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Study of Her2/Neu Expression in Colon Adenocarcinoma and Its Correlation with Clinicopathological Variables

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Abstract:
Objective*- Colon cancer is a prevalent human malignancy. HER2/neu is an important oncogene in breast cancer, but its prevalence and significance in colon cancer have been poorly documented. The aim of this study is to determine the rate and pattern of HER2/neu expression in colon carcinoma by immunohistochemistry (IHC).*
Materials and Methods*- twenty-nine colon carcinoma specimens were chosen. IHC for HER2/neu was Performed. Clinicopathologic data and IHC results were analysed.*
Results*- Most of the carcinoma cases was well differentiated and located in the left side. There was positive HER2/neu staining in a high percent of cases (7 Cases, 24.14%) with both cytoplasmic (5 cases, 17.24%) and membranous (2 cases, 6.90%) staining. There was positive HER2 staining in 28.57% of Stage II, in 57.14% of stage III and 14.28% of stage IV cases. There was positive HER2 staining in 3 (42.35%) of well differentiated and in 4 (57.14%) of moderately differentiated cases. There was no correlation between HER2/neu expression and age, sex, site and type of tumor.*
Conclusion *-The rate of HER2/neu expression in colon carcinoma is high. Because of more prominent membranous staining in higher stages and grades, Herceptin therapy could be helpful in patients with lymph node or distant metastases.*
Keywords: *Colon carcinoma, HER2/neu, Immunohistochemistry.*

I. Introduction

Colon cancer is the third prevalent cancer in men and women. It is a health problem worldwide. There are many researches about molecular and epidemiologic aspects of it. But there are only few articles about the HER2/neu expression in colon adenocarcinoma.

The HER 2/neu proto-oncogene is 1 of 4 epidermal growth factor receptor (EGFR)-related receptors, located on chromosome 17q21 and encodes a 185 kDa transmembrane protein with tyrosine kinase activity that functions as a growth factor receptor(15,16). ERBB2, a known proto-oncogene, HER2 is named because it has a similar structure to human epidermal growth factor receptor, or HER1. Neu is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor.

HER2/neu overexpression is already being established as a poor prognostic factor for breast carcinoma(17), moreover success of HER 2/neu-directed therapy in breast cancer has lead to evaluations of protein expression and gene amplification in different other malignancies like in prostate, ovarian and lung cancers as well as in several forms of gastrointestinal malignancies including colorectal cancer.

HER-2/neu testing has reached near standard-of-practice status in breast cancer. After a definite improvement in overall survival, this recently led to FDA-approval for the use of transtuzumab in HER2-positive metastatic gastric cancer (18). In contrast to breast cancer, there is evidence that in colorectal cancer cytoplasmic HER2 could in fact be associated with survival prognosis.

HER2 in overexpression rates colorectal carcinoma varied between zero and 84% in different studies (19) though the clinical significance of HER2 in these publications was not consistent. If further studies prove the role of HER2/neu expression in the development of colorectal carcinoma and/or prove its correlation with different clinicopathological data, HER2/neu can evolve as a new prognostic tool for colorectal carcinoma and

moreover monoclonal antibodies directed against HER2/neu like transtuzumab can be tried as an adjuvant therapy for these cases.

II. Material And Methods

This Analytical type study of 29 case of colorectal adenocarcinoma admitted in SMS medical & hospital, Jaipur, Rajasthan (India) proved to be adenocarcinoma.

Inclusion Criteria: All cases of adenocarcinoma colon & rectum.

Exclusion Criteria: Colorectal carcinoma other then adenocarcinoma.

After detailed history, clinical examination, radiological, endoscopic evaluation and tumor marker study clinical stage was assessed. After operation specimen was sent for histopathological examination and her 2/ neu staining.

Her2/Neu Staining:

There are multiple immunohistochemical kits available for her2-staining, but the polyclonal antibody-based HERCEPTEST (dako, glostrup, Denmark) was used.

The Scoring Of Her2/Neu

The tumour tissue with more than 10% cancer cells, which showed Staining for her-2/neu were classified as positive.

The staining pattern was seen as:

- Cytoplasmic only
- Membranous only and
- Membranous + cytoplasmic

Scoring was done on the basis of the percentage of positive cells: comprise four different outcomes:

Score 0 - no staining or staining in <10% of the tumor cells,
Score 1+ -10 – 40 %,
Score 2+ - 41 – 70 % and
Score 3+ - >70 %.

The relation between the her2/neu staining and clinicopathological variables were analysed using the chi-square test.

III. Results

A total of 29 colon adenocarcinoma cases were included in the study. All the specimens were surgical colon resections. The patients were comprised of 16 (55.17%) men and 13 (44.83%) women, and their mean age at surgery was 46.1 years (range, 23- 85).

Table1: Clinicopathologic characteristics of colon cancer cases

Sex	
Male	16
Female	13
Site	
Right	10
Left	19
Grade	
Well differentiated	13
Moderately differentiated	16
Poorly differentiated	0
Stage	
A	4
B	8
C	15
D	2

They were categorized as left sided including Recto –Sigmoid was most commonly involved present in 15 patients (51.72 %), followed by Ascending colon in 9 patients (31.03 %), Transverse colon in 2 patients (6.90 %), 1 patient (3.45 %) each of hepatic flexure, splenic flexure, and descending colon involvement. There were 29 cases with the histologic diagnosis of conventional adenocarcinoma (which includes 13 well-differentiated (44.83%) and 16 moderately-differentiated grades (55.17%) and no case of poorly differentiated grade. The stages of the tumors were as follows: Stage I : 4 (13.79 %) , stage II : 8 (27.59 %), stage III : 15 (51.72 %) and stage IV : 2 (68.97 %). Out of 29 patients in our study no nodes recovered from 4 (13.79 %) patients. Out of 25 patients in which nodes recovered, 16 (55.17 %) found to be metastatic and 9 (31.03 %) found to be reactive. Out of 29 patient, 8 patients are found to have significantly raised CEA i.e. >5ng/ml, rest of the 21 have normal CEA i.e. <5ng/ml.

HER2/Neu Staining

There were 7 (24.14%) HER2 positive and 22 (75.86%) HER2 negative cases (Fig. 1-3). 5 (17.24%) cases had cytoplasmic and 2 (6.90%) cases had membranous staining. patients have positive membranous staining, this might be because of more number of younger patients that too of well/mod. differentiated adenocarcinoma the chance of HER2/neu positivity may less as documented.

Table 2

% of staining	Cytoplasmic Only		Membranous only		Membranous +Cytoplasmic	
	No.	%	No.	%	No.	%
+1(10-40%)	5	17.24%	2	6.90 %	0	-
+2(41-70%)	0	-	0	-	0	-
+3(>70%)	0	-	0	-	0	-
Total	5	17.24%	2	6.90 %	0	-

Table 3 – Association of Her-2/neu with age

Age range	No. Of patients	Staining			
		Negative	+1(10-40%)	+2 (41-70%)	+3(>70 %)
< 30	4	3	1	0	0
31-40	7	5	2	0	0
41-50	**9**	8	1	0	0
51-60	5	4	1	0	0
>60	4	2	2	0	0
Total	29	22	7	0	0
Percentage		75.86 %	24.14 %	0	0

No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and age. (*P-value* = 0.596)

Table 4 – Association of Her-2/neu with sex

SEX	HER2/neu				TOTAL
	NEG	+1	+2	+3	
MALE	12	4	0	0	16
FEMALE	10	3	0	0	13
TOTAL	22	7	0	0	29

No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and sex. (*P-value* = 0.904)

Table 5 – Association of Her-2/neu with CEA level

CEA level	No. Of patients	Staining							
		Negative		+1(10-40%)		+2 (41-70%)		+3(>70 %)	
		No.	%	No.	%	No.	%	No.	%
Normal (<5ng/ml)	21	15	71.43 %	6	28.57 %	0	-	0	-
Significant(>5ng/ml)	8	7	87.5 %	1	12.5 %	0	-	0	-
Total	29			7		0	-	0	-

No statististically significant relationship was detected between scoring of HER2/neu expression in tumor cells and pre-operative CEA level. (p-value = 0.168)

Table 6 – Association of Her-2/neu with grade of tumor

Grade/Differ	No. Of patients	Staining							
		Negative		+1(10-40%)		+2 (41-70%)		+3(>70 %)	
		No.	%	No.	%	No.	%	No.	%
Well	13	10	76.92	3	23.08	0	0	0	0
Moderate	16	12	75.00	4	25.00	0	0	0	0
Poor	0	0	0	0	0	0	0	0	0
Total	29	22		7		0		0	

No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and tumor differentiation. (*p*-value = 0.904).

Table 7- association of Her-2/neu with T stage

Grade/Differ	No. Of patients	Staining							
		Negative		+1(10-40%)		+2 (41-70%)		+3(>70 %)	
		No.	%	No.	%	No.	%	No.	%
T1	0	0	-	0	-	0	-	0	-
T2	6	5	83.33%	1	16.67%	0	-	0	-
T3	20	14	70.00%	6	30.00%	0	-	0	-
T4	3	3		0		0	-	0	-
Total	29	22		7		0	-	0	-

Shows No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and pathological T-Stage. (*P-value* = 0.538)

Table 8- Association of Her 2/neu staining with lymph node status

Lymphnodes	No. Of cases	IHC staining							
		Negative		+1(10-40%)		+2 (41-70%)		+3(>70 %)	
		No.	%	No.	%	No.	%	No.	%
Metastasis	16	11	68.75%	5	31.25%	0		0	
Reactive	9	8	88.89%	1	11.11%	0		0	
Not recovered	4	3	75.00%	1	25.00%	0		0	
Total	29	22		7		0		0	

No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and lymph node status. (*P-value* = 0.528)

Table 8- Association of HER 2-neu with Stage of tumor in patients.

Lymph nodes	No. of cases	IHC staining							
		Negative		+1(10-40%)		+2 (41-70%)		+3(>70 %)	
		No.	%	No.	%	No.	%	No.	%
Stage I	4	4	100 %	0	0	0		0	
Stage IIA	5	3	60 %	2	40 %	0		0	
Stage IIB	2	2	100 %	0	0	0		0	
Stage IIC	1	1	100 %	0	0	0		0	
Stage IIIA	2	0	0	2	100 %	0		0	
Stage IIIB	11	10	90.91 %	1	9.09 %	0		0	
Stage IIIC	2	1	50 %	1	50 %	0		0	
Stage IVA	2	1	50 %	1	50 %	0		0	
Stage IVB	0	0	0	0	0	0		0	
Total	29	22		7		0		0	

No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and Stage of Tumor. (*p*-value = 0.388)

IV. Discussion

In this study of colorectal cancer from Northern part of India we couldn't demonstrate significant expression of HER2/neu in therapeutic range (2+ and 3+), that's why no significant association of HER2/neu was found with age, sex, grade, T- stage, Lymph node status, CEA and overall stage of tumor. Though this HER2/neu expression and its correlation with various clinicopathological variables had been documented in some of the studies from different part of the world, which are also found to be statistically significant.

This difference may be due to difference in technique (tissue fixation, slide storage procedure, antigen retrieval and incubation time), scoring, and antibodies (monoclonal/polyclonal). This may also be due to regional differences, younger age of presentation, vegetarian diet and more over most of the patients in our study were of well/moderate differentiation. More over the number of patients in our study were also less which might be a cause of this.

As regard to degree of differentiation similar to our study ELWY et al (13) found most cases (72%) to be moderately differentiated. Though No statistically significant relationship was detected between scoring of Her-2/neu expression in tumor cells and tumor cell differentiation (p-value = 0.259). As regard to lymph node status similar to our study Pappas et al (7) found positive lymph nodes in 18 patients, out of which 16 were HER2/neu negative and only 2 were found to be HER2/neu positive. The majority of HER-2/neu negative tumors had no lymph node involvement (67.3%). This correlation was not significant (p=0.12). ELWY et al (13) and McKay et al (17) also found no statistically significant relationship between scoring of Her-2/neu expression in tumor cells and lymph node metastasis (p-value >0.05).

As regard to CEA level similar to our study Change et al (28) also found no statistically significant relationship. we didn't find any statistically significant correlation of tumor stage with HER2, this may be 1) because of less number Of patients in our study, and/or 2) may be because of regional differences. Pappas et al (7) also didn't found any statistically significant correlation with HER2. At places where we found HER2/neu positive (+1) it was mostly cytoplasmic (17.24 %) as compared to membranous (6.90 %). In contrast to membranous HER2 overexpression, a significant proportion of colorectal tumors show cytoplasmic HER2 overexpression in most studies. (6, 7, 8) Half et al. studied HER2 receptor expression in colorectal cancer cell lines. They analysed protein expression in relation to mRNA levels, HER2 amplification, and clinicopathological variables. There was strong membranous staining in 5% of primary colorectal carcinomas. Cytoplasmic staining was found in 63.5% of primary tumors. Membrane, but not cytoplasmic localization, was strongly associated with HER2 gene amplification. They found a significant correlation between HER2 cytoplasmic staining and tumor differentiation (16).

V. Conclusion

In this study of colorectal cancer from Northern part of India we couldn't demonstrate significant expression of HER2/neu in therapeutic range (2+ and 3+), that's why no significant association of HER2/neu was found with age, sex, grade, T- stage, Lymph node status, CEA and overall stage of tumour, though this HER2/neu expression and its correlation with various clinicopathological variables had been documented in some of the studies from different part of the world, which are also found to be statistically significant.

This difference may be due to difference in technique (tissue fixation, slide storage procedure, antigen retrieval and incubation time), scoring, and antibodies (monoclonal/polyclonal). This may also be due to regional differences, younger age of presentation, vegetarian diet and more over most of the patients in our study were of well/moderate differentiation. More over the number of patients in our study were also less which might be a cause of this.

At places where we found HER2/neu positive (+1) it was mostly cytoplasmic (17.24 %) as compared to membranous (6.90 %). In contrast to membranous HER2 overexpression, a significant proportion of colorectal tumors show cytoplasmic HER2 overexpression in most studies.

So a large metacentric trial with standardised techniques is needed on a large number of tumours to confirm the role of HER2/neu in colorectal pathogenesis, and if the role of cytoplasmic HER2/neu found to be in positive in colorectal cancer, intracellular HER2-targeting compounds, for example lapatinib, might be a new treatment option for the patients having cytoplasmic HER2 overexpression.

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