

Expression of Ki67 and CerbB-2 in Gall Bladder, Oesophageal, Small Intestinal and Colorectal Adenocarcinomas: A Study from a Tertiary Care Hospital in Western U.P.

Cheena Garg¹, Arjun Agarwal², Ranjan Agrawal³

ABSTRACT

Introduction: Gall bladder cancer is the commonest biliary tract malignancy with poor prognosis. Oesophageal adenocarcinomas are aggressive with very poor survival. Adenocarcinomas of small intestine are rare, in comparison to colorectal carcinomas. We studied the expression of two commonly studied immunohistochemical markers Ki67 and CerbB-2 in gall bladder and gastrointestinal carcinomas, to find out their prognostic significance.

Material and Methods: Histopathologically confirmed cases of adenocarcinomas of gall bladder (19 cases), oesophagus (3 cases), small intestine (3 cases) and colorectal region (8 cases) were included. Formalin fixed paraffin embedded tissue blocks were retrieved and sections were studied immunohistochemically for expression of Ki67 and CerbB-2. Tumors of all stages and grades were studied.

Results: Our study revealed a significantly high Ki67 in neoplastic glands of gall bladder, oesophageal, small intestinal and colorectal adenocarcinomas as compared to the non neoplastic areas. The Ki67 labelling index was higher in moderately and poorly differentiated areas as well as mucinous and signet ring type adenocarcinomas. There was no relation with stage/depth of tumor invasion. CerbB-2 was significantly overexpressed in gall bladder and colorectal adenocarcinomas. Expression was higher in advanced stage and greater depth of invasion. There was no association with tumor grade or type. In esophageal and small intestine adenocarcinomas, expression was not related to either tumor stage or grade.

Conclusion: A panel of Ki67 and CerbB-2 in gastrointestinal and biliary tract malignancies may correlate well with different prognostic indicators in these malignancies, like tumor stage, histologic type and grade.

Keywords: Gall Bladder, Oesophagus, S.I., Colorectal, Ki67, CerbB2.

INTRODUCTION

Gall bladder cancer is the commonest biliary tract malignancy and carries a poor prognosis. Oesophageal adenocarcinomas also have an aggressive course with very poor survival. Adenocarcinomas of small intestine are rare, in comparison to colorectal carcinomas, especially in the industrialized world.

C-erbB2, a cell surface growth factor receptor, has gained popularity as a candidate for targeted therapy in different cancers. There is an increasing evidence that over-expression of tyrosine kinase growth factor receptors such as C-erbB2 may play an important role in the development of biliary tract

carcinomas.¹ Over- expression of C-erbB2 has been studied in various tumors like breast, gastric, colorectal, pulmonary, and gallbladder, and has become a valid indication for targeted therapy by trastuzumab in breast cancer.¹⁻³ HER2 (CerbB2) overexpression or amplification has been studied in patients with gastrointestinal tumors such as esophageal adenocarcinoma,⁴ gastric adenocarcinoma,³ and colorectal adenocarcinoma³

Ki-67 labeling index has been a marker of proliferation and long studied in several cancers. It has been found to be significantly higher in gall bladder malignancies in patients in age group <40 years (P = 0.027), and poorly differentiated tumors (P = 0.023).⁵⁻⁶

So far, not much work has been done in relation with these markers in gall bladder and gastrointestinal tract cancers. Only scanty data is available from India on the expression of these biomarkers in these malignancies. We studied the expression of these two commonly studied immunohistochemical markers Ki67 and CerbB-2 in gall bladder and gastrointestinal carcinomas, to find out their prognostic significance and potential etiological relationship.

MATERIAL AND METHODS

Histopathologically confirmed cases of adenocarcinomas of gall bladder, oesophagus, small intestine and colorectal region over a half yearly period in histopathology laboratory of Rohilkhand Medical college (Bareilly, UP), were included. Formalin fixed paraffin embedded tissue blocks were taken and desired sections were studied immunohistochemically for expression of Ki67 (Mouse Monoclonal antibody from Dako, Germany) and CerbB-2 (Dako, Germany). Positive control for Ki67 was a known case of acute lymphoblastic leukaemia, while that for CerbB2 was a known case of Her2neu positive breast cancer. Areas unstained with respective IHC in the stained slides were taken as negative

¹Assistant Professor, Department of Pathology, ²Assistant Professor, Department of Surgical Oncology, ³Professor, Department of Pathology, Rohilkhand Medical College and Hospital, Bareilly, UP, India

Corresponding author: Arjun Agarwal, 35/C8, Ashok Kiran Hospital, Rampur Garden, Bareilly, UP-243001, India

How to cite this article: Cheena Garg, Arjun Agarwal, Ranjan Agrawal. Expression of Ki67 and CerbB-2 in gall bladder, oesophageal, small intestinal and colorectal adenocarcinomas: a study from a tertiary care hospital in Western U.P. International Journal of Contemporary Medical Research 2019;6(9):11-15.

DOI: <http://dx.doi.org/10.21276/ijcmr.2019.6.9.17>

controls. Tumours of all stages and grades were studied.

CerbB2 evaluation of the results was done according to the criteria as recommended by the manufacturer using the scores from 0 to 3+. Score 0 is defined as no staining at all or membrane staining in < 10% of tumour cells. Score 1+ is defined as faint/barely perceptible membrane staining in > 10% of tumour cells. The cells are only stained in part of the membrane. 2+ is defined as weak to moderate staining of the entire membrane in > 10% of the tumour cells. And 3+ is defined as strong staining of the entire membrane in > 10%. Score of 0 and 1+ indicates a negative tumour like in breast carcinoma, while only 3+ is considered positive. 2+ is equivocal. Cytoplasmic staining was taken negative.

KI67 was expressed as MIB 1 Labelling Index. It is the number of positive nuclear staining cells out of total number of cells counted (upto 1000) multiplied by 100. (<10 is taken negative).

RESULTS

Total 33 cases were studied, of which 19 (57.6%) were gall bladder adenocarcinomas, 3 (9.1%) were oesophageal adenocarcinomas and small intestinal adenocarcinomas, each. Another 8 (24.2%) were colorectal adenocarcinomas. Mean age for gall bladder adenocarcinoma was 49.5 years,

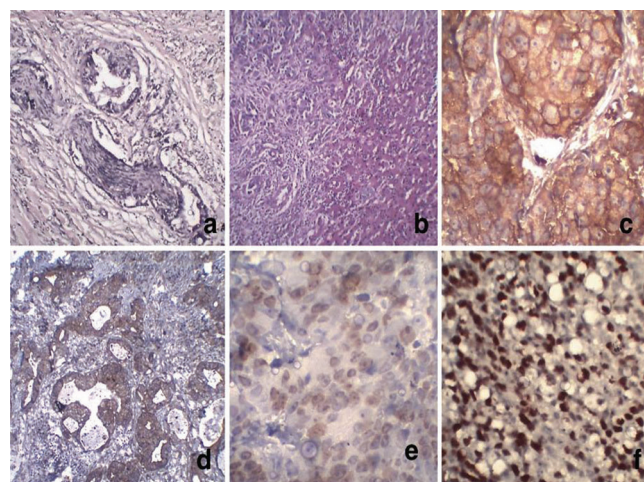


Figure-1: a: Shows well differentiated gall bladder adenocarcinoma with perineurial invasion (H&E x 100); b : Shows poorly differentiated GB adenocarcinoma with hepatic metastasis.(H&E x 100); c: Shows CerbB2 positivity (3+) in a poorly differentiated GB adenocarcinoma (IHC x 400); d: Shows CerbB2 positivity (3+) in a well differentiated GB adenocarcinoma in areas of hepatic metastasis. (IHC x 40); e: Shows strong Ki67 positivity (MIB1 LI= 66) in a poorly differentiated GB adenocarcinoma (IHC x 400); f: Shows strong Ki67 positivity (MIB 1 LI= 72) in a signet ring type GB adenocarcinoma (IHCx 400)

		Total	CerbB2 (No.and % of positive cases)	Ki67 no. of positive cases (mean MIB 1 LI)
Tumour stage/depth of invasion	Upto mucosa	0	0	0
	Upto ms propria	3	2(67%)	2 (26.5)
	Full thickness (all layers)	16	12(75%)	11(37.4)
	Total cases	19	14	13
	Lymphovascular/perineural invasion	5	2(40%)	3(40)
	Hepatic metastasis	5	4(80%)	4(52)
Tumour grade/Differentiation	Well differentiated	13	9(69%)	7(21)
	Moderately differentiated	2	1(50%)	2(35)
	Poorly differentiated	4	4(100%)	4(62)
	Total cases	19	14	13

Table-1: Immunohistochemical Expression of CerbB2 and Ki67 in GB adenocarcinoma

		Total	CerbB2 (no. of positive cases)	Ki67 no. of positive cases (mean MIB 1 LI)
Tumour stage	Upto mucosa	1	0	1(30)
	Upto ms propria	3	0	2(54.5)
	Full thickness	4	3(75%)	2(69)
	Total cases	8	3	5
	Omental metastasis	2	1(50%)	2(69)
Tumour grade	Well differentiated	5	2(40%)	2 (46)
	Moderately differentiated	1	0	1 (43)
	Poorly differentiated	2	1(50%)	2(71)
	Total cases	8	3	5

Table-2: Immunohistochemical Expression of CerbB2 and Ki67 in Colorectal adenocarcinoma

Tumor subtype	Total no. of cases having identifiable subtype	CerbB2 (no.and % of positive cases)	Ki67 No. of positive cases (Mean MIB1 LI)
Mucous secreting	4	1(20%)	1 (32)
Papillary	5	4 (80%)	5 (43)
Mucous secreting + papillary	2	0	1 (62)
Signet ring type	3	3(100%)	3 (64.3)

Table-3: CerbB2 and KI67 expression according to tumor histologic subtype

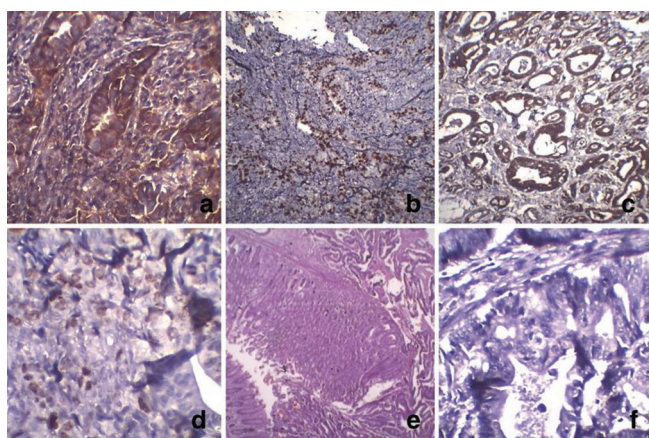


Figure-2: a: Shows moderately differentiated oesophageal adenocarcinoma with CerbB2 positivity (3+). (IHC x 400); b: Shows moderately differentiated oesophageal adenocarcinoma with strong Ki67 positivity (MIB 1 LI= 80%). (IHC x 40); c: Shows CerbB2 positivity (3+) in well differentiated transmural invasion of colorectal adenocarcinoma. (IHC x 100); d: Shows Ki67 positivity (MIB 1 LI= 62) in a poorly differentiated colorectal adenocarcinoma. (IHC x 400); e: Shows well differentiated small intestinal adenocarcinoma. (H&E x 40); f: Shows Ki67 negativity (MIB 1 LI= 8) in a well differentiated small intestinal adenocarcinoma. (IHC x 100).

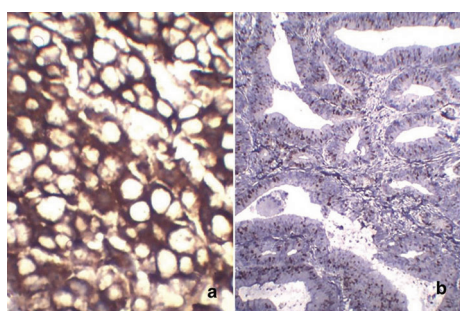


Figure-3: a: Shows CerbB2 positivity (3+) in signet ring type adenocarcinoma. (IHC x 100); b: Shows Ki67 positivity (MIB 1 LI= 80) in a mucopapillary type adenocarcinoma. (IHC x 40X).

oesophageal adenocarcinoma was 65 years, colorectal adenocarcinoma was 46.25 years and small intestinal carcinomas was 37 years.

Gall bladder and small intestinal adenocarcinomas were commoner in females (94.7% and 67%, respectively). Oesophageal cancers were all in males. While, colorectal carcinomas had equal sex distribution.

Overall, out of 33 adenocarcinomas, 20 were well differentiated, 6 moderate and 7 poorly differentiated histologically.

Five cases of gall bladder adenocarcinoma (Table 1) had lymphovascular / perineural invasion (Figure 1a), of which 4 were well differentiated while one was poorly differentiated. 5 cases of gall bladder malignancy showed hepatic metastasis (Figure 1b), of which 2 were moderately and 2 poorly differentiated while one was well differentiated.

Score 3 + was taken as positive in CerbB2 assessment. The number of cases expressing CerbB2 positivity (Figure 1c) were more in the categories of full thickness invasion (Table 1) (75% of total cases in that category i.e.12 out of

16), hepatic metastasis (4 out of 5, i.e. 80%) (Figure 1d) and poorly differentiated carcinomas (all 4 cases, 100%) as compared to others. However, the different results were statistically insignificant.

KI67 expression (mean MIB 1 Labelling Index) was found to be statistically significant according to tumour grade/differentiation (Table 1) (Figure 1e, f) (p value= 0.000). The expression of Ki67 among different tumour stages / depth of invasion was statistically insignificant (p value=0.561).

Tumor stage could not be assessed in oesophageal cancers as all the samples received were endoscopic biopsy specimens. None of the cases got resected at our institution, thus the lack of assessment of IHC in different stages. Only one out of three cases showed CerbB2 positivity (Figure 2a). All the three cases showed Ki67 positivity in the neoplastic area while the non neoplastic zones were negative (Figure 2b). Cases with moderate differentiation showed higher mean MIB1 LI (90) than poorly differentiated case (78). This result could not lead to a significant relationship as the number of cases were too small to be analysed statistically.

In colorectal carcinomas, CerbB2 was positive in cases of full thickness invasion (Table 2) (Figure 2c) (3 out of 4, i.e. 75%) as well as one case out of 2, of omental metastasis (50%). None of the cases of lower stage tumours showed CerbB2 expression. However, some well differentiated tumors did express CerbB2 along with a poorly differentiated one.

5 out of 8 cases of colorectal carcinoma expressed KI67 positivity. MIB 1 LI was higher in poorly differentiated cases (Table 2) (Figure 2d) as well as those with full thickness invasion and omental metastasis. However, the results were statistically insignificant (p value= 0.263 for tumour stage and 0.408 for grade).

Amongst the 2 cases of colorectal adenocarcinomas with omental metastasis, one each was well and poorly differentiated tumour.

One out of three cases of small intestine adenocarcinoma was positive for both CerbB2 as well as Ki67. This case was moderately differentiated. The remaining 2 cases were well differentiated (Figure 2e) without positive expression of CerbB2, however, one was 2+ and another 1+, none being 0. Ki 67 expression in these 2 cases was < 10% (Figure 2f) but both showed some expression, that was more than the non neoplastic areas.

CerbB2 was positive in majority cases of papillary and signet ring type carcinoma (Table 3) (Figure 3a), but the significance of this relation needs to be established by further studies. Whereas, MIB1LI was higher in mucopapillary (Table 3) (Figure 3b) and signet ring type carcinomas.

DISCUSSION

Ki67 and CerbB-2 overexpression has been studied extensively in several malignancies with an established prognostic or causative pathogenetic role. In India, gall bladder carcinomas are reported frequently in the Gangetic belt region.⁵ Pathogenesis involves several molecular alterations and altered gene expression.

Out of 33 cases studied, majority were gall bladder

adenocarcinomas, followed by colorectal and then oesophageal and small intestine. Patients were mostly in their middle ages except for oesophageal adenocarcinoma where mean age was 65 years. Females predominated the gall bladder adenocarcinoma cases, while males were commoner in oesophageal cases. Colorectal malignancy showed equal distribution while small intestinal adenocarcinomas were commoner in females. These trends were in concordance with the existing literature.

Majority of gall bladder carcinomas were well differentiated (13 out of 19) while only 4 were poorly differentiated. Ki67 was highly expressed in these poorly differentiated cases (mean MIB1 LI= 62) as compared to 21 in well differentiated ones ($p= 0.000$). Ki67 overexpression has been related to poor tumor differentiation in some of the previous studies as well.⁶

CerbB-2 has been related to advanced tumor stage⁷ in gall bladder adenocarcinomas. In our cases, 12 out of 16 cases (75%) which showed full thickness invasion, were positive for CerbB2 as compared to only 67% of those with muscular propria invasion. 80% of those with hepatic metastasis were also CerbB2 positive. The results, though in trend with the previous mentioned study, were statistically insignificant. This shows that more studies would be needed on a larger number of patients in our region, to validate the same.

There is also proven overexpression of Ki67 and CerbB-2 in esophageal adenocarcinomas.^{9,10} However, in our cases stage could not be assessed as all the samples received were endoscopic biopsy specimens. None of the cases got resected at our institution, thus the lack of assessment of IHC in different stages. Only one out of three cases showed CerbB2 positivity. All the three cases showed Ki67 positivity in the neoplastic area while the non neoplastic zones were negative.

Colorectal adenocarcinomas overexpress both Ki67 and CerbB-2¹¹ in comparison to non-neoplastic tissue. In our study, CerbB2 was positive in cases of full thickness invasion (3 out of 4, i.e. 75%) as well as one case out of 2, of omental metastasis (50%). None of the cases of lower stage tumours showed CerbB2 expression. 5 out of 8 cases of colorectal carcinoma expressed Ki67 positivity. MIB 1 LI was higher in poorly differentiated cases as well as those with full thickness invasion and omental metastasis.

Increased Ki67 has been found in poorly differentiated small intestine adenocarcinomas¹⁰, however, CerbB-2 was not overexpressed¹¹ in some studies. One out of our three cases of small intestine adenocarcinoma was positive for both CerbB2 as well as Ki67. This case was moderately differentiated. The remaining 2 cases were well differentiated without positive expression of CerbB2, however, one was 2+ and another 1+, none being 0. Ki 67 expression in these 2 cases was < 10% but both showed some expression, that was more than the non neoplastic areas. There is a need to carry out further study to evaluate the nature of expression of these markers in small intestinal adenocarcinoma, as the literature is very variable.

None of the studies so far, highlight the expression of Ki67

and CerbB2 in different types of adenocarcinomas. In our study, CerbB2 was positive in majority cases of papillary and signet ring type carcinoma, but the significance of this relation needs to be established by further studies. Whereas, MIB1LI was higher in mucopapillary and signet ring type carcinomas.

CONCLUSION

Evaluation of a panel of Ki67 and CerbB-2 in gastrointestinal and biliary tract malignancies correlate well with different prognostic indicators in these malignancies, like tumor stage, histologic type and grade. In the Indian scenario, where such studies are still meagre future studies with larger sample size on these lines may provide more important insights that may be utilized in the diagnosis and treatment of gastrointestinal and biliary tract malignancies.

REFERENCES

1. Nakazawa K, Dobashi Y, Suzuki S, Fujii H, Takeda Y, Ooi A. Amplification and overexpression of C-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. *J Pathol* 2005;206:356-65.
2. Chaube A, Tewari M, Garbyal RS, Singh U, Shukla HS. Preliminary study of p53 and C-erbB-2 expression in gallbladder cancer in Indian patients. *BMC Cancer*. 2006 10;6:126. Available from: [Http://www.biomedcentral.com/1471-2407/6/126](http://www.biomedcentral.com/1471-2407/6/126).
3. KoeppenHK, WrightBD, BurtAD, et al. Overexpression of HER2/neu in solid tumours: an immunohistochemical survey. *Histopathology*. 2001;38:96-104.
4. Reichelt U, Duesedau P, Tsourlakis M, et al. Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus. *Mod Pathol*. 2007;20:120-129.
5. Pandey M, Shukla M, Shukla V. Diet and gall bladder cancer. *Indian J Med Paediatr Oncol* 2008;29:6-7.
6. Doval DC, Azam S, Sinha R, Batra U, Mehta A. Expression of epidermal growth factor receptor, p53, Bcl2, vascular endothelial growth factor, cyclooxygenase-2, cyclin D1, human epidermal receptor-2 and Ki-67: Association with clinicopathological profiles and outcomes in gallbladder carcinoma. *J Carcinog* 2014;13:10-10.
7. Kumari N, Kapoor VK, Krishnani N, Kumar K, Baitha DK. Role of C-erbB2 expression in gallbladder cancer. *Indian J Pathol Microbiol* 2012;55:75-9.
8. Tiwari E, Pallipady A, Mishra S. Role of Immunohistochemistry in Early Diagnosis of Oesophageal Biopsies – a Review. *Int J of Recent Scientific Rsrch* 2015; 6:3375-3379.
9. Gowryshankar A, Nagaraja V, Eslick GD. HER2 status in Barrett's Esophagus & Esophageal Cancer: a meta analysis. *J Gastrointest Oncol* 2014;5:25-35.
10. Terada T. Malignant tumors of the small intestine: A histopathologic study of 41 cases among 1,312 consecutive specimens of small intestine. *Int J Clin Exp Pathol* 2012;5:203-209.
11. Farzand S, Siddique T, Saba K, Bukhari MH. Frequency of HER2/neu overexpression in adenocarcinoma of gastrointestinal system. *World J Gastroenterol* 2014;20:5889-5896.
12. Georgescu CV, Săftoiu A, Georgescu CC, Ciurea R,

Ciurea T. Correlations of Proliferation Markers, p53 Expression and Histological Findings in Colorectal Carcinoma. J Gastrointest Liver Dis 2007;16:133-139.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 08-08-2019; **Accepted:** 01-09-2019; **Published:** 16-09-2019