Original Article

Expression of Her-2/neu in Colon Carcinoma and Its Correlation with the Histological Grades and the Lymph Nodes Status

Pathology Section

MANMEET KAUR GILL, KALPANA JAIN, MRIDU MANJARI, TANVEER KAUR

ABSTRACT

Background and Objective: This study was done to check the expression of Her-2/neu in colon carcinoma and its correlation with the histological grades and lymph node metastasis. Colon cancer is the third prevalent cancer in men and women and it is a leading cause of morbidity and mortality in both. Her-2/neu is a useful antigenic marker in immunological studies of colon carcinoma and it can be used for predicting the prognosis and the treatment.

Materials and Methods: The present study was conducted on 40 cases of histopathologically proven colon carcinoma cases to check for Her-2/neu expression by using the novocastra kit. Both cytoplasmic as well as membranous staining were considered as positive for colon cancer.

Results: Her-2/neu was positive in 65% of the cases. It was seen in 68.75% cases of well differentiated, 53.84% cases of moderately differentiated and 100% cases of poorly differentiated conventional adenocarcinomas. Mucinous carcinomas showed more positivity (71.4%)for Her-2/neu as compared to conventional adenocarcinomas (64.5%). The positivity was more in the grade III tumours as compared to that in the other grades. All the colorectal carcinoma cases with metastatic nodes were positive for Her-2/neu staining.

Conclusion: Thus, it was concluded that colorectal carcinomas, especially those with lymph node metastasis, should be subjected to Her-2/neu expression studies, as the tumours which expressed Her-2/neu could carry a poor prognosis and therefore would require a different therapeutic approach, as these cases could respond to Trastuzumab (Herceptin) therapy.

Key Words: Colon carcinoma, Her-2/neu expression, Immunostaining

INTRODUCTION

Colon cancer is the third prevalent cancer in men and women and it is a leading cause of morbidity and mortality in both. The worldwide incidence rate of colon cancer is increasing to approximately 2% annually [1]. Environmental and dietary factors have also been considered to be important aetiological factors for 85-95% of all the colorectal cancer cases. Studies have documented higher incidence rates with increased dietary fat, particularly with diets which are rich in animal fat. A protective role of dietary fibres and supplemental calcium intake on colonic carcinogenesis has been suggested [2,3]. Epidemiological studies have reported that physical inactivity and obesity are associated with an increased risk of colorectal cancer [4]. Genetic susceptibility plays a significant role in colon cancer formation, as was evident from certain conditions which were associated with a high risk of colorectal cancer, such as family history, adenomatous polypi, familial polyposis, hereditary non-polyposis colon cancer syndrome and inflammatory bowel disease [5].

Growth factors are the protein products of genes which are called proto-oncogenes, which are fundamentally important for normal cells. The HER (Human epidermal growth factor receptor) family of receptors; HER1 (EGFr), HER2 (Her-2/neu or ErB2), HER3 and HER4 are membrane-bound. The G-protein receptors, when activated, drive multiple signal transduction pathways which regulate the cellular growth [6].

HER-2 is located on chromosome 17q21 and it encodes a 185kD transmembrane protein that lacks a natural ligand. HER-2 activation initiates signal cascades including the MAPK (Mitogen-activated protein kinase) and PI3K/AKT (3-kinase) pathways that are essential for cell proliferation and differentiation [7].

In tumour model systems, the overexpression of the Her-2/neu gene correlates with mitogenesis, malignant transformation, increased cell motility, invasion and metastasis [8]. Overexpression of Her-2/neu has been reported in many other epithelial malignancies, including cancers of the lung, prostate, bladder, pancreas and oesophagus and in sarcomas [6].

Materials and Methods

The present study was conducted on 40 cases of colon carcinoma which were received as surgically resected colon tissues. The relevant clinical history, family history, investigations and gross examination were recorded. The tissues were formalin fixed and paraffin embedded and sections of five microns thickness were cut and stained with haematoxylin and eosin for the histological typing and grading of the lesions. The sections were subjected to immunohistochemical staining for Her-2/neu to study its expression.

Immunohistochemistry (IHC) was performed on three-four micron thick sections which were made on poly-L-lysine-coated slides. Antigen retrieval was performed by heating the sections in citrate-buffer at pH 6.0 by using a pressure cooker. The monoclonal antibody, RTU-CB11 (Novocastra Laboratories) was used for Her-2/neu detection in a 1:40 dilution by using a standard streptovidin-biotin peroxidase method.

Results and interpretation of the IHC staining:

- The positive control tissue showed a brown coloured end product for Her-2/neu at the site of the target antigen in the cytoplasm and membrane of the cells.
- The negative control sections did not have any coloured product since there was no antigen antibody reaction.

- The tests which showed positivity in the form of a specific colour meant that the tissue had an antibody specific antigen.

IHC SCORING [6]

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
- Membranous cytoplasmic and
- Membranous

The intensity of the staining was categorized as;

- Weak
- Moderate and
- Strong

Scoring was done on the basis of the percentage of positive cells:

- +1 → 10-40%
- +2 → 41-70% and
- +3 → > 70%

The data was compiled and analyzed statistically.

RESULTS

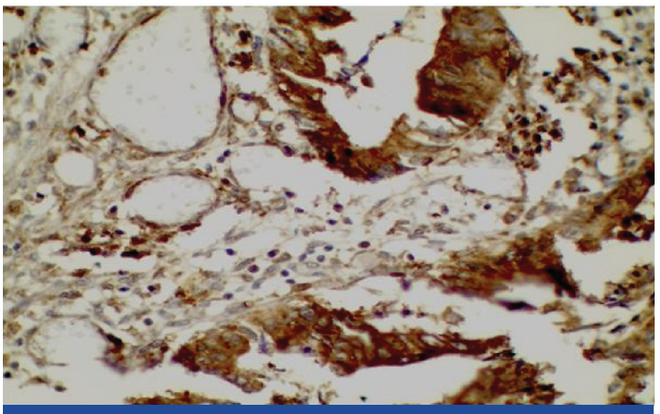
A total of 40 colorectal carcinoma cases were included in the study. Of the total 40 cases, 24(60%) were males and 16(40%) were females. A peak incidence was seen in the 5th to 7th decades of life. The youngest patient was 19 years old and the oldest was 88 year of age. The lesions were present in the right colon in 17 cases and in the left colon and rectum in 23 cases.

The profile of colorectal carcinoma comprised of a maximum number of cases 31(77.5%) of conventional adenocarcinoma. Mucinous adenocarcinoma was seen in 7(17.5%) cases. One case each of carcinoid and signet ring cell carcinoma were seen. The conventional adenocarcinomas comprised of 31 cases, out of which, 16 cases were of well differentiated (grade I) tumour, 13 cases were of moderately differentiated (grade II) tumour and two cases were of poorly differentiated (grade III) tumour.

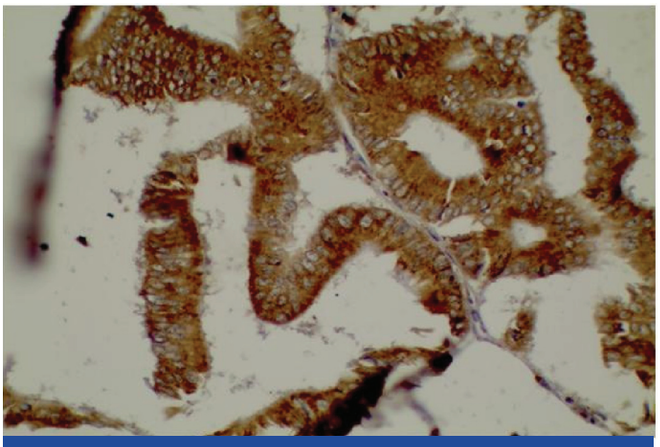
Immunohistochemical staining for Her-2/neu was performed on all the 40 cases of colorectal carcinoma, the scoring was done and the results were interpreted in terms of the intensity, pattern and the percentage of Her-2/neu staining.

There were 26 (65%) Her-2/neu positive cases and 14 (35%) were Her-2/neu negative cases. 30% showed +3 staining, 12.5% showed +2 staining and 22.5% of the cases showed +1 positivity for Her-2/neu.The intensity of the staining in most of the cases varied from moderate (50%) to strong (12.5%), with weak staining in only one case. The pattern of staining in a majority of the cases was cytoplasmic, with only three cases of membranous-cytoplasmic staining, out of which two were of conventional adenocarcinoma with grade I and one case was of mucinous adenocarcinoma. No case showed a pure membranous pattern of staining [Table/Fig-1 and 2].

Of the total 16 cases of grade I conventional adenocarcinoma, 11 cases showed positivity, whereas in grade II, out of 13 cases, seven cases were positive and there were only two cases of grade III, both of which showed positivity. This shows that the positivity was more in the grade III tumours (100%) as compared to the other grades [Table/Fig-3]. It was evident that mucinous carcinoma showed more positivity (71.4%) as compared to conventional adenocarcinoma (64.5%).



[Table/Fig-1]: Positive Her-2/neu staining (strong, cytoplasmic, 51%, +2) in adenocarcinoma (IHC, 400×)



[Table/Fig-2]: Positive Her-2/neu staining (strong, membranous cytoplasmic, >70%, +3) in adenocarcinoma (IHC, 100×)

Percent of Staining	Grade I	% age	Grade II	% age	Grade III	% age
Negative	5	31.25	6	46.16	0	0
+1(10-40%)	4	25.00	2	15.38	0	0
+2(41-70%)	1	6.25	1	7.70	1	50
+3(>70%)	6	37.5	4	30.76	1	50
Total	16	100	13	100	2	100

[Table/Fig-3]: Percent of Her-2/neu staining in different grades of tumor

In 20 patients, no nodes were recovered from the histopathological specimen which was sent for evaluation. Out of 20 cases in which the nodes were recovered, 50% (ten nodes) were metastatic and 50% (ten nodes) were reactive. All the cases with metastatic nodes were positive for the Her-2/neu staining. In the cases with reactive nodes, only four cases of colon carcinoma out of ten were positive for the Her-2/neu staining. There was a correlation between the increasing Her-2/neu score and the metastatic lymph nodes. This finding was statistically significant, with a p-value of 0.015 (Chi-square test) [Table/Fig-4].

It was noticed that as the age of the patient increased, the percentage positivity of the Her-2/neu staining increased and this finding was statistically significant, with a p-value of 0.002 (Chi-square test) [Table/Fig-5].

Lymph Node	No. of Cases	IHC Staining			
		–ve	+1	+2	+3
Metastatic	10	0	2	4	4
Reactive	10	6	3	0	1
Not Recovered	20	8	4	1	7
Total	40	14	9	5	12

[Table/Fig-4]: Comparison of Her-2/neu staining and lymph node status

Age at Presentation	No. of Cases	Percent of Staining			
		Negative	+1 (10-40%)	+2 (41-70%)	+3 (>70%)
<30	6	0	2	4	0
31-50	11	5	2	1	3
51-70	19	9	4	0	6
>70	4	0	1	0	3
Total	40	14	9	5	12
Percentage	100	35	22.5	12.5	30

[Table/Fig-5]: Correlation of Her-2/neu staining and age

DISCUSSION

Colon cancer is the third prevalent cancer in men and women and it accounts for nearly 15% of all the diagnosed cancers. The worldwide incidence rates of colon cancer are increasing to approximately 2%, annually [1]. Immunohistochemistry refers to the process of localizing proteins in the cells of a tissue section, thus exploiting the principle of antibodies binding specifically to antigens in biological tissues. Immunohistochemistry is relatively inexpensive, widely available, easy to preserve and less time consuming and it requires a routine microscope.

Although the tumour is diagnosed histopathologically on light microscopy, various immunological markers are expressed by colorectal carcinomas and depending on them, the treatment and the prognosis differs. The various immunological markers for colon carcinomas are MUC1 and MUC3, p53, keratin (positivity for keratin 20 and negativity for keratin 7), CEA, Her-2/neu, CDX2, tumour associated glycoprotein (TAG-72), cathepsin, villin, hCG and PLAP (Placental alkaline phosphatase).

Her-2/neu is a useful marker which can be used to predict the outcome of colon cancers. Its overexpression correlates with a poor prognosis. Moreover, it is used to predict the patient response to adjuvant chemotherapy and endocrine therapy and to select the patients for immunotherapy with a targeted monoclonal antibody therapy [9,10]. Intuitively, the patients who overexpress HER-2 should respond to Trastuzumab (Herceptin) therapy, independent of the tissue of origin of the cancer. Herceptin has been shown to inhibit the colony formation of the HCA-colon cancer cell line and the HCA-7 tumour xenografts.

The Her-2/neu protooncogene is amplified and as a result, is overexpressed in 25 to 30% of the human breast cancers. In breast cancer staining, it was interpreted as follows: 0 = no staining; +1 = weak or incomplete membranous staining; +2 = moderate or complete membranous staining of at least 10% of the invasive tumour cells; and +3 = strong membranous staining of at least 10% of the invasive tumour cells. The cases which were interpreted as 0 or +1 were considered as negative, and the cases which were interpreted as +2 or +3 were considered as positive.

In the present study, 24(60%) were male patients and 16(40%) were females. Ghaffarzadegan, as well as Schuell, found a similar sex distribution in their studies [6,11].

Our observations of the peak incidence of colorectal carcinoma in the 5th to 7th decades of life and the mean age of the patients being 53.9 years (SD ±16.7) with a range of 19 to 88 years, were almost the same as observed by Ghaffarzadegan and Neklason [6,12].

Tavangar and associates reported 45.6% cases with involvement of the right colon and 54.4% cases with involvement of the left colorectal region [13]. Similarly, in the present study, the lesions were present in the right colon in 17(42.5%) cases and in the left colorectal region in 23(57.5%) cases.

One of the important features of HER2 staining in colon cancer in our study, was the pattern of the staining. There was high percentage of positive staining (65%), out of which 57.5% were cytoplasmic, with only 7.5% of membranous-cytoplasmic staining. No case showed a pure membranous pattern of staining. The intensity of the staining in most of the cases varied from moderate (50%) to strong (12.5%), with weak staining in only one case (2.5%). Out of the 65% positive cases, 30% showed +3 staining, 12.5% showed +2 staining and 22.5% of the cases showed +1 positivity for Her-2/neu. It was noticed that as the age of the patients increased, the percentage positivity of the Her-2/neu staining increased and this finding was statistically significant, with a p-value of 0.002 (chi-square test). There was no correlation between the Her-2/neu expression and sex and the site and type of the tumour.

Ghaffarzadegan et al studied Her-2/neu protein overexpression in 69 cases of colon adenocarcinoma to assess the frequency, pattern and the intensity of the staining and the clinical significance of these events. There was positive Her-2/neu staining in 59.4% cases, with cytoplasmic staining in 65.9% and membranous cytoplasmic staining in 34.1% of the cases. As in our study, they found no correlation between Her-2/neu expression and sex and the site and type of the tumour [6].

Half et al studied HER2 receptor expression in colorectal cancer cell lines. They analyzed the protein expression with respect to the mRNA levels, HER2 amplification, and the clinicopathological variables. There was strong membranous staining in 5% of the primary colorectal carcinomas. Cytoplasmic staining was found in 63.5% of the primary tumours. They found a significant correlation between the HER2 cytoplasmic staining and the tumour differentiation [14]. Their results were very similar to our results, especially the correlation with grade, as our grade III tumours showed 100% positive Her-2/neu staining.

Tavangar et al reported that overall, 12 (21.8%) patients showed positive Her-2/neu staining out of 55 patients. There was a significant correlation ($P<0.05$) between a more advanced stage of the disease and the prevalence of the Her-2/neu over-expression. There was a positive correlation between the size of the tumour and the Her-2/neu over-expression ($P<0.05$). The tumour grade also positively ($P<0.001$) correlated with the Her-2/neu expression (i.e. most of the tumours which over-expressed Her-2/neu were of the moderate or poorly differentiated types), which was also observed in our study [13].

Nathanson et al studied Her-2/neu expression and gene amplification in colon cancer. They used a quantitative PCR/ ligase detection reaction (LDR) technique, FISH and immunohistochemistry.

They found that among 139 cases which were evaluated by immunohistochemistry (IHC), Her-2/neu overexpression was seen in five cases (3.6%). They concluded that the low prevalence of the Her-2/neu gene amplification and the protein overexpression suggested that this oncogene played an infrequent role in the development and progression of colon cancer [15]. We guessed that they considered only the membranous staining as overexpression, which could be the reason for the low positivity.

Schuell et al examined 77 colorectal cancer cases for the presence of the Her-2/neu oncoprotein by immunohistochemistry. In only 4% of the cases (three specimens), the Her-2/neu membranous expression was observed in the therapeutic range (2+ and 3+), 70% were Her-2/neu negative and 26% barely showed immunostaining (1+). There was no significant association with the tumour grade, gender, localization of the primary tumour or survival [11].

The frequency of the Her-2/neu overexpression, as was detected by IHC in colorectal cancer, was reported to be in the range of 0-83% [16,17].

The broad range of the Her-2/neu overexpression might be due to

1. The varying patterns of staining (cytoplasmic/membranous/both) by which overexpression was defined.
2. Difference in the fixation of the tumour tissues.
3. The diversity of the antibody and the IHC procedure which was used. There was lack of standardization of the detection system.

In the present study, out of the 40 cases, in 20 (50%) cases, the lymph nodes were recovered and ten out of them were metastatic. All the cases of colorectal carcinoma with metastatic nodes were positive for Her-2/neu staining. In the cases with reactive nodes, only four cases of colon carcinoma out of ten were positive for Her-2/neu staining. There was a correlation between the increasing Her-2/neu scores and the metastatic lymph nodes. This finding was statistically significant, with a p-value of 0.015 (Chi-square test).

Park et al studied the clinical significance of the Her-2/neu expression in colon cancer. They examined the Her-2/neu expression by immunohistochemistry. They found overexpression of Her-2/neu in 12.5% of the patients. Tumours with a positive Her-2/neu status showed higher rates of nodal metastases, which was similar to the findings of our study and a poor mean survival [18]. Again we guessed that the reason for the low positivity was that they considered only the membranous staining as positive (according to the guidelines for breast cancer).

Li et al noticed that the Her-2/neu expression was positive in 49 out of 317 colon cancer samples (15.5%) and that it was negative in the remaining 268 samples (84.5%). Only seven samples were strongly positive. The Her-2/neu expression correlated with the tumour size and distant metastasis (both $P < 0.05$), but not with the other clinicopathological features which were assessed [19].

McKay et al studied the C-erbB2 protien expression in a large cohort of colorectal tumours and lymph node metastases. C-erbB-2 was found to be expressed in 81.8% of the tumours. They did not find any correlation between c-erbB2 staining and lymph node metastases [20].

Lazaris et al identified a 36% expression rate of Her-2/neu as a predictor of a poor outcome [21]. Similarly, Park et al reported a 47% protein expression rate by using a polyclonal antibody and correlated the overexpression with a higher incidence of post-operative recurrence [22]. A phase II trial in conjunction with the National Cancer Institute demonstrated low levels of Her-2/neu overexpression (8%); however, when these patients with metastatic colorectal cancer were treated with herceptin in combination with irinotecan, 5 of the 7 patients who overexpressed it, responded to the therapy [23].

In the present study, overexpression of Her-2/neu was seen in 65% of the colorectal carcinoma cases and so, our results could be applied to the meta-analyses of the prevalence of Her-2/neu overexpression in colorectal cancer patients and also to develop new chemotherapeutic regimens for these patients.

CONCLUSION

Thus, it was concluded that conventional adenocarcinoma as well as non-conventional colorectal carcinoma, especially with lymph node metastasis, should be subjected to evaluation of the Her-2/neu expression, as the tumours which express Her-2/neu carry a poor prognosis and require a different therapeutic approach. These cases respond to Trastuzumab (Herceptin) therapy, which has been shown to inhibit the colony formation of the HCA-colon cancer cell line and the HCA-7 tumour xenografts. At same time, Her-2/neu was used to predict the patients' response to adjuvant chemotherapy and endocrine therapy and to select the patients for immunotherapy with targeted monoclonal antibody therapy.

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AUTHOR(S):

1. Dr. Manmeet Kaur Gill
2. Dr. Kalpana Jain
3. Dr. Mridu Manjari
4. Dr. Tanveer Kaur

PARTICULARS OF CONTRIBUTORS:

1. MD, Pathology, Assistant Professor, Department of Pathology, AIMSR, Bathinda, India.
2. DNB, Pathology, Department of Pathology, AIMSR, Bathinda, India.
3. MD, Pathology Department of Pathology, SGRD, Amritsar, India.
4. BDS, Department of Periodontics, CDSH, Rau-Indore, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Manmeet Kaur Gill,
H.N. 22265/A, Shant Nagar, Bathinda,
Punjab, India.
Phone: 9876610985
E-mail: drmanmeet27@yahoo.co.in

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