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RESEARCH ARTICLE

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.

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ABSTRACT

Our study revealed a significantly high expression of Ki67 in neoplastic epithelial 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 mucopapillary and signet ring type adenocarcinomas. There was no particular relation with stage of tumor spread/depth of tumor invasion. CerbB-2 was significantly overexpressed in gall bladder and colorectal adenocarcinomas. Expression was higher in tumors of advanced stage and greater depth of invasion. There was no significant association with tumor grade or type. In esophageal and small intestine adenocarcinomas, expression was not related to either tumor stage or grade.

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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. We studied the expression of two commonly studied immunohistochemical markers Ki67 and CerbB-2 in gastrointestinal adenocarcinomas to find out their prognostic significance and potential etiological relationship.

MATERIALS 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. Archived formalin fixed paraffin embedded tissue blocks were retrieved 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 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 (reference) using the scores from 0 to 3+. Score 0 is defended as no staining at all or membrane staining in < 10% of tumour cells. Score 1+is defined as faint/barely perceptable 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. Hofmann et al., 2008 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). Shimura et al. 2016.

RESULTS AND OBSERVATIONS

Table 1. Distribution of cases of adenocarcinoma according to site

Site	No. of cases	Percentage (%)
Gall bladder	19	57.6
Esophagus	3	9.1
Colorectal	8	24.2
Small intestine	3	9.1
Total	33	100

Table 2. Mean age of distribution

Site	Mean age (yrs)
Gall bladder	49.5
Esophagus	65
Colorectal	46.25
Small intestine	37

Table 3. Sex distribution of cases

Site	Males	Females	Total
Gall bladder	1	18	19
Esophagus	3	0	3
Colorectal	4	4	8
Small intestine	1	2	3
Total	9	24	33

Out of 5 cases of gall bladder adenocarcinoma with lymphovascular / perineural (Figure 1) invasion 4 were well differentiated while one was poorly differentiated. 5 of the cases showed hepatic metastasis (Figure 2) , 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 3) were more in the categories of full thickness invasion (75% of total cases in that category i.e.12 out of 16), hepatic metastasis (4 out of 5,i.e. 80%) (Figure 4) 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 (Figure 5,6) (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 7). All the three cases showed Ki67 positivity in the neoplastic area while the non neoplastic zones were negative (Figure 8). Cases with moderate differentiation showed higher mean MIB1 LI than poorly differentiated case. This result could not lead to a significant relationship as the number of cases were too small to be analysed statistically. CerbB2 was positive in cases of full thickness invasion (Figure 9) (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 (Figure 10) 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). 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 11) 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 12) but both showed some expression, that was more than the non neoplastic areas. Table 11: CerbB2 and KI67 expression according to tumor subtypeCerbB2 was positive in majority cases of papillary and signet ring type carcinoma (Figure 13), but the significance of this relation needs to be established by further studies. Whereas, MIB1LI was higher in mucopapillary (Figure 14) 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.[1] 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[2].

CerbB-2 has been related to advanced tumor stage[3] 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.[4,5.]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-2[7] 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%).

Evaluation of Ki67 and CerbB-2 in gastrointestinal malignancies is important to establish causative and prognostic relationships, especially in the Indian scenario, where such studies are still meagre. 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 adenocarcinomas[6], however, CerbB-2 was not overepressed[7] in some studies.

Table 4. Distribution of cases according to degree of tumour differentiation (corresponds to tumour grade)

Site	Well differentiated	Moderately differentiated	Poorly Differentiated	Total
Gall bladder	13	2	4	19
Esophagus	0	2	1	3
Colorectal	5	1	2	8
Small intestine	2	1	0	3
Total	20	6	7	33

Table 5. Distribution of cases according to depth of tumour invasion (corresponds to pathological tumour stage)

Site	Upto mucosa	Upto muscularis propria	Full wall thickness (all layers)	Total
Gall bladder	0	3	16	19
Esophagus biopsy	3	-	-	3
Colorectal	1	3	4	8
Small intestine	1	1	1	3
Total	5	7	21	33

Table 6. Distribution of cases as per tumour metastasis and lymphovascular/perineural invasion

Site	Lymphovascular/perineural invasion	Metastasis
Gall bladder	5	5
Esophagus	-	-
Colorectal	0	2
Small intestine	0	0

Table 7. Immunohistochemical Expression of Her2Neu / CerbB2 and Ki67 in GB adenocarcinoma

		Cerbb2 (no. & % of positive cases)	Ki67 no. Of positive cases (mean mib 1 li)
Tumour stage/depth of invasion	Upto mucosa	0	0
	Upto ms propria	2(67%)	2 (26.5)
	Full thickness	12(75%)	11(37.4)
	Total positive cases	14(73.7%)	13
	Lymphovascular/perineural invasion	2(40%)	3(40)
	Hepatic metastasis	4(80%)	4(52)
Tumour grade/differentiation	Well differentiated	9(69%)	7(21)
	Moderately differentiated	1(50%)	2(35)
	Poorly differentiated	4(100%)	4(62)
	Total positive cases	14(73.7%)	13

Table 8. Immunohistochemical Expression of Her2Neu / CerbB2 and Ki67 in Oesophageal adenocarcinoma

		CerbB2 (no. of positive cases)	Ki67 No. of positive cases(Mean MIB1 LI)
Tumour grade	Well differentiated	0	0
	Moderately differentiated	1	2 (90)
	Poorly differentiated	0	1(78)
	Total positive cases	1	3

Table 9. Immunohistochemical Expression of Her2Neu / CerbB2 and Ki67 in Colorectal adenocarcinoma

		Cerbb2 (no. Of positive cases)	Ki67 no. Of positive cases (mean mib 1 li)
Tumour stage	Upto mucosa	0	1(30)
	Upto ms propria	0	2(54.5)
	Full thickness	3(75%)	2(69)
	Total positive cases	3(37.5%)	5
	Omental metastasis	1(50%)	2(69)
Tumour grade	Well differentiated	2(40%)	2 (46)
	Moderately differentiated	0	1 (43)
	Poorly differentiated	1(50%)	2(71)
	Total positive cases	3(37.5%)	5

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.

Table 10. Immunohistochemical Expression of Her2Neu / CerbB2 and Ki67 in Small Intestine adenocarcinoma

		CerbB2 (no. of positive cases)	Ki67 No. of positive cases (Mean MIB 1 LI)
Tumour stage	Upto mucosa	0	0
	Upto ms propria	0	0
	Full thickness	1 (100%)	1 (50)
	Total positive cases	1(33%)	1
Tumour grade	Well differentiated	0	0
	Moderately differentiated	1 (100%)	1(50)
	Poorly differentiated	-	-
	Total positive cases	1(33%)	1

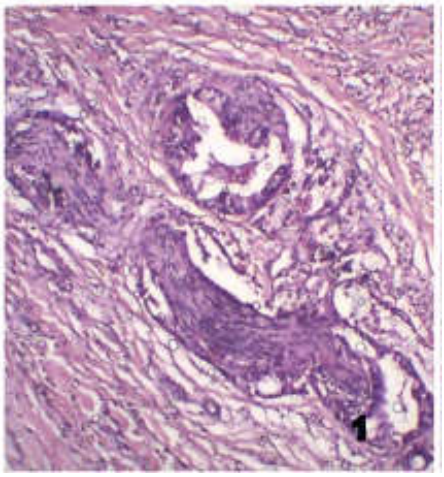

Figure 1. Shows well differentiated gall bladder adenocarcinoma with perineurial invasion (H&E x 100)

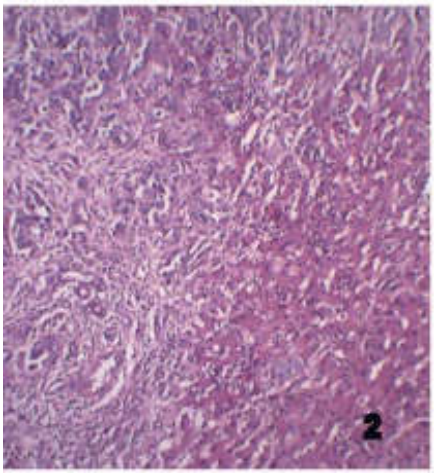

Figure 2. Shows poorly differentiated GB adenocarcinoma with hepatic metastasis.(H&E x 100)

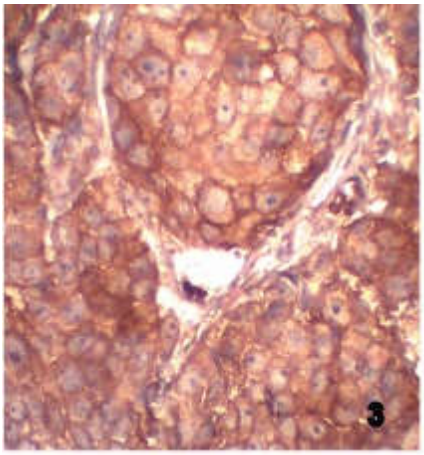

Figure 3. Shows CerbB2 positivity (3+) in a poorly differentiated GB adenocarcinoma (IHC x 400)

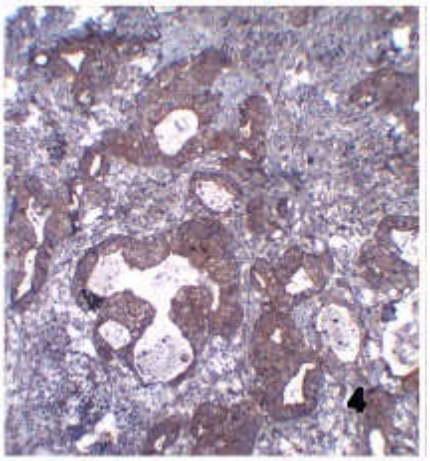

Figure 4. Shows CerbB2 positivity (3+) in a well differentiated GB adenocarcinoma in areas of hepatic metastasis (IHC x 40)

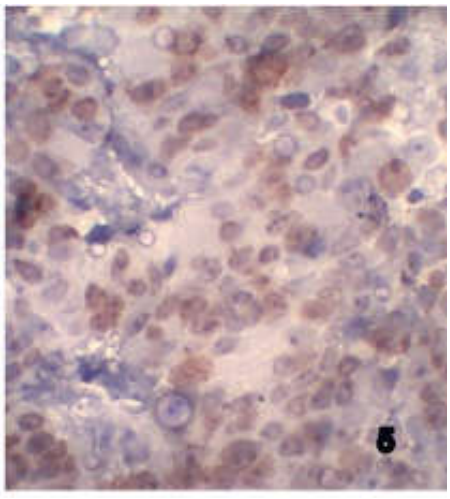

Figure 5. Shows strong Ki67 positivity (MIB1 LI= 66) in a poorly differentiated GB adenocarcinoma (IHC x 400)

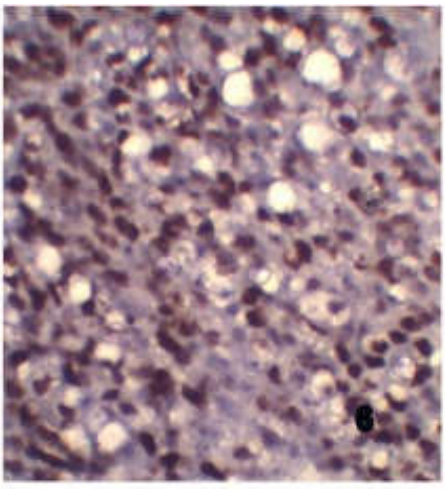

Figure 6. Shows strong Ki67 positivity (MIB 1 LI= 72) in a signet ring type GB adenocarcinoma (IHCx 400)

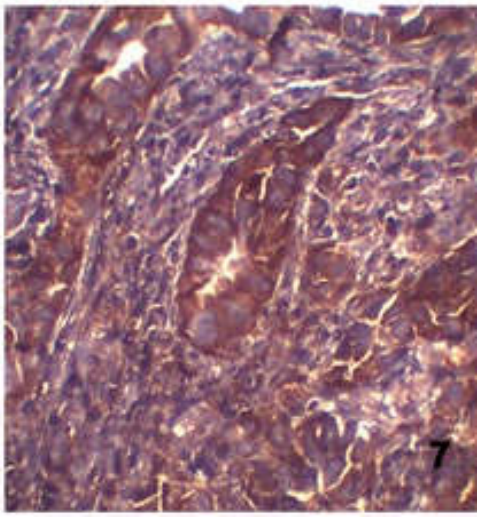

Figure 7. Shows moderately differentiated oesophageal adenocarcinoma with CerbB2 positivity (3+). (IHC x 400)

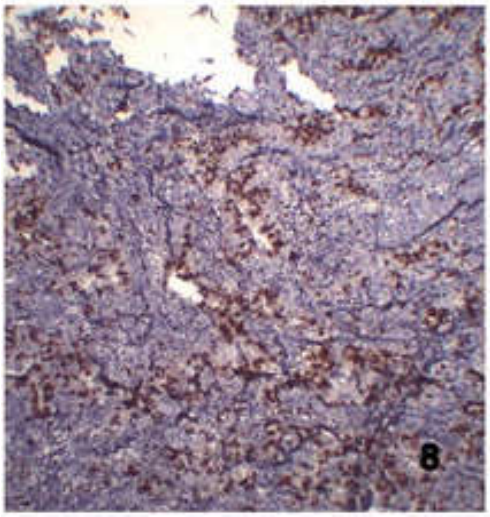

Figure 8. Shows moderately differentiated oesophageal adenocarcinoma with strong Ki67 positivity (MIB 1 LI= 80%). (IHC x 40)

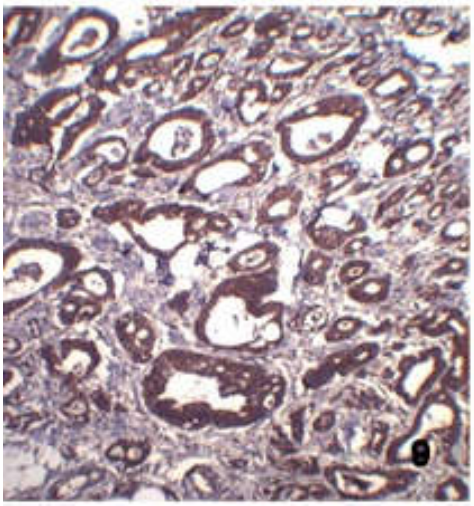

Figure 9. Shows CerbB2 positivity (3+) in well differentiated transmural invasion of colorectal adenocarcinoma. (IHC x 100)

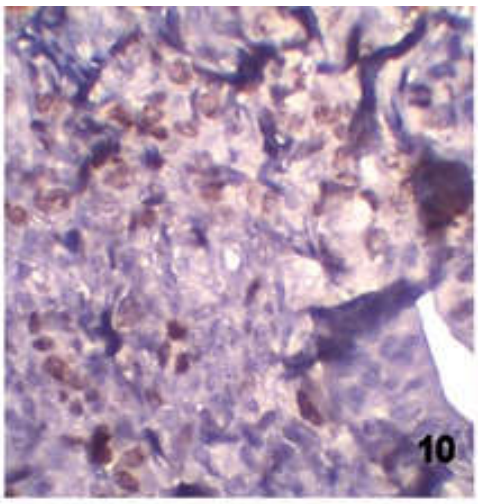

Figure 10. Shows Ki67 positivity (MIB 1 LI = 62) in a poorly differentiated colorectal adenocarcinoma. (IHC x 400)

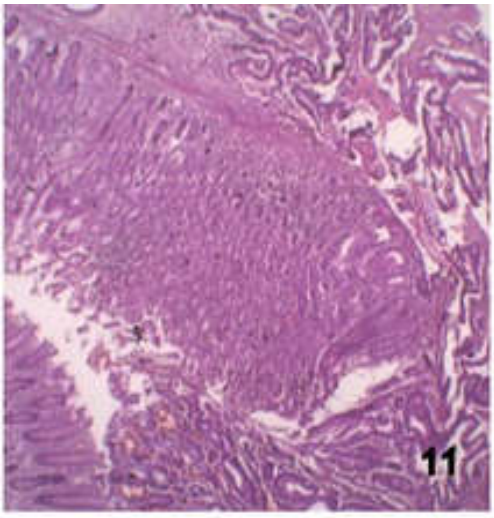

Figure 11. Shows well differentiated small intestinal adenocarcinoma. (H&E x 40).

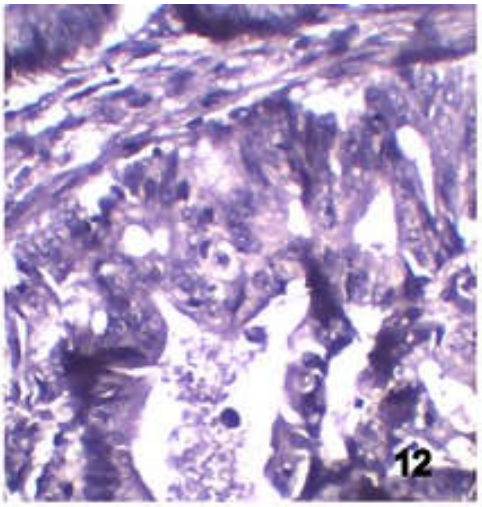

Figure12. Shows Ki67 negativity (MIB 1 LI= 8) in a well differentiated small intestinal adenocarcinoma. (IHC x 100)

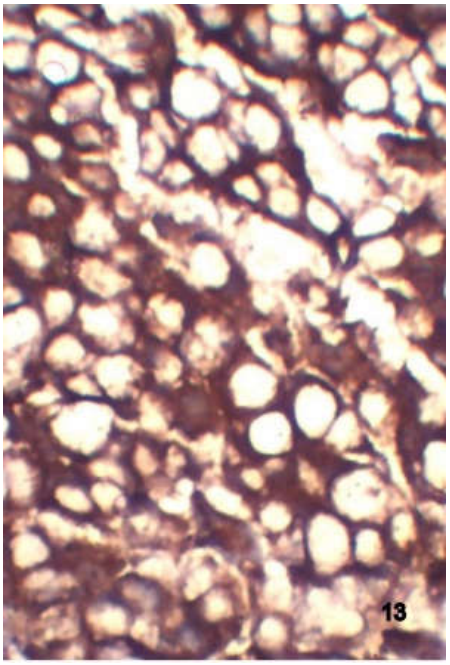

Figure 13. Shows CerbB2 positivity (3+) in signet ring type adenocarcinoma. (IHC x 100)

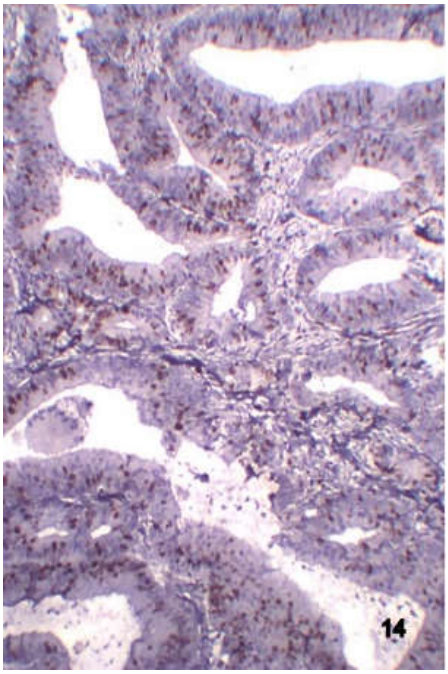

Figure 14. Shows Ki67 positivity (MIB 1 LI= 80) in a mucopapillary type adenocarcinoma. (IHC x 40)

Conclusion

Evaluation of Ki67 and CerbB-2 in gastrointestinal malignancies is important to establish causative and prognostic relationships, especially in the Indian scenario, where such studies are still meagre.

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