Immunohistochemical expression of Ki-67 in gall bladder carcinoma

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Abstract

Introduction: Gall bladder carcinoma accounts for 0.3-0.7% of all cancers and has a peak incidence among women aged 70 and 79 years. Recently many molecular markers like Ki-67, cyclin D1, MUC1 and p53 with prognostic impact on GBC have been described. Ki-67 is a proliferative marker which is expressed in all active phases of the cell cycle and it can be detected in the nucleus of tumour cells indicating its proliferative activity.
The aim of this study is to evaluate the expression of Ki-67 in carcinoma of gall bladder by immunohistochemistry and to correlate the mean Ki-67 labelling index with the various histological grades of gallbladder carcinoma.
Materials and Methods: All the histopathologically proven cases of gall bladder malignancy from July 2014 to June 2017 (n=44) were examined for Ki-67 expression immunohistochemically.Ki-67 labelling index was calculated. ANOVA test and independent 't' test were applied to see correlation of Ki-67 expression with various grades of gall bladder carcinoma.
Results: The Ki-67 labelling index was minimum in well-differentiated adenocarcinoma of gall bladder and maximum in poorly differentiated adenocarcinoma of gall bladder. The p value amongst these groups was found to be statistically significantly different.
Conclusion: 88.64% of Gall bladder carcinoma was positive for Ki-67 and the Ki-67 labelling index increased significantly as the histological grade increased.

Keywords: Ki-67, Ki-67 labelling index, MIB labelling index, Gall bladder carcinoma.

Introduction

According to autopsy reports Gall bladder carcinoma is the most common carcinoma of the biliary tract and accounts for 80 to 90% of biliary cancers worldwide.[1] Gall bladder carcinoma accounts for 0.3-0.7% of all cancers and has a peak incidence among women aged between 70 and 79 years.[2] Due to the biological behaviour of the tumour and diagnostic delay the prognosis is poor.[3] The exact etiology is unknown, but association of gallstones with an increased risk for GBC has been reported.[4] Treatment either surgical or palliative in patients with GBC rely mostly on prognostic factors such as the T stage which is defined by the depth of invasion in the gallbladder wall.[5] Recently many molecular markers like Ki-67,[6] cyclin D1,[7] MUC1,[8] p53.[9,10] with prognostic impact on GBC have been described.

Ki-67 is a proliferative marker which is expressed in all phases of the cell cycle except G0 and can be detected in the nucleus of proliferating tumour cells.[11] The Ki-67 monoclonal antibody was originally generated by immunizing mice with the nuclei of the Hodgkin lymphoma cell line L428. The name Ki in Ki-67 protein refers to the city of origin (Kiel) and the number 67 came from the clone number in the 96-well plate. Initially this antigen was not characterized and was referred to mainly as the Ki-67 antigen. Later it was found to be a protein whose primary structure could be deduced from the corresponding c DNA, but revealed no homology to any known polypeptide and its function remained indistinct so the initial name Ki-67 was kept.[12]

The aim of the present study was to evaluate the expression of Ki-67 in gallbladder carcinoma by immunohistochemical method and to correlate the mean Ki-67 labelling index with the various histological grades of gallbladder carcinoma.

Materials and Methods

This study was carried out in the Pathology Department of Sri Ram Murti Smarak Institute of Medical Sciences, Bareilly. All histologically proven cases of gall bladder carcinoma, received in the department of pathology from July 2014 to June 2017 were included in the study.

The study was approved by the institute's ethical committee. Immunostaining was carried out using antibody to Ki67 antigen (clone MIB-1, isotype IgG1). All slides with fixed tissue sections were first deparaffinised with xylene and then rehydrated with graded alcohols. Antigen retrieval was done by boiling the slides in EDTA solution of pH 6.0 at 100°C for 2.5 minutes. Peroxide block and protein block were applied for 10 minutes each. Then the slides were incubated with the primary anti Ki -67 antibody. After rinsing with buffer, incubation was done in biotinylated rabbit anti-mouse IgG for 30 minutes. Then the sections were washed and incubated with streptavidin-biotinylated

horseradish peroxidase complex for 30 minutes and reacted with diaminobenzidine chromogen. The slides were counter stained with haematoxylin. The Ki-67 expression was calculated counting the percentage of positively stained tumour cell nuclei out of the total cells counted (n=1000); >20% of stained cells were considered positive.[2] The collected data were entered in excel sheets and analyzed. Statistical tests were applied for the data as applicable. One way anova test and independent t test were applied to see the correlation of Ki-67 expression with different grades of gall bladder carcinoma. A p value <0.05 was considered as significant.

Observation and Results

There were 44 patients of gall bladder carcinoma with an age range of 20 to 89 years. The peak incidence of Gall bladder carcinoma was seen in 40-49 years of age. Mean age was 50.43±13.3 (Table 1). In this study there were 34 female patients (77.27%) and 10 (22.73%) male patients. Male to Female ratio was 1:3.4. Gall bladder carcinoma was more common in females in each decade wise age group distribution except >70 years of age where equal distribution was present. Among the studied patients the most common site of malignancy was fundus of gall bladder seen in 38 patients (86.36%) followed by the body of gall bladder in 4 patients (9.09%) and neck of gall bladder in 2 patients (4.55%).Gall stones were present in 32 patients (72.73%) and absent in 12 patients (27.27%). In the present study 39 patients (88.64%) were of adenocarcinoma NOS, 4 patients (9.09%) were of mucinous adenocarcinoma and 1 patient (2.27%) of adenosquamous carcinoma. 16 patients (36.36%) were of well differentiated, 21 patients (47.73%) were of moderately differentiated and 7 patients (15.91%) were of poorly differentiated histological grade. In the present study Ki-67 positivity was present in 34 patients (87.18%) of adenocarcinoma NOS (n=39), 4 patients (100.0%) of mucinous adenocarcinoma (n=4) and 1 patient (100.0%) of adenosquamous histological type (n=1). Ki-67 positivity was present in 12 patients (75.0%) of Well differentiated (n=16), 20 patients (95.24%) of moderately differentiated (n=21) and 7 patients (100.0%) of poorly differentiated histological grade (n=7) (Table 2). The Mean Ki-67 labelling Index was 27.0±4.2 in cases of well differentiated adenocarcinoma, 32.5±6.1 in cases of moderately differentiated adenocarcinoma and 33.14±2.5 in cases of poorly differentiated adenocarcinoma. The difference in the mean Ki-67 labelling index in the different grades of carcinoma was statistically significantly different. p=0.010 (Table 3, Fig. 1-4).

**Table 1: Age Range of the Patients**

Age in years	No. of patients (n=44)	Percentage
20-29	2	4.55%
30-39	5	11.36%
40-49	17	38.64%
50-59	6	13.64%
60-69	10	22.73%
70-79	2	4.55%
80-89	2	4.55%
Mean±SD	**50.43±13.3**	

Table 2: Correlation of Ki-67 expression with histological grade

Histological grade (No. of cases)	Ki-67 Positive cases	Ki-67 Negative cases
Well Differentiated (16)	12 (75.0%)	4 (25.0%)
Moderately Differentiated (21)	20 (95.24%)	1 (4.76%)
Poorly Differentiated (7)	7 (100.0%)	0.0

**Table 3: Correlation of mean Ki-67 labelling index with histological grade**

| Histological grade	Mean Ki-67 labelling index	p-Value
Well Differentiated	27.0±4.2	**0.010**
Moderately Differentiated	32.5±6.1	
Poorly Differentiated	33.14±2.5	
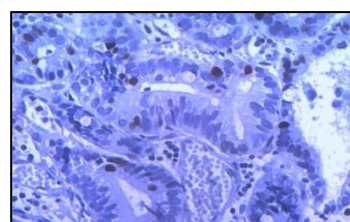
Fig. 1: Ki-67 immunopositivity in well differentiated adenocarcinoma gallbladder(40X).
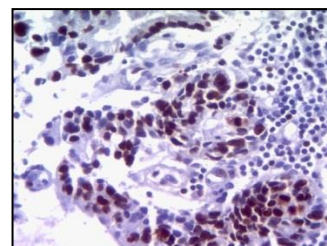
Fig. 2: Ki 67 immunopositivity in moderately differentiated adenocarcinoma gallbladder (40X)
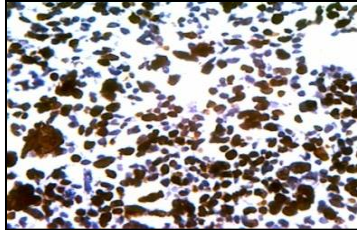
Fig. 3: Ki-67 immunopositivity in poorly differentiated adenocarcinoma gall bladder (40X)
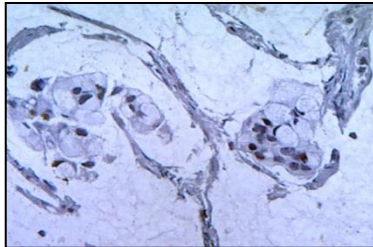
Fig. 4: Ki-67 immunopositivity in mucinous adenocarcinoma gallbladder (40X)

Discussion

Gallbladder cancer is the most common malignancy of the biliary tract according to autopsy studies. It accounts for 80%–95% of all biliary tract cancers worldwide and ranks sixth among all gastrointestinal cancers. There is a striking variability in the global rates for gallbladder cancer due to the differences in the environmental exposure and intrinsic genetic predisposition to cancer.[13] There is often a rapid and silent progression rendering it inoperable by the time it is detected. An early diagnosis and surgical resection may lead to a better outcome.[14] Even with surgery most patients progress to metastatic disease necessitating improvement in adjuvant therapies.[15] Five-year survival is 21–69% in patients of gall bladder cancer who have undergone curative resection,. Nevertheless, 85% of T3/T4 tumors have an overall survival of only 2–8 months as demonstrated by the French Surgical Association.[16]

Factors predicting poor prognosis are T stage[17] (defined by depth of invasion in Gall bladder wall), lymph node involvement and high grade and the decision regarding use of surgery or palliative treatment depends on these factors. Gall bladder cancer is often diagnosed incidentally after performing laparotomy or laproscopy for benign gall bladder disease.

In the early 1980s, a German group originally identified the Ki-67 antigen, by using a mouse monoclonal antibody against a nuclear antigen from a Hodgkin's lymphoma-derived cell line. It is a non-histone protein and was named after the researchers' location, Ki for Kiel University, Germany, with the 67 label referring to the clone number on the 96-well plate.[18] Studies have identified the involvement of Ki-67 in the early steps of polymerase I-dependent ribosomal RNA synthesis has been identified by several studies. Although Ki-67 is important for cell division and indicates the cells in the growth fraction of a tumour yet its exact function is not well known. Except for the resting phase G0, Ki67 antigen is expressed throughout during G1, S, G2, and M phases of cell cycle.Ki-67 levels are low in G1 and S phases and their peak level occurs in mitosis.[19,20]

There is a sharp decrease in Ki-67 levels later in the mitotic phase (anaphase and telophase). The measurement of Ki-67 is done on paraffin sections by an immunohistochemical method, using the MIB-1 antibody. Ki-67 scoring is done by calculating the percentage of tumor cells with nuclear staining.[21] Ki-67 activity has been associated with the proliferating cells growth fraction.[22] MIB a monoclonal antibody is now the preferred antibody to the Ki-67 antigen for use in immunohistochemistry and Ki-67 is considered to be nuclear matrix protein proliferation antigen.[23] Often, the clinical course of cancer is correlated with the fraction of Ki-67 positive tumor cells i.e. Ki-67 labelling index.[12]

In the present study there were a total of 44 patients. The age range of the patients was 40 to 69 years with the mean age of presentation being 50.43±13.3 years. Male to Female ratio was 1:3.4(Table 1).Similar results were seen in a study by Khan I et. al[24] where cases ranged from 28 to 72 years with peak incidence in 41 to 50 years age group and male to female ratio was 1:3.8. Similar results were found in a study conducted by Hamdaniet. al [25] on 198 patient where the mean age was 55 years, with a range of 28-82 years and fifth decade as the peak age of presentation. Mallik I A[26] found that most patients of gall bladder carcinoma were women (77%) and mean age was 55 years (+/-11 years) which is consistent with the present study.

Gall stones were present in 32 patients (72.73%) and absent in 12 patients (27.27%) in our study. Gallstones are associated with gall bladder carcinoma in mostly 70 to 80 % cases as reported by Chaudhary A et. al.[27] In a study by Khan I et. al.,[24] gallstones were found in 45 out of 63 gall bladder carcinoma patients (71.42 %).

Most common site of carcinoma gallbladder was fundus (n=38; 88.36%) followed by body (n=4, 9.09%) and neck (n=2; 4.55%) in present study. Ghosh Y et. al.[28] also concluded that fundus was the most common site of carcinoma gallbladder.

In our study 39 patients (88.64%) were of adenocarcinoma NOS, 4 patients (9.09%) were of mMucinous adenocarcinoma and 1 patient (2.27%) of adenosquamous histological type. Albores-Saavedra J et. al.[29] has also mentioned Adenocarcinomas being most frequent histological subtype of gallbladder carcinoma representing approximately 90-95% of all cases. In contrast, adenosquamous or squamous cell carcinomas are rare. Another study conducted by

Hundalet. al.[1] also states that adenocarcinoma is the most common histological type, accounting for 98% of all gall bladder carcinoma.

In present study 16 patients (36.36%) were of well differentiated, 21 patients (47.73%) were moderately differentiated and 7 patients (15.91%) were of poorly differentiated histological grade. A study by Brandt Rauf P et. al.[30] also showed that the most common neoplasm was adenocarcinoma, out of which most common were moderately to well differentiated (40-50%), while poorly differentiated adenocarcinoma accounted 30% of cases.

Ki-67 positivity was present in 34 patients (87.18%) of adenocarcinoma NOS (n=39), 4 patients (100.0%) of mucinous adenocarcinoma (n=4) and 1 patient (100.0%) of adenosquamous histological type (n=1) in our study. Roa EI et. al.[31] also concluded in his study that a staining index of more than 20% was seen in 75% of gallbladder cancer samples.

Ki-67 positivity was present in 12 patients (75.0%) of well differentiated adenocarcinoma (n=16), 20 patients (95.24%) of moderately differentiated adenocarcinoma (n=21) and 7 patients (100.0%) of poorly differentiated adenocarcinoma histological grade (n=7) (Table 2) in present study. The Mean Ki-67 labelling Index % was 27.0±4.2 in well differentiated adenocarcinoma (WD), 32.5±6.1 in moderately differentiated adenocarcinoma (MD), 33.14±2.5 in poorly differentiated adenocarcinoma (PD), and the variation in the Ki-67 labelling index among different grades of carcinoma was statistically significant, p=0.010 (Table 3). Similar association was found in a study conducted by Parul G et. al.[32] which showed Ki-67 labelling index increasing from Well differentiated (25.50±6.73%) to Moderately differentiated (29.40±8,36%) and Poorly differentiated (37.50±3.77%) histological types of Gallbladder carcinoma. Lee S[33] stated PCNA and MIB-1 indices in chronic cholecystitis were significantly lower than those obtained in moderately differentiated and poorly differentiated adenocarcinoma of the gallbladder (P < 0.001). Similarly, cases of ampullary and gallbladder CIS had significantly lower PCNA and MIB-1 indices than the invasive carcinoma cases (P < 0.001). The poorly differentiated adenocarcinomas of the gallbladder had higher mean MIB-1 indices but reduced patient survival when compared with the moderately differentiated carcinomas.

Conclusion

We conclude that majority of Gall bladder carcinoma (88.64%) were positive for Ki-67 staining and the mean Ki-67 labelling index increases as the grade increases.

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