

Original Research Article

Her-2/neu Revisited - The Role of Her2neu in Colorectal Carcinoma: A Three Year Study on Colonoscopic Biopsies

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Abstract

Introduction: Finding newer modalities of treatment is the need of the hour for Colorectal carcinoma as the incidence of is on the rise. One such is the finding of overexpression of "Her-2/neu" a biological marker, with "Targeted therapy" which can open new avenues of treatment. *Objectives:* 1) To study the histopathology of neoplastic lesions in colonoscopic biopsies. 2) To determine the proportion of Her-2/neu overexpression in Colorectal Carcinomas. 3) To study association between Her-2/neu over expression with grade and lymph node status - wherever possible. *Methods:* All the histologically confirmed Colorectal Adenocarcinomas were subjected to Immunohistochemical staining for detection of Her-2/neu overexpression. A "3+" complete membrane staining considered positive. *Results:* In the present study, Immunohistochemical staining of confirmed Adenocarcinomas with Her-2/neu for overexpression revealed a total of 16 (23.5%) cases as Positive, which were histologically low grade tumours, with a statistically significant p value of 0.0012. *Conclusion:* The incidence of colorectal carcinoma is on the rise at the rate of 2% annually. Herceptin, an antibody directed against Her-2/neu antigen can be a novel therapy for the poor prognostic groups, which constituted around of 23.5% of cases in our study.

Keywords: Colonoscopy; Colorectal carcinoma; Her-2/neu; Immunohistochemistry.

Introduction

Colorectal cancer is the third prevalent cancer in men and women and remains one of the major leading causes of death worldwide, despite advances in surgical techniques and adjuvant chemotherapeutic regimens [6]. Colonoscopy is

currently considered to be gold standard for cancer surveillance [3].

Her-2/neu Expression in Colorectal Carcinoma

Her-2/neu, a Proto oncogene, when mutated promotes tumor cell growth and migration.

The Overexpression often portends a worse prognosis [10]. With a higher rates of nodal metastases [1]. Hence, development and availability of monoclonal antibodies against the protein [9] needed.

Trastuzumab' a monoclonal antibody directed against Her-2/neu might potentially serves as a promising drug in colorectal cancers and also seems to be a valuable prognostic marker within the multimodal treatment of advanced colorectal cancer [6].

Over expression of her-2/neu correlates with poor prognosis and hence could respond to Trastuzumab (Herceptin) therapy [1,2,4] in all the Positive cases.

The study stands unique as a very few Indian studies have been undertaken on this topic and with a positive hope of opening doors to the newer modalities of treatment.

Aims and Objectives

1. To study the histopathology of the colonic neoplasms in colonoscopic biopsies.
2. To determine the proportion of Her-2/neu over expression in colorectal carcinomas.
3. To study association between Her-2/neu over expression with grade and lymph node status – wherever possible.

Materials and Methods

The present study was undertaken in the Department of Pathology, of a tertiary health care centre, during the period August 2012 to August 2014.

One hundred and fifty nine biopsies from patients attending the Gastroenterology OPD in the tertiary health care centre, who presented with lower gastrointestinal tract symptoms, were studied.

Inclusion criteria: All the colonoscopic biopsies taken from terminal ileum to rectum, received in the Department of Pathology, Bangalore.

Exclusion criteria: 1) poorly fixed/unfixed specimens

- 2) Patients with past history of breast cancer.

All colonoscopic biopsy specimens were collected in 10% neutral buffered formalin processed and embedded with the mucosal surface being uppermost. 4µ thick serial sections were prepared and stained with H & E.

Detailed study of the sections was done under light microscope and diagnosis rendered accordingly.

Further, all cases confirmed as colorectal carcinoma, were subjected to Immunohistochemical staining for detection of Her-2/neu expression.

- *Positive control:* Sections of a breast carcinoma previously found to be positive for Her-2/neu (with score of 3+)
- *Negative control:* Duplicate sections of the same sample in which primary antibody had been excluded and replaced with PBS.

Table 1: IHC Scoring as given by Li et al. [9]

Criteria	Score
No immunostaining or membrane staining in <10% of tumor cells.	0 (Negative)
Incomplete membrane staining of >10% of tumor cells.	1 + (Negative)
Weak to moderately complete membrane staining of >10% tumor cells.	2 + (Weakly Positive)
Moderate to strongly complete membrane staining of >10% tumor cells.	3 + (Positive)

In the present study, a score of "3+" was considered as "positive".

Statistical Analysis:

Statistical analysis was carried out using Graph Pad software, 2x2 contingency tables and Chi-square test with Yates correction was used to calculate "p" value to arrive at statistical significance.

"p" value of <0.05 was considered statistically significant.

Results and Observations

During the period of the present study; August 2012 to August 2014, in the Department of Pathology of a tertiary health care centre, Bangalore, one hundred and fifty nine colonoscopic biopsy specimens were examined.

Among the 159 colonoscopic biopsies studied – "68" were non-neoplastic, "23" were benign and "68" were malignant lesions.

As shown in Figure 1: in the present study, out of 68 malignant lesions, 24 cases (35.3%) were Well differentiated Adenocarcinomas, 25 cases (36.8%) were Moderately differentiated Adenocarcinomas, 9 cases (13.2%) were Poorly differentiated, 8 cases (11.8%) were Mucin secreting Adenocarcinomas and 2 cases (2.9%) were Signet ring cell carcinomas.

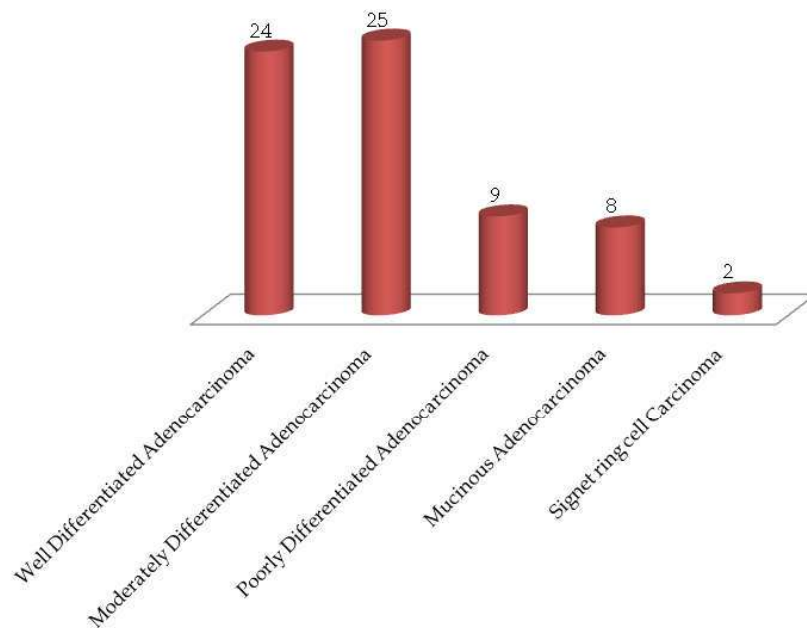


Fig. 1: Distribution of Neoplastic malignant tumors

Table 2: Age Distribution in Malignant lesions

Age group	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
Well differentiated adenocarcinoma	1	3	7	2	6	5	0	0
Moderately differentiated adenocarcinoma	0	1	6	6	4	6	1	1
Poorly differentiated adenocarcinoma	0	1	0	3	2	2	1	0
Mucin secreting adenocarcinoma	0	1	2	3	1	1	0	0
Signet ring cell Carcinoma	0	0	0	1	1	0	0	0
Total	1	6	15	15	14	14	2	1

As shown in Table 2, Malignant lesions were seen in a wide age range of 11 to 90 years. Maximum clustering of cases were noted between 31-50 years age group, showing a trend of occurrence in younger age group in recent years.

Sex distribution

In the present study, majority affected were males (61.8%) and remaining were females (38.2%).

Her-2/Neu Expression in Colorectal Cancers

Table 3: Percentage of Her-2/neu expression

Score	Number	Percentage
0	19	27.9%
1+	18	26.5%
2+	15	22.1%
3+	16	23.5%

Maximum number of cases (19) were of Score 0 (27.9%).

“3+” Score, with complete membrane staining, was taken as positive, which was found in 16 cases constituting 23.5% of the total number of malignant cases.

Table 4: Comparison of Her-2/neu expression with Grade of tumor

Grade	0	1+	2+	3+	p-value
Well differentiated adenocarcinoma	1	2	6	15	0.0012
Moderately differentiated adenocarcinoma	6	11	8	0	
Poorly differentiated adenocarcinoma	5	3	0	0	
Mucin secreting adenocarcinoma	6	1	1	1	0.3574
Signet ring cell carcinoma	1	1			

In the present study, it was noted that the maximum positivity for Her-2 neu over expression was seen in the well differentiated adenocarcinoma group of tumors.

Correlation between lower grades of tumor (Well and Moderately differentiated adenocarcinomas) and Her-2/neu over expression was found to be statistically significant, with a p value of 0.0012.

Whereas, correlation between the High grade tumors (Poorly differentiated, Mucin secreting and signet ring cell carcinoma) and Her-2/neu over expression was found to be statistically insignificant, with a p value of 0.3574.

19 colon resection specimens among the 68 malignant tumors were received out of which, 10 cases showed metastatic deposits in the lymph nodes recovered.

Table 5: Correlation between Lymph node status and Her - 2neu positivity

Lymph node status	No. of patients	HER - 2 positive	p -value
N0 - NX	9	3	2812
N+	10	0	

No correlation was found between the Her-2/neu score and lymph node status. P-value was statistically insignificant (0.2812).

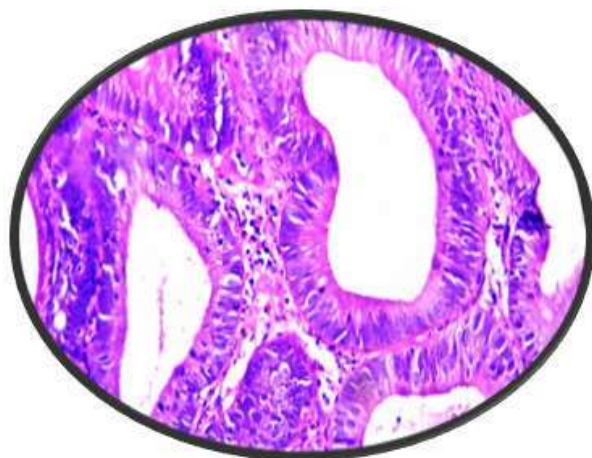


Fig. 2: Well differentiated Adenocarcinoma (H&E 40x)

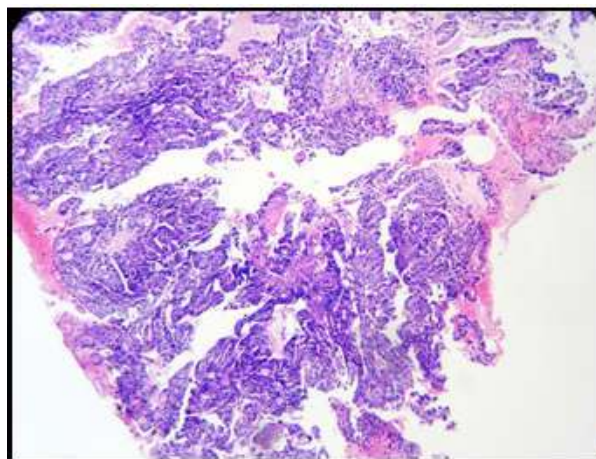


Fig. 4: Poorly differentiated Adenocarcinoma (H&E 40x)

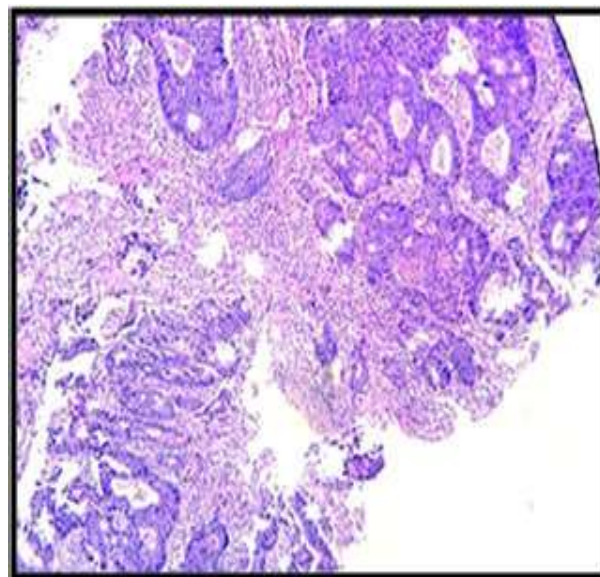


Fig. 3: Moderately differentiated Adenocarcinoma (H&E 40x)

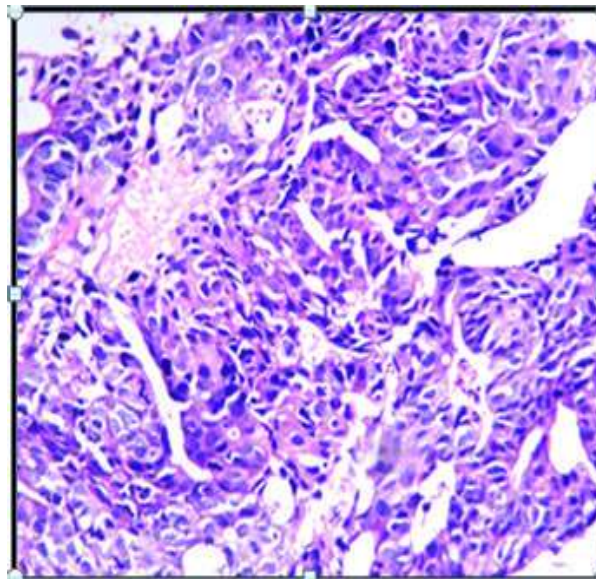


Fig. 5: Poorly differentiated Adenocarcinoma (H&E 400x)

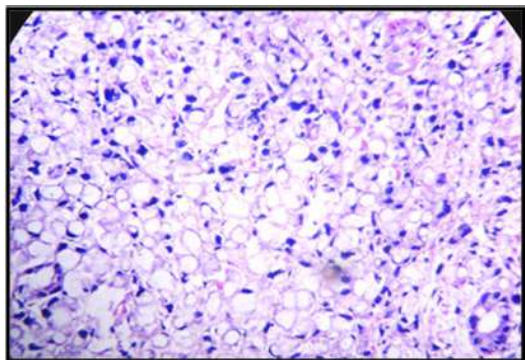


Fig. 6: Signet ring cell carcinoma (H&E 100x)

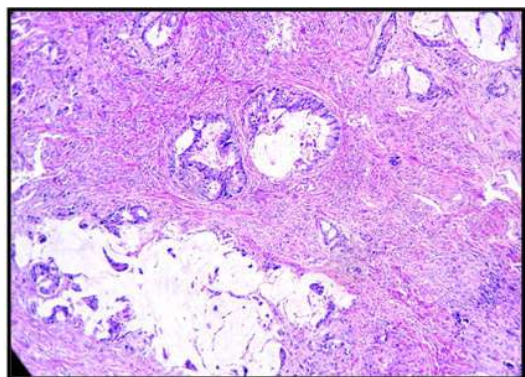


Fig. 7: Mucinous Adenocarcinoma (H&E 100x)

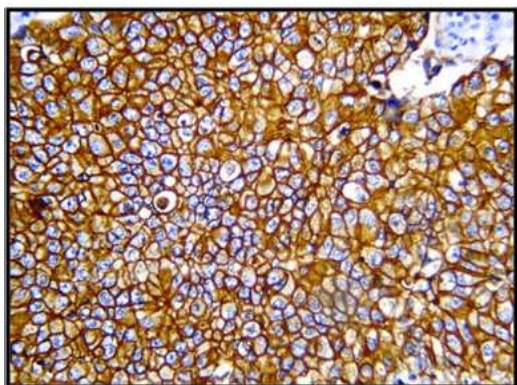


Fig. 8: Her-2/neu 3+ positive control - Breast carcinoma (IHC, Her-2 400x)

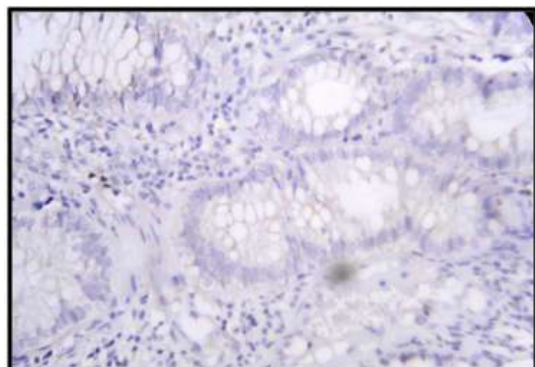


Fig. 9: Her-2/neu Score of "0" - Colorectal Carcinoma (IHC, Her-2 400x)

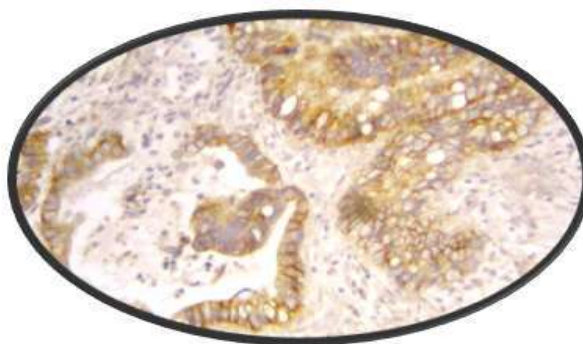


Fig. 10: Her-2/neu score of "1+" - Colorectal carcinoma (IHC, Her2 100x)



Fig. 11: Her-2/neu score of "2+" - Colorectal carcinoma (IHC, Her-2 100x)

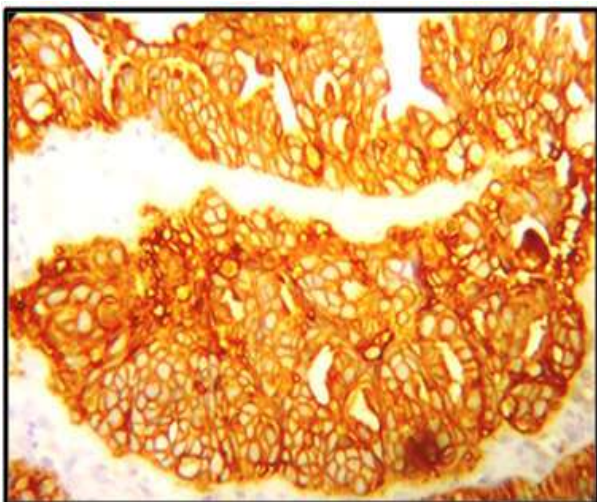


Fig. 12: Her-2/neu score of "3+" - Colorectal carcinoma (IHC, Her-2 400x)

Discussion

The spectrum of colonic lesions are best diagnosed with colonoscopic biopsies, for their conclusive diagnosis [11].

In the present study, conducted in department of Pathology of a tertiary health care centre, 159 colonoscopic biopsies were received, in the period between August 2012 and August 2014.

Of the 159 cases, 68 (42.8%) were non-neoplastic, 23 (14.4%) were benign neoplastic and 68 (42.8%) were malignant neoplastic cases. This finding corresponds to the study series of R.Teague et al. [5], Sidney J et al. [12] and Rajbhandari M et al. [11] wherein the non-neoplastic lesions were maximum in occurrence (Table 6).

But, in the present study, equal incidence of non-neoplastic and malignant neoplastic lesions was seen, due to the fact that ours is an Oncology referral center.

Neoplastic lesions (Benign)

Adenomatous polyps, precursor lesions, are the most common neoplasms found during Colorectal Cancer (CRC) screening, the removal of the which may prevent and reduce mortality [13].

In the present study, there were totally 23 neoplastic benign cases, out of which 18 were tubular adenomas (78.3%), 3 were villous adenomas (13%), 1 case of Tubulovillous adenoma (4.3%) and 1 case of Benign spindle cell lesion (4.3%).

Malignant lesions:

In the present study of 68 malignant lesions, clustering of cases was observed between 31-70 years of age. Maximum number of cases observed in (31-50) years age group, showing a shift in trend of occurrence of colorectal carcinoma in younger age group. Mean age of presentation was 49.4 years.

Majority of the malignant cases were males 41(61.8%) and the rest were females 26 (38.2%).

The most common site of occurrence of the malignant tumors was Rectum.

All these findings are in accordance with the study series of Sudarshan et al. [14] and Laishram RS et al. [15] (Table 7).

Histological grade of tumor

In the present study, out of 68 malignant lesions, 24 cases (35.3%) were Well differentiated Adenocarcinomas, 25 cases (36.8%) were Moderately differentiated Adenocarcinomas, 9 cases (13.2%) were Poorly differentiated, 8 cases (11.8%) were Mucin secreting Adenocarcinomas and 2 cases (2.9%) were Signet ring cell carcinomas.

The most common histological grade in the present study was moderately differentiated Adenocarcinoma.

The present study is in accordance with the study series of Laishram RS et al. [15] and Shyamal Kumar et al. [16], wherein the most common histological grades were Well differentiated and Moderately differentiated (Table 8).

Table 6: Showing distribution of all lesions in comparison with other studies

Study	No. of cases	Non neoplastic	Benign Neoplastic	Malignant Neoplastic
R.H.Teague et al. [5]	57	25 (43.9%)	15 (26.3%)	17 (29.8%)
Sidney J. et al. [12]	212	130 (61.3%)	42 (19.8%)	40 (18.9%)
Rajbhandari M et al. [11]	126	93 (73.8%)	8 (6.3%)	25 (19.8%)
Present study	159	68 (42.8%)	23 (14.4%)	68 (42.8%)

Table 7: Comparison of age and sex distribution of malignant lesions with other studies

Study	No. of cases	Mean age	No. of male patients	Most common location of tumor
Sudarshan et al. [14]	233	43 years	134 (57.5%)	Rectum -192(82.4%)
Laishram RS et al. [15]	54	47.5 years	29 (53.7%)	Rectum- 29 (53.7%)
Present study	159	49.4 years	41 (61.8%)	Rectum- 38 (55.9%)

Table 8: Showing comparison of Histological grade of malignant lesions with other similar studies

Study	No. of cases	Well differentiated	Moderately Differentiated	Poorly Differentiated	Mucin secreting	Signet ring cell carcinoma
Laishram RS et al. [15]	54	17 (31.48%)	20 (37.04%)	17 (31.48%)	-	-
Shyamal Kumar et al. [16]	180	51 (28.3%)	49 (27.2%)	20 (11.1%)	32 (17.8%)	28 (15.6%)
Present study	68	24 (35.3%)	25 (36.8%)	9 (13.2%)	8 (11.8%)	2 (2.9%)

Her-2/neu scores

In the present study, Maximum number of cases showed Score of “0” – 19 (27.9%).

Score of “3+” was taken as positive, which constituted 16 cases (23.5%).

Percentages of positive cases were 23.5%.

Table 9: Showing Her-2/neu scores

Score	Number	Percentage
0	19	27.9%
1+	18	26.5%
2+	15	22.1%
3+	16	23.5%

Table 10: Comparison of Her-2/neu over expression with other studies

Author	Study	Conclusions
Scheull et al. [2]	77 CRC cases	3% showed positivity
Nathanson et al. [17]	139 CRC cases	Overexpression in 4%
Kavanagh et al. [8]	132 CRC cases	2% positivity
Park et al. [3]	137 CRC cases	65 cases (47.4%) over expressed Her-2/neu
Ghaffarzadegan et al. [7]	69 cases	59.4% showed over expression
Tavangar et al. [18]	55 cases	Over expression in 12 cases (22%)
Present study	68 cases	Over expression in 23.5%

Abbreviation: CRC is colorectal carcinoma

Table 10 shows the variability in the Her-2/neu over expression in colorectal carcinomas.

The present study is in accordance with the study of Tavangar et al., which showed over expression in 22% cases.

Present study showed Her-2/neu positivity in 23.5% of cases.

Table 11: Comparison of Her-2/neu expression with Grade of tumor

Grade	0	1+	2+	3+	p-value
Well differentiated Adenocarcinoma	1	2	6	15	0.0012
Moderately differentiated adenocarcinoma	6	11	8	0	
Poorly differentiated adenocarcinoma	5	3	0	0	0.3574
Mucin secreting adenocarcinoma	6	1	1	1	
Signet ring cell carcinoma	1	1			

In the present study, it was noted that the maximum positivity for Her-2 neu overexpression was seen in the well differentiated adenocarcinoma

group of tumors.

Correlation between lower grades of tumor (Well and Moderately differentiated adenocarcinomas) and Her-2/neu over expression was found to statistically significant, with a p value of 0.0012.

Whereas, correlation between the High grade tumors (Poorly differentiated, Mucin secreting and signet ring cell carcinoma) and Her-2/neu over expression was found to be statistically insignificant, with a p value of 0.3574.

This was found to be in accordance with the study series of Seo et al. [6].

19 colon resection specimens among the 68 malignant tumors were received in the Department of Pathology.

Out of the 19 specimens, 10 cases showed metastatic deposits in the lymph nodes recovered.

Table 12: Correlation between Lymph node status and Her – 2/ neu positivity

Lymph node status	No. of patients	HER – 2 positive	P-value
N0 - NX	9	3	2812
N+	10	0	

No correlation was found between the Her-2/ neu score and lymph node status. P-value was statistically insignificant (0.2812).

This finding is in accordance with the study conducted by McKay et al., where they did not find any correlation between Her-2 neu staining and lymph node metastases [19].

Other studies conducted by Park et al. and Gill et al. [1], showed that tumors with a positive Her-2/neu status had higher rates of nodal metastases. But, in present study no such correlation was observed and it is in accordance with a recently conducted study in 2014 by Seo et al. [6].

Summary

The present study titled Her-2/neu Revisited - the role of Her-2/neu in Colorectal carcinoma, A three year study on colonoscopic biopsies, was conducted in a tertiary health care centre, Bangalore, between August 2012 and August 2014.

- A total of 159 colonoscopic biopsies were studied.
- Out of the 159 cases, 68 (42.8%) were non neoplastic lesions, 23 (14.4%) were benign lesions and 68 (42.8%) were malignant

lesions.

- Malignant lesions encountered were only Adenocarcinomas. 24 (35.3%) cases of Well Differentiated Adenocarcinoma, 25 (36.8%) cases of Moderately Differentiated Adenocarcinoma, 9 (13.2%) cases of Poorly Differentiated Adenocarcinoma, 8 (11.8%) cases of Mucin secreting Adenocarcinoma and 2 (2.9%) cases of Signet ring cell carcinoma.
- Maximum number of malignant lesions were observed in (31-50) years age group and Mean age of presentation was 49.4 years.
- Of the 68 malignant lesions 41 (61.8%) cases were males and most common site of occurrence was Rectum (55.9%).
- Well differentiated and moderately differentiated were the most common histological grades.
- Percentage of 3+ Her-2/neu positive cases were 23.5% and maximum positivity was seen in Well differentiated group of tumors.
- Correlation between lower grades of tumor (Well differentiated and Moderately differentiated) and Her-2/neu over expression was found to be statistically significant, with a p value of 0.0012.
- No correlation was found between Her-2/neu score and Lymph node status, with p value of 0.2812, which is statistically insignificant.

Conclusion

The incidence of Colorectal carcinoma is rising at the rate of 2% annually and patients have developed resistance to chemotherapeutic agents like Oxaliplatin and Irinotecan.

Hence, there is an ongoing search for newer therapeutic modalities, like monoclonal antibodies.

The success of Her-2/neu directed therapy in breast cancer has led to its evaluation in other tumor types and hence in the present study the percentage of over expression in colorectal carcinomas was evaluated.

Her-2/neu protein is credibly expressed in colorectal cancers in present study and exhibits relatively distinct localization patterns in these tumors especially where the histological grade is low or moderate.

Therefore, Her-2/neu might constitute a

new adjuvant monoclonal antibody targeted immunotherapy in selected cases.

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