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HER 2/neu as a theranostic marker in colorectal carcinoma and its relationship with histopathological correlates-A pilot study

G. Priyanka[1], Leena Dennis Joseph[2,*], G. Barathi[3], Monica Kumbhat[4]

[1]UG Student, [2]Professor, [3]Assistant Professor, [4]PG Student, Dept. of Pathology, Sri Ramachandra University, Chennai

***Corresponding Author:**
Email: leenadj@gmail.com

Abstract

Introduction: Colorectal carcinoma(CRC) is one among the many leading causes of cancer related mortality worldwide. The survival rate of patients with CRC is better because of advancements made in the treatment modalities, in terms of surgery and chemotherapeutic options. This study is done to know the immunoexpression of Her 2/neu in CRC and its correlation with histologic grades. Her 2/ neu is considered to be a beneficial Immunohistochemical (IHC) marker for colon cancer which can be used for predicting the prognosis and for targeted therapeutic modality.

Materials and Method: The present pilot study was done to understand the expression of Her 2 /neu in histologically proven 25 cases of adenocarcinoma colon using monoclonal anti Her 2 /neu antibody (Biogenix). Positivity was recorded as per the consensus panel recommendations on Her 2/neu scoring.(1) The clinical profile of the patients were obtained from the archives in the Pathology department of our institution.

Results: This study had a total of 25 hemicolectomy specimens of colorectal carcinoma. Of the total 25 cases, 14 cases (56%) were males and 11 cases (44%) were females. Forty percentage of patients were in the age group of 50-60 years. Histopathologically, most of them were adenocarcinomas. Her 2/neu expression was negative in all the 25 cases of colorectal carcinoma in our study. The control tissue was Her 2 /neu positive breast carcinoma with complete membrane staining in the tumor cells(3+).

Conclusions: Her 2/neu is considered as a therapeutic modality in advanced and metastatic CRC cases. This was a pilot study and we need to perform the IHC stain on more cases to understand its expression in our population.

Keywords: Colorectal carcinoma, Her 2/neu, Immunohistochemistry

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Introduction

Colorectal carcinoma(CRC) is one of the most prevalent cancers in men and women and is among the leading cause of morbidity and mortality. Even with excellent surgical and chemotherapeutic regimens, CRC remains as one of the leading causes of cancer related mortalities worldwide(2) Further advances have been made with identification of biological markers for targeted therapy. Treatment with Anti Epidermal growth factor(EGFR)monoclonal antibody with the use of drugs like cetuximab and panitumumab, has been documented to show a progression free survival in advanced CRC cases with wild type KRAS. Even with these advances, many patients respond poorly to treatment and at times their response is totally unpredictable. Thus extensive research is on, to discover new molecular biomarkers which can improve outcome, therapeutic response and potential targets in CRC patients.

The Vascular Endothelial growth factor(VEGF) A is an angiogenic factor that is produced by the tumor cells, the function of which is neoangiogenesis. This mechanism promotes metastasis by promoting the leakage of blood vessels for vascular invasion(3) VEGF expression has been shown to be upregulated in many tumors and is of prognostic value. Treatment with bevacizumab, antibody against VEGF, shows significant improvement in patient outcome. Recently Her 2/neu was found to be closely associated with VEGF expression.

The Her 2 /neu oncogene, also called c-erbB2 codes for a transmembrane tyrosine kinase receptor which is, homologous to EGFR. This receptor is involved in the growth and progression of the malignant cells. Over expression of this protein is demonstrated in 25 to 35% of breast cancers.(4) Treatment of these patients with trastuzumab (herceptin), an anti Her 2/neu monoclonal antibody has been shown to reduce the bulk of tumor, improve response to chemotherapy, thus improving survival in primary and metastatic breast cancers.(5) The role of herceptin is evaluated in CRC also for therapeutic benefits for the patients.

Material and Method

Institutional Ethics clearance was obtained for the study. The cases were selected from the pathology case files. Twenty five hemicolectomy specimens were taken in the study group. Clinical profile of these patients were recorded from the case sheets. All the Hematoxylin and Eosin stained slides of these cases were reviewed and appropriate slide was selected, which had both normal colonic tissue and tumor. The block corresponding to the selected slide was used for cutting sections for the immunohistochemistry(IHC).

Antibody against Her 2/neu (Biogenix) was used for immunohistochemical staining of the sections. The stained slides were reviewed by two pathologists and the results were interpreted and recorded as per the current recommendations and scoring systems. Positivity was demonstrated as complete membrane staining of the tumors cells.

Results

This study included a total of 25 hemicolectomy specimens of colorectal carcinoma. Of the total 25 cases, 14 cases (56%) were males and 11 cases (44%) were females. In this group 40% of patients were in the age group of 50-60 years. Nine cases had the growth in the right colon and 13 cases in the left colon. Three of them had lesion in the transverse colon. Histopathologically, 22 cases were adenocarcinoma (Fig. 1), two cases were signet ring carcinomas (Fig. 2) and one was a mucinous carcinoma (Fig. 3). Five cases were of Grade 1 adenocarcinoma, 16 cases were Grade 2 adenocarcinoma, and one case was a Grade 3 adenocarcinoma. In the Signet ring carcinoma, one showed Grade 1 and the other was Grade 3. The mucinous tumour was Grade 3.

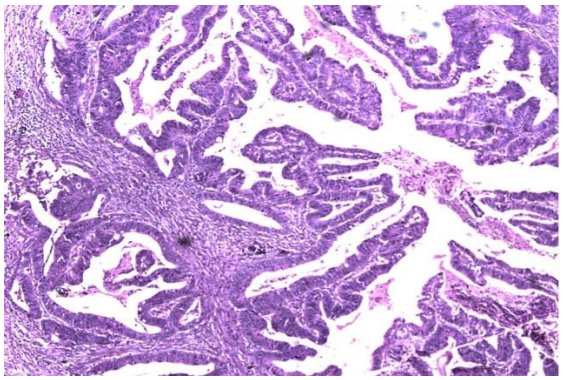

Fig. 1: Moderately differentiated adenocarcinoma(H&Ex100)

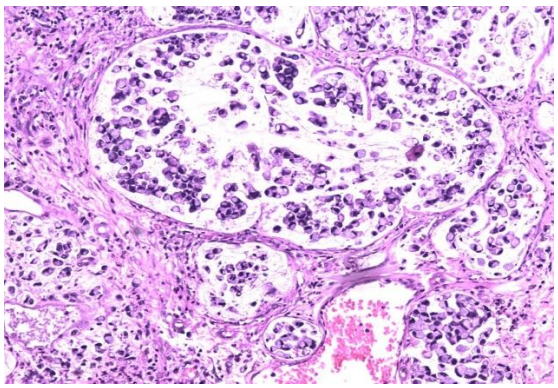

Fig. 2: Signet ring cells in adenocarcinoma(H&Ex100)

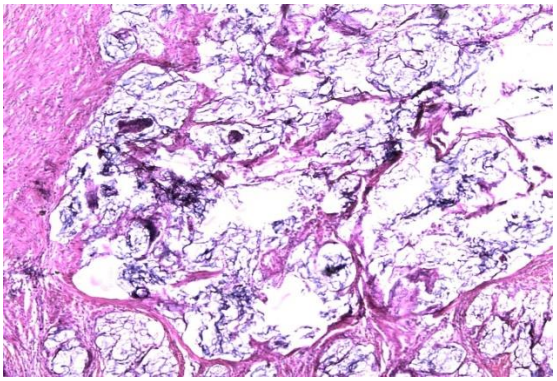

Fig. 3: Mucinous carcinoma(H&Ex100)

HER 2 NEU expression was negative in all our 25 cases of colonic carcinoma in our study(Fig. 4). The control tissue was a HER 2 positive breast cancer with complete membrane staining in the tumor cells(Fig. 5)

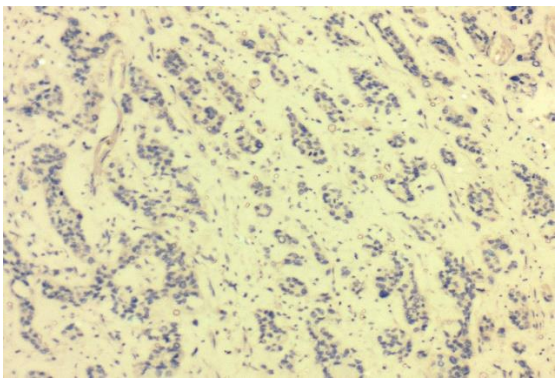

Fig. 4: Her 2/neu negativity in tumor cells(IHCx100)

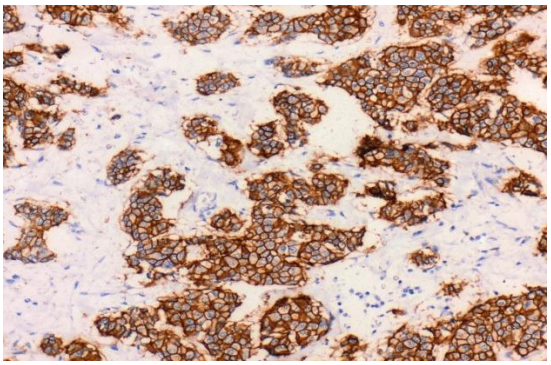

Fig. 5: Positive control showing 3+her2/neu positivity(IHCx100)

Discussion

Globally colorectal carcinomas accounts for 10% of all the incident cancers, with several histological types carrying varied prognostic features. Among them signet ring type carries a poor prognosis. Advanced stage disease needs an intense chemotherapy, with targeted therapy being more focussed on targeting the cancer cells. Monoclonal antibodies targeting the VEGF such as Bevacizumab and EGFR such as Cetuximab are already in use for CRC therapy. Cancer

mortality is higher in economically advanced countries because of high fat and red meat diet. A large number of evidence demonstrates that Colorectal carcinomas are associated with accumulation of genetic alterations like K-RAS, APC, SMAD-4, p53, Her 2/neu gene etc.

The Her2/neu oncogene belongs to tyrosine kinase family which includes EGFR or Her -1, Her- 2, Her- 3 and Her-4. This gene can be traced to the long arm of the chromosome 17q21 and this encodes a 185 kD transmembrane protein that lacks a natural ligand .Dimerization of this gene upon activation regulates intracellular signal transduction through 'Mitogen Activated Protein Kinase', 'Phosphoinositide -3-kinase', 'phospholipase-C etc. This finally results in the expression of proteins that enhances cellular proliferation and differentiation.

Her 2/neu overexpression is also observed in other malignancies like, ovarian, breast gastric, colorectal, lung and also prostate cancers. Amplification of this gene is responsible in preventing cellular apoptosis. Transtuzumab (Herceptin) is a new monoclonal antibody that is targeted against this Her 2/neu gene. This antibody causes an increase in level of p27, a protein that halts cellular proliferation. Pertuzumab is another drug that is recently approved by FDA in these patients. This drug inhibits the dimerization of HER 2.

In CRC, studies on Her2/neu status shows a positivity ranging from 2.7%[6] to 47.7.[(7)] This wide range could be explained by the different scoring systems used. In many studies even the 2+ positivity was counted as positive, contributing to a higher positivity. Another difficulty in comparing IHC studies is the variety of available antibodies. Various preanalytical methods, variations in the laboratory techniques like duration of formalin fixation. The technique of Antigen retrieval and incubation time may also affect the outcome of the staining process.

Heppner et al found a 1.6% Her 2/neu positivity, amongst 1645 colorectal carcinomas cases included in their study. Her 2/neu positivity was significantly correlated with lymph node metastasis and displayed a tendency to poorer overall survival.[(8)] In our study, the sample size needs to be improved to understand the expression of this marker. Since this is a pilot study, we got a negative result in all our cases. We Plan to extend our study to more number of cases. However in most of the studies, the percentage positivity is very low.

Qingguo Li et al studied the expression of Her 2/neu and VEGF in colon cancer and evaluated the expression with clinicopathological parameters and prognosis.[(9)] They found positivity in only 15.5% of their CRC cases and negativity in the remaining 84.5% cases. In our study, all the cases were negative with Her 2/neu staining. Demirbas et al reported an association between Her 2/neu overexpression and tumor size(>5 cms), along with differentiation grade, vascular and lymphatic invasion.[(10)]

Other than IHC other techniques for assessing Her 2/neu status are also described in literature. Seo et al studied the incidence and clinical implications of Her 2/neu status in primary CRC. The Her 2/neu status was determined by performing dual colour silver in situ hybridisation(SISH) and Immunohistochemistry.[(11)] In their study, they found out that Her 2 /neu gene amplification was more in CRCs located in the rectum than in the right and left colon. In our study, the expression was negative in all the cases, irrespective of the location of the tumor. According to Seo et al, Her 2 status did not predict patient prognosis, but they inferred that it forms a basis for future studies on patient selection for Her 2 /neu targeted therapy.

Conclusions

Her 2/neu gene amplification and protein overexpression has a role in the prognosis of the patients. It can also be a theranostic tool for CRC patients. Variable expression was noted in the literature, however all our cases were negative. We intent to continue the study on more cases of Colorectal carcinoma and to compare it with the patient survival data.

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