Original Research Article

New Indian Journal of Surgery
Volume 9 Number 5, September - October 2018
DOI: https://dx.doi.org/10.21088/nijs.0976.4747.9518.21

Role of Ki-67 as a Prognostic Marker in Cancer Gall Bladder- a Study from an Endemic Region

Vaibhav Raj Gopal[1], Surender Kumar[2], Nuzhat Hussain[3], H.S. Pahwa[4], Abhinav Arun Sonkar[5], Awanish Kumar[6], Mithlesh Bhargav[7]

[1]Senior Resident [2]Professor [4]Professor [5]Professor and Head [6]Professor, Department of Surgery, King George's Medical University, Lucknow, Uttar Pradesh 226003, India. [3]Professor and Head, Department of Pathology, Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh 226003, India. [7]Senior Resident, Department of Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh 226003, India.

How to cite this article:

Vaibhav Raj Gopal, Surender Kumar, Nuzhat Hussain et al. Role of Ki-67 as a Prognostic Marker in Cancer Gall Bladder- a Study from an Endemic Region. New Indian J Surg. 2018;9(5):653-57.

Abstract

Introduction: The etiopathogenesis of gallbladder cancer is a multistep process and hardly any of the existing biomarker(s) have a positive relation with the clinico-pathological parameters and outcome. Ki-67, a proliferative marker, is a fair prognostic indicator in cancers like the breast. The present study was conducted to assess its validity for the same and its influence on the treatment in gallbladder cancer. *Methodology*: Histopathological examination and immunohistochemistry for Ki-67 was performed on 41 patients of GBC and 31 cases of gallstone disease in the age of 40-70. Follow up was done at 3 months by history and physical examination. Student's unpaired t-test, ANOVA test, Chi square test and Pearson correlation coefficient analysis were applied to assess the relation of mean Ki-67 value to the TNM status, pathological grade, clinicopathological variables, stage and postoperative outcome at 3 months. *Results*: Mean Ki-67 levels were significantly raised in the malignant cases (40.00±17.42) as compared to benign (2.22±1.54, p=0.0001). Significantly higher Ki-67 levels were noted in gallbladder cancer patients with deranged liver function tests ($p<0.05$). Mortality within 3 months was associated with significantly higher Ki-67 (45.66±21.11, p=0.0001). Similarly, mean Ki-67 was significantly high in patients with nodal metastasis (47.05±17.23, p=0.03). However, no significant difference was noted in relation to the tumour (T) ($p>0.05$) and distant metastatic (M) status (p=0.77) and the stage (p=0.21). *Conclusion:* Higher Ki-67 index suggests an advanced disease in the form of nodal metastasis and jaundice. It has an independent and inverse impact on the prognosis. Due to its overexpression in gallbladder cancer, it can be exploited for targeted therapy.

Keywords: GBC; Gall Bladder Cancer; Ki-67; IHC; Immunohistochemistry.

Corresponding Author: Surender Kumar, Professor, Department of Surgery, King George's Medical University, Lucknow, Uttar Pradesh 226003, India.

E-mail: dr_kukku@yahoo.co.in

Received on 15.05.2018, **Accepted on** 09.06.2018

Introduction

Gallbladder cancer is an insidious onset disease of gastrointestinal tract. Symptomatology is by and large non-specific and includes weight loss, dyspepsia, pain and jaundice and this is the reason for its late diagnosis and hence poor outcomes. Till date none of the investigations either radiological or biochemical are confirmatory for diagnosis except for the histopathology. Non- specific markers include CA 19-9, CA-125 and CEA[1] and none of them have a prognostic relationship with the disease. It is endemic in the entire Gangetic belt [2,3,4]. Worldwide it has greater prevalence in western parts of South America [5,6] (Chile and Peru).

Starting from simple cholecystectomy to palliative chemotherapy, cancer gall bladder has a wide range of treatment options depending upon the stage at diagnosis and clinical presentation. Surgical resection is employed up-to stage III and stage IV is classified as an unresectable tumour that is primarily dealt with, palliation.

Gallbladder cancer development is a multistep process, but hardly any of the existing biomarker(s) have a positive relation with the clinico-pathological parameters and outcome except for EGFR [7,8,9]. Since current existing prognostic criteria have a limited role, the present study was focussed to assess the validity of

Ki-67 as a prognostic indicator and its influence on the treatment of GBC in Indian patients.

Methodology

A total of 72 patients in the age of 40-70 were enrolled in this prospective study conducted over a period of two years. Out of these, 41 (32 females and 9 males) constituted radiology (USG abdomen and CECT scan) proven cases of carcinoma gallbladder and 31 (25 females and 6 males) were radiology (USG abdomen) proven cases of gallstone disease. Those unwilling to participate or had already received treatment in the form of surgical resection and chemotherapy were excluded.

TNM status was checked on CT, and those appearing to have rescetable disease were subjected to extended cholecystectomy but if, intra-operatively the disease was found to be unresectable or metastatic(distant nodal or peritoneal) then the procedure was abandoned and a biopsy was taken. 19 cases underwent extended cholecystectomy and a simple biopsy was taken in the remaining during exploration. Control group included gallstone disease patients. 25 underwent laparoscopic and 6 had open cholecystectomy. After histological confirmation of malignancy, cases were planned for adjuvant/ palliative platinum based chemotherapy: gemcitabine 1g (day 1 and 8) plus cis-platin 50mg/m^2 (day 1,2,3) cycled every 21 days for six times.

Gall bladder tissue obtained from both the test and control was formalin fixed and sent to the Department of Pathology, RML-IMS, Lucknow for tissue study. Histopathological confirmation of the disease was done using haematoxylin and eosin staining. Control cases were dealt with similarly. Immuno-staining for Ki-67 marker was done. Final stage of the cancer was derived after histological evaluation.

The test and the control cases were subjected to follow up at 3 month and were checked for symptom recurrence by history and physical examination. 22 were asymptomatic, 4 were symptomatic and 15 expired within3 months in the test group. Among the symptomatic, all had abdominal pain as their major complain. None complained of jaundice and abdominal distension or any other symptom. Out of 15 expiries, two had a secondary cause of death i.e. hypertensive stroke and myocardial infarction, rest all expired due to the disease itself. Postoperative outcome in the control group was uneventful.

Student's unpaired t-test, ANOVA test, Chi square test and Pearson correlation coefficient analysis were applied to know the relation of mean Ki-67 to the: TNM status, pathological grade, clinic-pathological variables and stage and post-operative status at 3 months.

Results

Table 1: Comparison of Ki-67 between cases and controls

Samples	Ki-67 (%) (Mean±SD)
Cases(n=41)	40.00±17.42
Control(n=31)	2.22±1.54
p-value[1]	0.0001

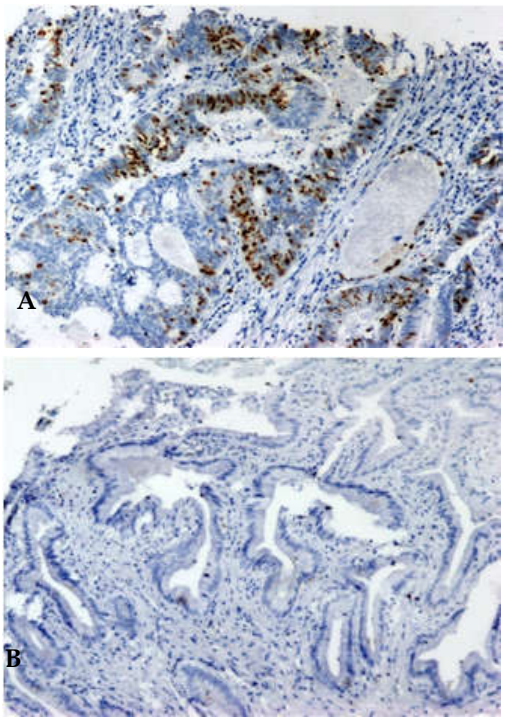


Fig. 1: Photomicrograph depicting immunostaining for Ki-67
(a) gall bladder adenocarcinoma(test)-well differentiated type, Ki-67 index 40% **(b)** chronic cholecystitis(control), Ki-67 index 2%

Table 2: Association of Ki-67 with clinico-pathological parameters

Symptom	Mean Ki-67 ± SD	p Value
Pain (n=38)	39.50±17.35	0.43
Lump(n=16)	41.56±16.90	0.65
Jaundice(n=11)	51.81±18.34	0.02
Pathological diagnosis		
Moderately -differentiated adenocarcinoma (n=17)	45.29±20.95	
Well-differentiated adenocarcinoma (n=24)	37.21±13.78	0.10
Biochemical parameters	**Ki-67 correlation coefficient**	
Serum Total bilirubin	0.98	0.0001
Serum Direct bilirubin	0.47	0.002
Serum ALP	0.36	0.02

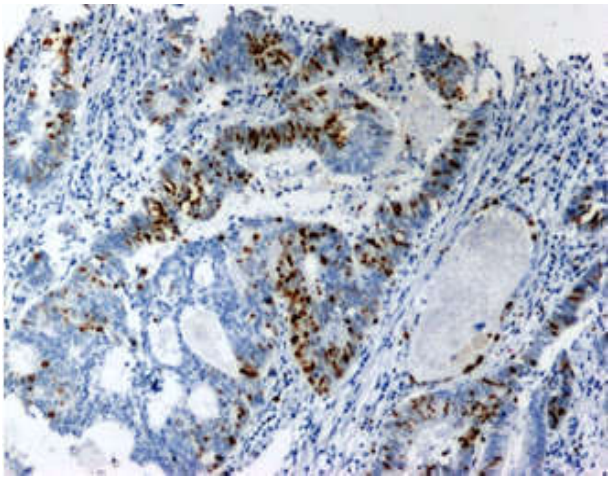
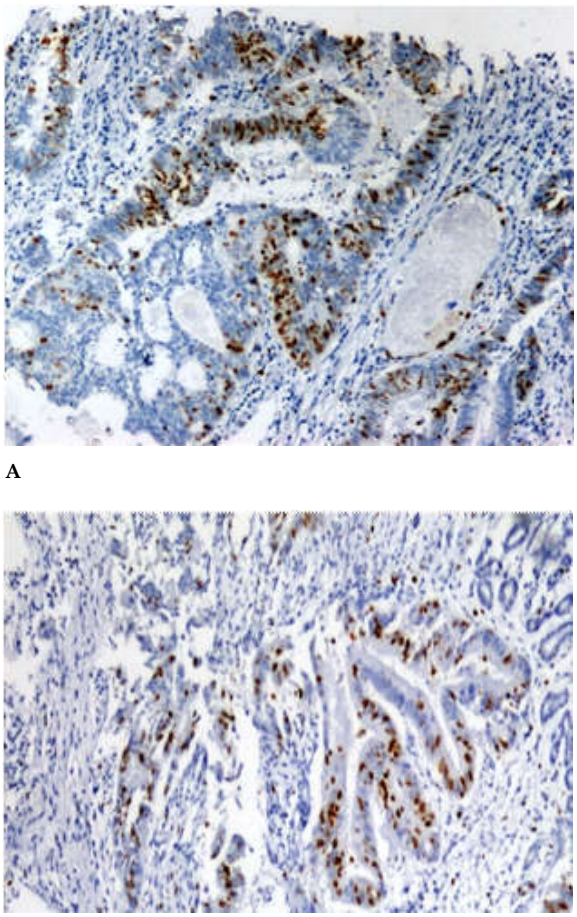


Fig. 2: Photomicrograph depicting dense immuno-staining for Ki-67 index in an icteric patient (Ki-67 index 40%)

Table 3: Comparison of Ki-67 with the TNM (tumour, nodal, metastatic) status of the disease

TNM status		Mean Ki-67±SD	p Value
T	T2 (n=8)	31.21±17.26	p>0.05
	T3 (n=33)	42.12±17.04	
N	N0(n=14)	31.07±17.34	
	N1 (n=10)	40.50±13.00	0.03
	N2 (n=17)	47.05±17.23	
M	M0(n=28)	39.46±16.00	0.77
	M1(n=13)	41.15±20.83	
Stage	II (n=5)	32.00±21.67	0.21
	III (n=13)	35.76±12.55	
	IV (n=23)	44.13±18.38	



A

B

Fig. 3a: Slide showing dense immuno-staining for Ki-67 from a case with distant nodal metastasis (N2), Ki-67 index 40%. **(b)** Immuno-staining for Ki-67 from a case with regional node involvement (N1), Ki-67 index 15%

Table 4a: Relation of postoperative status at 3 month with Ki-67 index, M-status and stage

Status at 3 month	Ki-67 (%) (Mean±SD)
Expired (n=15)	45.66±21.11
Symptomatic (n=4)	37.50±5.00
Asymptomatic (n=22)	16.49±19.77
p-value	0.0001

Table 4b: Relation of postoperative status at 3 month with Ki-67 index, M-status and stage

	Expired		Symptomatic		Asymptomatic		p-value
Metastasis	**No.**	**%**	**No.**	**%**	**No.**	**%**	
							0.01
M0 (n=28)	6	21.4	3	10.7	19	67.9	
M1 (n=13)	9	69.2	1	7.7	3	23.1	
Stage	**No.**	**%**	**No.**	**%**	**No.**	**%**	0.002
II (n=5)	0	0.0	0	0.0	5	100.0	
III(n=13)	1	7.7	1	7.7	11	84.6	
IV(n=23)	14	60.9	3	13.0	6	26.1	

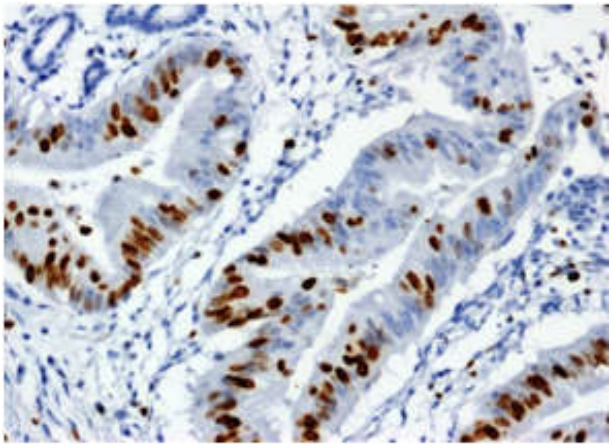


Fig. 4: Photomicrograph depicting Ki-67 immuno-staining in a patient who expired within 3 months with proliferation index 70%

Discussion

Ki-67, a proliferation marker, is present in higher concentration in the actively dividing cells. Its presence varies according to the stage of the cell cycle. It appears in the synthetic phase and increases progressively through the G1 and early M1 phase to finally disappear in the G0 phase [10].

Mean Ki-67 value was significantly raised in the malignant specimens (40.00) as compared to the benign (2.22); p-value= 0.0001. According to a study conducted by C Soon Lee et al. (1998), mean Ki-67 indices and mean PCNA value in chronic cholecystitis were significantly lower than those obtained in both moderately and poorly differentiated adenocarcinoma of the gallbladder [11].

GBC patients who had jaundice had a higher Ki-67 index, p=0.02. This was in coherence with the weak positive correlation of deranged liver function test profile with Ki-67 index. No significant difference was observed with abdominal pain and lump.

The grade of cellular differentiation had no significant relation with the degree of cellular proliferation. Out of the 41 recruited cases, 17 had moderately differentiated and 24 had well differentiated adenocarcinoma. Their association with mean Ki-67 index was statistically insignificant, p=0.10. Hidalgo et al. (2004) also stated an insignificant relation of cellular differentiation grade with both p53 and Ki-67 index [12]. Similarly tumour invasiveness in the organ wall (T-status) is not significantly related to a high Ki-67 index, p>0.05.Hidalgo et al. (2004) in their study also concluded that Ki-67 antigen expression was not related to the organ wall invasion (parietal infiltration) [12].

GBC that has a higher Ki-67 value has a higher tendency to spread to the adjoining and distant lymph nodes (Para-aortic, inter aorto-caval). A higher Ki-67 index is directly proportional to lymphatic metastatic potential. The mean value of Ki-67 is highest for N2 disease (47.05) and the data is statistically significant, p=0.03. Hui Met al. (2002) also showed a significant relation between a high Ki-67 index and lymphatic invasion (p=0.007) [13].

Distant nodal spread and jaundice [14,15,16] in GBC patients are suggestive of advanced disease and so the higher Ki-67 values associated with them seems to be relevant. Cellular proliferation in carcinogenesis of gall bladder cancer is not affecting the depth of invasion within the organ wall but is associated more with trespassing via lymphogenous route.

Metastatic cancer (M1) and stage IV disease is having statistically insignificant relation with a high Ki-67 index (p=0.77, p=0.21). Now when the mortality at 3 months is compared separately with Ki-67 index, stage IV and metastatic disease (M1), result is statistically significant (p=0.0001, p=0.002, p=0.01). Majority of cases who expired had inoperable disease intra-operatively and so they underwent only biopsy, except for 2 cases who had operable disease and underwent extended cholecystectomy. Both these 2 cases had a secondary cause of death i.e. hypertensive stroke and myocardial infarction. Shrestha M et al. (1998) have stated that a high Ki-67 index in patients with GBC have worse postoperative prognosis ($p < 0.05$) [17]. However, K KAI et al. (2013) and Hidalgo et al. (2004) have stated that Ki-67 index has little role in deciding the postoperative outcome [12,18].

Metastatic cancer, stage IV disease and a higher Ki-67 index are independently associated with a dismal outcome at 3 months but the former two separately do not have a significant relation to Ki-67. A higher Ki-67 does not necessarily mean a higher stage disease since stage comprises three components:- tumour status (T), lymph node status (N) and metastatic status (M) and Ki-67 associates significantly only with the nodal status (N) of the disease..

Conclusion

A higher Ki-67 index is associated with lymphatic metastatic potential and deranged liver function test parameters. It has no association with the stage of the disease and so cannot affect the treatment protocol. But its value is significantly increased in the GBC patients and hence can be used for targeted therapy in future. Targeted therapy against Ki-67 in an in-vitro 3D model for ovarian cancer has shown positive results [19].But further studies will be needed in this regard. EGFR as a molecule for targeted therapy has already shown promising outcomes [20]. Mortality at 3 months is also significantly associated with a higher Ki-67 value, hence it can be taken as one of the factor for deciding the outcome of the disease. But the overall prognosis is

multifactorial depending on clinical presentation, stage, cellular differentiation and Ki-67 index alone cannot be responsible.

References

1. Shukla VK, Sharma D, Gurubachan, Dixit VK, Usha. Diagnostic value of serum CA-242, CA 19-9, CA 15-3, CA-125 in patients with carcinoma of gall bladder. Trop Gastroenterol 2006;27:160-5.
2. Randi G, Franceschi S, La Vecchia C. Gallbladder cancer worldwide: geographical distribution and risk factors. Int J Cancer. 2006 Apr 1;118(7):1591-602.
3. SayeedUnisa, PalepuJagannath, VinayDhir, Chiranjeeva Khandelwal, LalatenduSarangi and Tarun Kumar Roy. Population-based study to estimate prevalence and determine risk factors of gallbladder diseases in the rural Gangetic basin of North India. HPB(Oxford). 2011 Feb; 13(2):117-25.
4. Kapoor VK, McMichael AJ. Gallbladder cancer: an Indian' disease. Natl Med J India. 2003;16:209-13.
5. Izarzugaza MI, Fernandez L, Forman D, Sierra MS. Burden of gallbladder cancer in Central and South America. Cancer Epidemiology. 2016 Sep;44 Suppl 1:S82-S89.
6. Arroyo GF, Gentile A, Parada LA. Gallbladder cancer: South American experience.Chin Clinical Oncology. 2016 Oct;5(5):67.
7. Sergio Renato Pais-Costa, José Francisco de Matos Farah, Ricardo Artigiani-Neto, Sandro Jose Martins, Alberto Goldenberg. Evaluation of P53, E-cadherin, Cox-2, and EGFR protein immuno-expression on prognosis of resected gallbladder carcinoma. Brazilian archieves of digestive surgery 2014 September;27(2):126-32.
8. Kumar N, Khan MA, Kumar N; Rigvardhan, Ranjan R, Hazra N. Epidermal growth factor receptor expression in carcinoma gallbladder: A prospective study in Indian scenario. J Cancer Res Ther. 2016 Apr-Jun;12(2):959-62.
9. Kaufman M[1], Mehrotra B, Limaye S, White S, Fuchs A, Lebowicz Y, Nissel-Horowitz S, Thomas A. EGFR expression in gallbladder carcinoma in North America. Int J Med Sci. 2008 Sep 22;5(5):285-91.
10. Bruno S, Darzynkiewicz Z. Cell cycle dependent expression and stabilityof the nuclear protein detected by Ki-67 antibody in HL-60 cells. Cell proliferation, January 1992;25(1):31-40.
11. C. Soon Lee. Differences in cell proliferation and prognostic significance of proliferating cell nuclear antigen and Ki-67 antigen immunoreactivity in in situ and invasive carcinomas of the extrahepatic biliary tract. Cancer 1996;78:1881-7.
12. Luis A Hidalgo Grau, Josep M Badia, Carmen Admella Salvador, Teresa SolerMonsó, JosepFeliuCanaleta, Josep M GubernNogués et al.Gallbladder carcinoma: the role of p53 protein overexpression and Ki-67 antigen expression as prognostic markers. HPB (Oxford). 2004; 6(3):174-180.
13. Hui AM, Shi YZ, Li X, Sun L, Guido T, Takayama T, Makuuchu M. Proliferative marker Ki-67 in gallbladder carcinomas: high expression level predicts early recurrence after surgical resection. Cancer Lett. 2002 Feb 25;176(2):191-8.
14. Hawkins WG, DeMatteo RP, Jarnagin WR, Ben-Porat L, Blumgart LH, Fong Y. Jaundice predicts advanced disease and early mortality in patients with gallbladder cancer. Ann SurgOncol. 2004 March;11(3):310-5.
15. Yang XW, Yuan JM, Chen JY, Yang J, Gao QG, Yan XZ, Zhang BH[1], Feng S, Wu MC. The prognostic importance of jaundice in surgical resection with curative intent for gallbladder cancer. BMC Cancer. 2014 Sep 3;14:652.
16. Yang XW, Yang J, Li L, Man XB, Zhang BH, Shen F, Wu MC. Analysis of the relationships between clinicopathologic factors and survival ingallbladder cancer following surgical resection with curative intent. PLoS One. 2012;7(12):e51513.
17. Shrestha ML, Miyake H, Kikutsuji T, Tashiro S. Prognostic significance of Ki-67 and p53 antigen expression in carcinomas of bile duct and gallbladder. J Med Invest. 1998 Aug;45(1-4):95-102.
18. KeitaKai, Masanori Masuda, Takao IDE, Yukari Takase, Atsushi Miyushi, Kenji Kitahara et al. Mitotic count reflects prognosis of gallbladder cancer particularly among patients with T3 tumor. MolClinOncol. 2013 July; 1(4):633-638.
19. Ramtin Rahmanzadeh, Prakash Rai, Jonathan P. Celli, Imran Rizvi, Bettina Baron-Lühr, Johannes Gerdes, and Tayyaba Hasan. Ki-67 as a molecular target for therapy in an in vitro 3D model for ovarian cancer. Cancer Res. 2010 Nov 15;70(22):9234–42.
20. Ymera Pignochino, Ivana Sarotto[†], Caterina Peraldo-Neia, Junia Y Penachioni, Giuliana Cavalloni, Giorgia Migliardi, Laura Casorzo, Giovanna Chiorino, Mauro Risio, Alberto Bardelli, Massimo Aglietta , Francesco Leone. Targeting EGFR/HER2 pathways enhances the antiproliferative effect of gemcitabine in biliary tract and gallbladder carcinomas. BMC Cancer 2010;10:631.