecystectomy.

Majority of patients irrespective of group were females. Though, proportion of males in premalignant group was higher (31.9%) as compared to that in malignant (21.2%) and control (11.5%) groups. Age of patients ranged from 14 to 78 years. Majority of patients in malignant group (n=66; 77.6%) and premalignant group (n=28; 59.6%) were aged >40 years of age whereas half of patients in control group were aged ≤40 years (n=11; 50%). Statistically, there was a significant difference among groups with respect to age (p=0.020).

Table 2: Distribution of cases according to Diagnostic Type in each group

SN	Type	No. of cases	Percentage
1.	Malignant (n=85)		
	Adenocarcinoma (Well differentiated)	34	40.0
	Adenocarcinoma (Moderately differentiated)	40	47.1
	Adenocarcinoma (Poorly differentiated)	5	5.9
	Adenocarcinoma sarcomatoid differentiation	1	1.2
	Adenocarcinoma with Xanthogranulomatous cholecystitis	5	5.9
2.	Premalignant&premalignantlikelesions (n=47)		
	Dysplasia	1	2.1
	Xanthogranulomatouscholecystitis	31	66.6
	Antral metaplasia	14	29.8
	Intestinal metaplasia	1	2.1
3.	Control (n=22)		
	Chronic cholecystitis	22	100

(Table -2) According to the type of tumour in malignant lesion predominantly are adenocarcinoma well differentiated (40%) and adenocarcinoma moderately differentiate (47%) and in premalignant and malignant like lesions predominantly are xanthogranulomatous cholecystitis (66.6%).

Table 3: Ki-67 Li Expression level in different groups

SN	Group	Expression					
		<10%		10-25%		>25%	
		No.	%	No.	%	No.	%
1.	Malignant (85)	64	75.3	14	16.5	7	8.2
2.	Premalignant (47)	47	100	0	0	0	0
3.	Control (22)	22	100	0	0	0	0

H=19.52; p<0.001 (Kruskall-Wallis test)

Ki-67 Li expression (Table 3) was observed to be <10% in 75.3% of malignant and all the cases in premalignant and control groups. In malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range 10-25% and remaining 7 (8.2%) had Ki-67 Li expression >25%. On evaluating the differences among groups statistically, the difference was found to be significant (p<0.001).

Table 4: Association between Ki-67 positivity and survival (n=49)

SN	Status	Total No.	Alive		Died	
			No.	%	No.	%
1.	Positive	11	4	36.7	7	63.6
2.	Negative	38	20	52.6	18	47.4
Total		49	24	49.0	25	51.0

χ^2=0.903; p=0.342 (NS)

(Table 4) Mortality rate up to a maximum follow up period of 19 months was 63.6% among Ki-67/Li positive and 47.4% among Ki-67/Li negative patients. Although survival rate was higher among Ki-67/Li negative as compared to Ki-67/Li positive patients yet this difference was not significant statistically (p=0.342).

Table 5: Association of Ki-67/Li Expression with different clinicopathological parameters

SN	Parameter	Total	No.	%	Statistical significance	
1.	Age				OR (95% CI)	'p'
	≤40 Years	49	4	8.2	Ref.	0.176
	>40 Years	105	14	13.3	1.73 (0.54-5.56)	
2.	Gender					
	Male	35	4	11.4	Ref.	0.957
	Female	119	14	11.8	1.03 (0.32-3.37)	
3.	Adenocarcinoma type					
	Well/Moderately differentiated	74	18	24.3	Ref.	0.654
	Other types	11	2	18.2	0.69 (0.14-3.50)	
4.	Adenocarcinoma subtypes					
	NOS	72	17	23.6	Ref.	0.853
	Mucinous	6	2	33.3	1.90 (0.32-11.38)	
	Papillary	6	1	16.7	0.76 (0.08-7.01)	
	Signet ring	1	0	0	-	
5.	Tumor Diameter					
	<2cm	16	4	25.0	Ref.	0.675
	>2 cm	45	9	20.0	0.75 (0.20-2.88)	
6.	Nodal involvement					
	No	43	9	20.9	Ref.	0.853
	Yes	16	3	18.8	0.87 (0.20-3.73)	
7.	Surrounding tissue involvement					
	No	41	9	22.0	Ref.	0.982
	Yes	18	4	22.2	1.02 (0.27-3.86)	
8.	Gall stones					
	No	42	12	28.6	Ref.	<0.001
	Yes	87	3	3.4	0.09 (0.02-0.34)	
9.	Chemotherapy					
	No	17	7	29.4	Ref.	0.559
	Yes	32	7	21.9	0.40 (0.11-1.44)	
10.	Clinical condition					
	Improved	32	2	11.8	Ref.	<0.001
	Deteriorated	17	9	52.9	16.88 (3.02-94.17)	
11.	Chief Complaint					
	Abdominal pain only	67	6	9.0	Ref.	0.313
	Others	83	4	4.8	1.94 (0.52-7.19)	

None of the associations (Table 5) except that between clinical condition and Ki-67/Li expression was observed (p<0.001). The odds of Ki-67/Li were higher for patients aged >40 years, females, with well/moderately differentiated carcinoma, Mucinous subtype, tumor diameter <2 cm, negative nodal status, gall stone positivity, having surrounding tissue involvement, involvement up to liver, without gall stones, deteriorated clinical status, complaints other than abdominal pain alone.

Discussion

Ki-67 is a nuclear protein that is present in all cell cycle phase except the G0 and early G1 phase and making it is a good marker for cell cycling [11,12]. Ki-67 has a prognostic and/ or predictive value in different tumour types. The use of antibodies to Ki-67 is a reliable and accurately assessing the growth fraction of neoplasms [13]. Ki-67 expression was studied in categoric form by substituting the mitotic counts-morphologic feature in the many tumour. Recent studies established the fact that an increased expression of Ki-67 indicates a better survival in few tumour as these tumours have better response to radiotherapy [14], as irradiation destroys preferentially the quick dividing cells[15,16]

In our study Ki-67 Li (Level of index) expression was observed to be <10% in 75.3 % of malignant and all the cases in premalignant and control groups. In malignant group, a total of 14 (16.5%) patients had Ki-67 Li expression in range of 10-25% and remaining 7 (8.2%) had Ki-67 Li expression of >25%. Our study is in concordance with the study of *Wang X. et al.* [17] who studied 30 cases of cholangiocarcinoma out of which 29 cases ie 96.7% were positive for Ki-67. *Spyratos F et al.* [18] analysed Ki-67 index in 5 cut offs (10%, 15%,17% (median), 20%, 25%) and showed that the optimal Ki-67 cut off was 25% and the mitotic index was the proliferative variable that best discriminated between low and high MIB-1 (Ki-67) samples. A MIB-1 cut off of 25% adequately identified highly proliferative tumours. Conversely with a MIB-1 cut off of 10% are low proliferation tumours. They concluded that MIB-1 (Ki-67) index should be combined with some other routinely used proliferative marker, such as mitotic index. *Lee CS* [19] observed that Ki-67 indices in chronic cholecystitis were significantly lower than those obtained in both in moderately and poorly differentiated adenocarcinoma of gall bladder which is in concordance with our study in which there was no positive expression of Ki-67 (ie>10%) index in control group (chronic cholecystits) was seen. *Hidalgo Grau LA et al*.[20] calculated Ki-67 expression by MIB-1 index : the % of positively stained tumour cell nuclei out of the total tumour cell counted (n=1000); >20% of stained cells was considered positive. They observed that out of 29 gall bladder carcinomas, 24 cases (58.5%) were positive for MIB-1 index.

In our study the association between Ki-67 positivity and survival could be studied only in 49 cases because follow up could be maintained in 49 patients only. In 49 cases, mortality rate up to maximum follow up period of 19 months, was 63.6% among Ki-67 Li positive and 47.4% among Ki-67 Li negative. Although survival rate was higher among Ki-67 Li negative as compared to Ki-67 Li positive patients. *Hidalgo Grau LA.et al.*[20] observed that five year survival of patient with a MIB-1 positive index was 9.2% as opposed to 27.7% for those with a negative index.

In our study, comparing the association of Ki-67 Li expression with different clinicopathological parameters, we observed that in the patients with age >40 years, 14 (13.3%) cases, and <40 years 4 (8.2%) cases showed expression of Ki-67. On exploring the association with gender, we found that there were 14 females(11.8%) out of 119 and 4 males out of 35 (11.4%) who were positive for Ki-67 expression. Among the well and moderately differentiated adenocarcinoma 18 cases (24.3%%) out of 74 cases of gall bladder carcinoma were positive for Ki-67 expression while other type had 2 cases positive for KI-67 expression (18.2%) out of 11 gall bladder carcinomas. Out of 16 cases with tumor size of <2cm, 4 cases (25%) were positive for Ki-67 expression while in remaining 45 cases with tumor size of >2cm, 9 cases were positive for the same (20%).In our study, nodal involvement was present in 16 cases, out of which 3 cases (18.8%) had Ki-67 expression and 9 out of 43 (20.9%) cases without nodal involvement shows Ki-67 expression. Out of 87 cases with gall stones, 3 cases (3.4%) showed positive Ki-67 expression and among 42 cases without gall stone, 12 cases (28.6 %) showed positive expression.

Among 17 patients who were not on chemotherapy, 7 (29.4%) showed Ki-67 expression while out of 32 who were on chemotherapy, 7 (21.9%) showed positive Ki-67 expression. *Doval DC et al*.[21] observed that Ki-67 Li was significantly higher in patients in age group <40 years (p=0.027) and poorly different-iated tumour (p=0.023). *Xuan YH et al.*[22] observed that Ki-67 expression in carcinoma was more prevalent in the advanced stage and in older patients. In addition staining intensity was higher in advanced stage and poorly differentiated carcinoma. *Wang X et al*. [17] observed that in lymph node metastasis group and clinical stage III group, Ki-67 Li was significantly higher than that in non lymph node metastasis as well as in early stage group. They also found Ki-67 Li of moderately or poorly differentiated cholangioca-rcinoma was significantly higher than that of well differentiated carcinoma.

Conclusions

The conclusion of my study is that,Ki-67 Li was higher (i.e. >25%) in only malignant group. No other group (premalignant or control) showed positivity more than 25%. There was no significant association between Ki-67 expression and variants of adenocarcinoma (p=0.813). The Ki-67 Li expression was higher for patients aged >40 years, females, with well/ moderately differentiated adenocarcinoma and tumor diameter <2 cm. The final conclusion is that Ki-67 is a marker of aggression of histopathologic lesions.

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