



## Original Research Article

## Frequency of overexpression of Her2/neu in colorectal adenocarcinomas– A hospital based cross-sectional study

Neha Ratan B<sup>1,\*</sup>, Vijayalaxmi V Suranagi<sup>1</sup>

<sup>1</sup>Dept. of Pathology, Jawaharlal Nehru Medical College, KLE University, Belagavi, Karnataka, India



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## ABSTRACT

**Introduction:** Colorectal malignancies stand out to be one of the chief causes of morbidity and mortality in the world. Although the total incidence of colorectal malignancy in India n population has marginally increased, it is now a common cause of cancer mortality among Indians.

The oncogene HER2/neu belongs to the tyrosine kinase receptor family. The favorable outcome of HER2/neu in management of breast carcinoma patients has directed to evaluation of gene amplification protein overexpression, and anti-tumor activity of Herceptin in various tumor types one amongst which is colorectal carcinomas. The objective of the study was to evaluate the frequency of overexpression of HER2/neu in colorectal adenocarcinomas (CRC) and its association with various histomorphological and clinicopathological parameters.

**Materials and Methods:** A total of 52 cases of colorectal adenocarcinomas including colonoscopic biopsies & resected specimens were studied. Paraffin embedded sections were stained with Hematoxylin & Eosin and histological and clinicopathological parameters were assessed. In IHC, antigens were retrieved by Heat Induced Epitope Retrieval method and HER2/ neu status was determined using a semiquantitative scoring system. The scores were then correlated with histological grade, type and clinicopathological parameters.

**Results:** The HER2/neu overexpression was noted in 53.8% of our cases and was seen to be commonly expressed by moderately differentiated tumours and conventional/ NOS histological type.

There was a statistically significant correlation observed between HER2/neu status and tumour grade ( $p=0.001$ ), tumour type ( $p=0.008$ ). There was no significant correlation between age, gender or localisation or stage of tumour.

**Conclusion:** Histological grade and histological type of CRC showed a significant correlation with HER2/neu overexpression, hence can be used as a predictive tool for determining the prognosis of the patients.

Patients who overexpress HER2/neu could become potential candidates for this monoclonal antibody based targeted immunotherapy thus reduce overall mortality and morbidity of CRC worldwide.

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### 1. Introduction

Colorectal malignancies ranks to be one of the major causes of morbidity and mortality in global populaion. Colorectal carcinoma (CRC) is projected to be third most common cancer in men (746,000 cases, 10.0% of the total) and the second most common amongst women (614,000 cases, 9.2%

of the total) worldwide in the year 2012. In India, CRC is the fifth most common cause of cancer mortality. According to GLOBOCAN, in India the incidence of colorectal carcinoma in the year 2012 was 6.3% and mortality was 7.1%.<sup>1</sup>

Similar to other epidermal growth factor receptors HER1, HER3, and HER4, The human epidermal growth factor receptor (HER 2/neu) is also located on chromosome 17q21. They belong to the tyrosine kinase receptor

\* Corresponding author.

E-mail address: [neha11788@gmail.com](mailto:neha11788@gmail.com) (N. Ratan B).

family. The high impact rate of HER2/neu in management of carcinoma breast has objectified the evaluation of the same in multiple tumor types including colon, rectum and stomach adenocarcinomas. HER2/neu overexpression could be used as a reliable immunomarker. Patients who overexpress HER2/ neu could be chosen for targeted immunotherapy using neoadjuvant monoclonal antibody. Chemotherapy with Herceptin is found to improve the prognosis of the patients.<sup>2</sup>

However, there are very few studies which have evaluated the use of HER2/ neu expression in patients with gastric and intestinal cancers. In the present study we aim to determine the frequency of overexpression of HER2/neu in colorectal adenocarcinomas, to study the association between HER2/neu overexpression with age and gender of the patient and grade, type and stage of the tumour and to study the various histomorphological changes in colorectal adenocarcinomas.

## 2. Materials and Methods

A total of 52 specimens which included all resected specimens and colonoscopic biopsy specimens of colorectal adenocarcinoma received and diagnosed as colorectal adenocarcinoma since January 2013-December 2015 in Department of Pathology were considered in the study. The identities of our subjects were concealed and on procuring permission from the institutional ethical committee, the study was performed. Poorly fixed, inadequate tissue and adenocarcinoma of upper gastrointestinal tract, small intestine and gastrointestinal stromal tumors were excluded from the study.

Demographic details of the patients such as age and sex were obtained from patient's record and histopathological requisition form. For prospective study, the samples were collected in 10% neutral buffered formalin and processed. For retrospective study, paraffin tissue blocks were retrieved from the archives, sections of 4-5  $\mu$  thick were cut and then stained using Haematoxylin and Eosin (H&E) and subjected to histomorphological analysis and were graded as well differentiated (grade I), moderately differentiated (grade II) and poorly differentiated (grade III) depending on the percentage of tumour showing gland formation.<sup>3</sup> Histological typing of the tumour was done using 2010 WHO histological classification of tumours of colon and rectum.<sup>3</sup>

All cases diagnosed as adenocarcinoma were subjected to immunohistochemistry (IHC) for determining HER2/neu over-expression. In IHC, antigens were retrieved by Heat Induced Epitope Retrieval method (HIER) in BIOGENEX EZ - RETRIEVER System V.3 microwave using trisaminomethane – ethylenediaminetetraacetic acid (TRIS EDTA) buffer at pH 9. BIOGENEX Pre-diluted rabbit monoclonal Anti-Human HER2/ neu/ErbB2[EP3] antibodies were used for staining.

Initially known cases of breast carcinoma acted as our positive external controls and glands looking normal acted as negative controls as per non availability of positive controls for colorectal adenocarcinoma. However, cases that stained strong membrane positive in our study were also used as controls for remaining cases.

The tumour tissue expressing Her2/neu in more than 10% cancer cells were classified as positive

The staining grade, intensity and pattern was seen as follows:<sup>4</sup>

Cytoplasmic, Membranous cytoplasmic and Membranous

The intensity of the staining was categorized as; Weak, Moderate and Strong Grading was done on the basis of the percentage of positive cells:<sup>4</sup>

G0 – NO STAINING, G1 +1 10-40%, G2 +2 41-70% and G3 +3 > 70%.<sup>4</sup>

HER2/neu expression in tumour cells were scored as per criteria used in a Gastric Cancer Trial.<sup>5</sup>

### 2.1. Statistical methods

Descriptive and inferential statistical analysis has been carried out in the present study using student t test (two tailed, independent) and Chi-square/ Fisher Exact test Qualitative data analysis and a p value  $\leq 0.05$  was considered statistically significant. The data was analysed using Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 and Microsoft word and Excel have been used to generate graphs, tables etc.

## 3. Results

A total of 52 samples were studied for Her2/neu expression and various clinicopathological and histomorphological data were tabulated.

### 3.1. HER2/neu Immunohistochemistry

A total of 52 cases were assessed for HER2/neu status .24(46.1%) cases were negative(score 0 and score 1), 16(30.8%) cases were equivocal(score 2) and12(23.1%) cases were positive(score 3). Hence overexpression was noted in 53.8% cases. (Table 1)

Most of the cases 23(44.2%) showed no expression. 7(13.5%) cases showed grade 1 and 11(21.2%) showed grade 2 and 3 expression.

12(23.%) cases showed strong positivity, 12(23.1%) showed moderate positivity and 3(5.8%) show weak staining.

Various pattern of staining was studied in 32(61.5%) cases. Partial membrane staining was seen in 12(23.1%) cases and complete membrane staining was noted in 4(7.7%) cases. Cytoplasmic staining alone was observed in 2(3.8%) cases and also was observed along with 10 (19.2%)

partial membrane staining and 4(7.7%) complete membrane staining.(Table 2)

A final scoring system was followed and scores were given and results were tabulated.

21(40.4%) cases were score 0 followed by score 2 16(30.8%), 12(23.1%) score 3 and 3(5.8%) score 1.(Figures 1, 2, 3 and 4)

### 3.2. Correlation of histopathological and clinicopathological parameters with HER2/neu final score

The HER2/neu scores were compared with the site of tumour. In this study, colon tumours commonly scored S0 and S2 while rectal tumours were scored S0, however there was no statistically significant correlation between the site of tumor and Her2/ neu expression.

Histological grades of colorectal adenocarcinomas were further compared with the HER2/ neu score. It was seen that a significant correlation existed between histological grade and HER2/ neu status. In our study Score 3 was commonly expressed by grade II tumors and score 0 by grade III tumors.(Table 3)

A significant relation was observed between the HER2/neu expression and the histological type of the tumour. Score 3 was most common in the conventional NOS subtype and least common in signet ring cell with mucinous subtypes. Of the 3 cases of cribriform comedo type only one case of cribriform comedo showed score 3 however other two cases showed scores 0 and 2 respectively.(Table 4)

A statistically significant correlation was not noted between HER2/ neu scores and gender and age of patient (P=0.75, 0.433 respectively).

A statistically significant correlation was not observed between HER2/neu scores and AJCC stage of patient (P=0.076). Score 3 was seen expressed by Stage II and III.(Table 5)

There was no significant correlation between lymph node metastasis with HER2/ neu status (P=0.245).

### 3.3. Histomorphological parameters

Amongst various histological types a majority of tumors 34(65.4%) were classified under NOS category and least belonged to signet ring cell type 2(3.8%) cases. There were two cases of mucinous carcinoma with signet cell component and one case of signet cell carcinoma with mucinous component (1.9%).

Grade I was observed in 8(20.5%) cases, 21 (53.8%) cases were grade II and 10(25.6%) cases were grade III.

Neutrophilic infiltration was observed 32 of 52 cases. Majority of the cases 22(42.3%) showed both intraglandular and intraepithelial patterns of infiltration.

Lymphocytic infiltration was also present in all the cases studied and was classified as mild, moderate and severe

degrees. Most of the cases 27(51.9%) showed moderate degree of infiltration. Necrosis was noted in 43(82.6%) of our cases and 2(3.8%) cases showed extensive necrosis. A total of 32(61.5%) cases of CRC were assessed for lymph node status of which 22(42.3%) were metastatic. Perineural invasion was noted in 15(28.8%) and 6(11.5%) showed lymphovascular invasion.(Table 6)

### 3.4. Clinicopathological parameters

Of the total 52 cases 25(48.1%) cases were those of resected specimens and 27(51.9%) cases were those of colonoscopic biopsies.

Number of colon specimens studied was marginally higher 27(51.9%) cases than rectal 25(48.1%) cases.

Left sided lesions were 31(59.6%) cases compared to right sided 21(40.4%) cases.

The most commonly affected age group was 40-50years(32.7%) cases and least common was >80years, 1.9%. The most young patient was 22years and the most old patient was 85years old.

The study showed male predilection. 21(40.3%) of the cases were female and 31(59.6%) cases were males. Male: female ratio was 1.5:1.

Most of the patients came with complaints of constipation associated with pain abdomen 32(61.5%) cases and 20(38.5%) cases came with the complaint of per-rectal bleed.

Most of the patients consumed non-vegetarian diet 39(75%) cases and vegetarians constituted 13(25%) cases.

Most of the cases 25(48.7%) showed polypoid/cauliflower growth followed by ulcerative 17(32.7%) cases and annular 10(19.0%) cases.

Majority of the cases 20(80%) fell in stage III AJCC followed by stage I 3(12%) and stage II 2(8%).(Table 6)

**Table 1: HER2/neu status in CRC**

HER2/neu status	No. of patients	%
Negative	24	46.1
Equivocal	16	30.8
Positive	12	23.1
Total	52	100.0

## 4. Discussion

Colo rectal cancer (CRC) is one of the chief cause s of cancer-related mortality in the world, accounting for approximately 10% of all human cancers.<sup>6</sup>

Early diagnosis of CRC, successful surgical treatment, clinico -pathological prognostic factors and response to adjuvant therapy have contributed to improved outcome in affected patients. Therefore, identification of markers associated with carcinogenesis, tumor growth, invasion and metastasis has been crucial to developing potential

**Table 2:** Characteristics of HER2/ neu expression in CRC

Pattern N=52(100%)	Cytoplasmic 2(6.3%)	Membranous		Complete Cm 4(12.5%)	Cm+cy 4(12.5%)
		Partial Pm 12(37.5%)	Pm+Cy 10(31.5%)		
Intensity N= 52(100%)	Nil 20(38.5%)	Weak 3(5.8%)		Moderate 17(32.7%)	Strong 12(23.1)
Percentage(grade) N=52(100%)	Grade 0(nil) 23(44.2%)		Grade1 (<10%) 7(13.5%)	Grade2 (40-70%) 11(21.2%)	Grade3 (>70%) 11(21.2%)
Final Score N=52(100%)	Score 0 21(40.4%)	Score 1 3(5.8%)		Score 2 16(30.8%)	Score 3 12(23.1%)

Pm- Partial membrane, Cy – cytoplasmic, Cm- complete membrane

**Table 3:** Correlation of histological grade of CRC with HER2/neu status

Grade	HER2/neu Score				Total
	So	S1	S2	S3	
Grade I	3(37.5%)	0(0%)	2(25.0%)	3(37.5%)	8(100.0%)
Grade II	3(14.3%)	0(0%)	10(47.6%)	8(38.1%)	21(100.0%)
Grade III	9(90.0%)	0(0%)	1(10.0%)	0(0%)	10(100.0%)
Total	15(38.5%)	0(0.0%)	13(33.3%)	11(28.2%)	39(100.0%)

P=0.001\*\*, significant, Fisher Exact test

**Table 4:** Correlation of histological type of CRC with HER2/neu status

Histological Type	HER2/neu Score				Total
	So	S1	S2	S3	
N	14(66.7%)	0(0%)	11(68.8%)	9(75%)	34(65.4%)
M	2(9.5%)	2(66.7%)	1(6.3%)	0(0%)	5(9.6%)
MC	1(4.8%)	0(0%)	2(12.5%)	2(16.7%)	5(9.6%)
C	1(4.8%)	0(0%)	1(6.3%)	1(8.3%)	3(5.8%)
M+SC	2(9.5%)	0(0%)	0(0%)	0(0%)	2(3.8%)
S	1(4.8%)	0(0%)	1(6.3%)	0(0%)	2(3.8%)
S+MC	0(0%)	1(33.3%)	0(0%)	0(0%)	1(1.9%)
Total	21(100%)	3(100%)	16(100%)	12(100%)	52(100%)

P=0.008\*\*, significant, Fisher Exact test

N- NOS, M- Mucinous, MC- Mucinous component, C- Cribriform- Comedo, SC – Signet ring cell component, S- Signet ring cell

**Table 5:** AJCC Stage distribution of patients studied in relation to Final score

AJCC Stage	Final Score				Total
	So	S1	S2	S3	
Stage I	3(25%)	0(0%)	0(0%)	0(0%)	3(12%)
Stage II	0(0%)	0(0%)	0(0%)	2(50%)	2(8%)
Stage III	9(75%)	2(100%)	7(100%)	2(50%)	20(80%)
Total	12(100%)	2(100%)	7(100%)	4(100%)	25(100%)

P=0.076+, significant, Fisher Exact test

therapeutic intervention.<sup>3</sup>

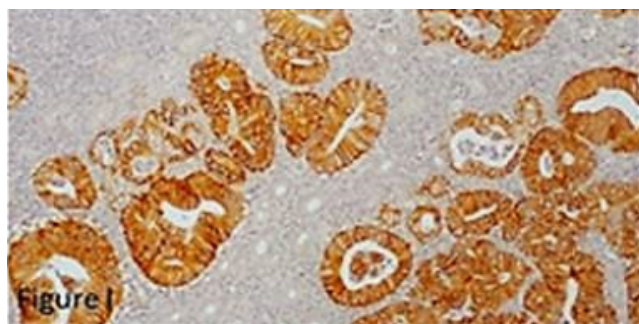
HER-2/neu expression has been reported earlier in CRC and is considered beneficial in targeted therapies in the same.<sup>7</sup> In the present study we evaluated the frequency of overexpression of HER2/ neu in CRC and the association of the same with histological grades, histological types, stage of colorectal adenocarcinoma and also studied various histo-morphological and clinico-pathological parameters.

Several studies have reported different frequencies of HER2/neu protein overexpression and amplification in colorectal adenocarcinoma. According to previous data, overexpression of HER2/neu in colorectal cancer ranges from 0%-83%.<sup>2</sup>

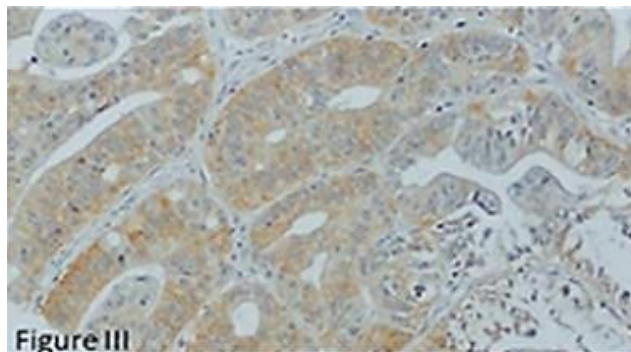
Our study included a total of 52 cases of colorectal adenocarcinomas which were graded and scored using a semiquantitative scoring system. In our study we considered all the cases showing score 2 and score 3 overexpression

**Table 6:** Histomorphological features noted in cases of CRC.

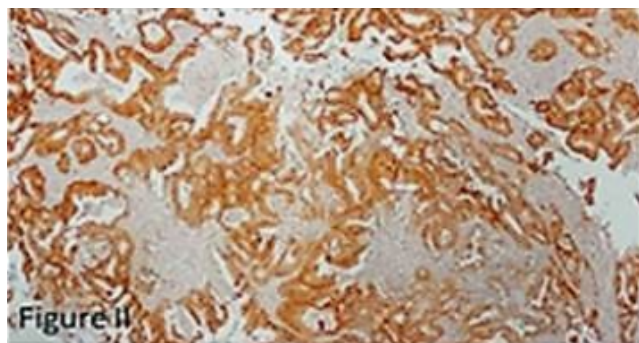
S. no	Histomorpholigal parameters	Location	Number of cases	%
1	Neutrophils N=32(100%)	Intraglandular & Intraepithelial	22	42.3
		Intraglandular Intraepithelial	4 6	7.7 11.5
2	Invasion N=21(100%)	Lymphovascular	6	11.5
		Perineural	15	28.8
3	Lymph Node status N=32(100%)	Metastatic	22	73.3
		Reactive	8	26.6
		None	26	17.3
4	Desmoplastic reaction N=52(100%)	Mild	17	3.7
		Moderate	7	13.5
		Dense	2	3.8
		None	9	17.3
5	Necrosis N=52(100%)	Mild	23	44.2
		Moderate	18	34.6
		Extensive	2	3.8
6	Lymphocytic infiltration N=52(100%)	Mild	12	23.1
		Moderate	27	51.9
		Severe	13	25



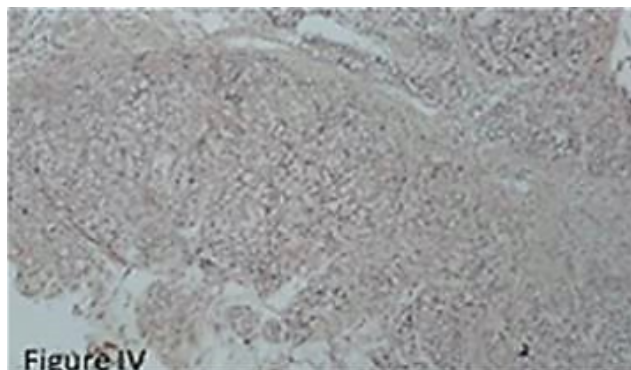
**Fig. 1:** Showing strong membrane positivity in >70% tumour cells – Score 3. (IHC, x200)



**Fig. 3:** Showing moderate partial membrane staining in 40% tumour cells - Score 2. (IHC, x400)



**Fig. 2:** Showing Moderate membranous cytoplasmic stain in >70% tumour cells – Score 2 ( IHC, x40)



**Fig. 4:** Showing negative staining in signet ring cell carcinoma Score 0.(IHC, x 100)

for HER2/ neu based on previous studies done on breast and gastric carcinoma as there is no standardized scoring system developed for colorectal carcinoma.<sup>8</sup> which could be a possible explanation of such high variation in the frequency of overexpression in previous studies.<sup>5</sup>

In the present study, 53.8% cases overexpressed HER2/neu of which 23.1% were positive and 30.8% were equivocal. A study done in Lahore reported 31% cases equivocal which was in agreement with our study and 31% positive which was slightly higher compared to our study.<sup>2</sup> But there was a wide range of variation among different other studies such as studies conducted by AL-Kuraya et al,<sup>9</sup> found amplification in 0% of 98%. Ross and McKenna studied the expression of HER-2/ neu in tumors of gastrointestinal tract and found that HER2/ neu protein overexpression or gene amplification in only 25% of all gastrointestinal malignancies.<sup>10</sup>

These variations in studies could be explained by technical variability in the performance of IHC staining. Also, IHC analysis is vulnerable to differences due to variability in tissue fixation, processing, choice of primary antibody, detection system, epitope retrieval, interpretation and reporting.<sup>11</sup>

The cases which showed expression of Her2/neu exhibited various patterns which we again separately analyzed in our study and found that 50% cases showed membranous staining and 50% cases showed cytoplasmic staining. Of the cases which showed membranous staining 25% cases showed complete membrane staining while 23.1% cases showed partial membrane staining. 43.7% cases showed membranous- cytoplasmic staining pattern.

Our results were consistent with another Egyptian study in which 23% cases exhibited complete membrane pattern.<sup>8</sup> Yet another study found complete membrane expression in only 12.5% of the patients which was discordant to our study.<sup>12</sup> A study done in Austria also observed partial membrane staining in 26% of their cases which again supported our study.<sup>13</sup> Another study done previously also observed membranous- cytoplasmic stain positivity in 52% of their cases which again is in agreement with our study.<sup>14</sup> An Indian study<sup>15</sup> showed 57.5% cytoplasmic staining pattern in their cases which was in agreement with our study while a study conducted by Half et al<sup>16</sup> and another study observed cytoplasmic staining in 63.5% and 65.9% cases respectively which was higher than that of our study while in another study only 34.1% of their cases expressed membranous cytoplasmic staining which was lower compared to our study.<sup>4</sup>

The significance of cytoplasmic HER2 is still unclear. In colorectal cancer, studies suggest that cytoplasmic HER2 could be used to predict tumor pathogenesis and prognosis. A reliable explanation could be that cytoplasmic HER2 forming homodimers activates the intracellular domain of tyrosine kinase.<sup>17</sup>

This could be the reason for the use of lapatinib in trials, an intracellular tyrosine kinase inhibitor, or other intracellular HER2 targeting compounds, which could be considered breakthrough in the treating CRC patients.<sup>18</sup>

Foda, et.al. also showed a significant agreement between cytoplasmic HER2 overexpression by IHC and gene amplification by FISH analysis suggesting that, unlike breast cancer, HER2 gene amplification in CRCs is not restricted to membranous expression. As a possible explanation, other mechanisms could exist and govern the ultimate localization of HER2 protein expression in CRC cases with HER2 gene amplification.<sup>19</sup>

The studies of Braut et al.<sup>20,21</sup> on EGFR (another member of the epidermal growth factor receptor family) expression in glottic cancer also showed a significant correlation between cytoplasmic staining of EGFR and gene amplification in cancer tissue. They concluded that their findings could be a new indicator of EGFR signaling in glottic cancer. In the majority of HER2 positive cancers, HER2 protein overexpression is the result of gene amplification.<sup>22</sup> They also observed few of the cases which were negative. This could be attributed to, unusual HER2 genotypes, such as polysomy of chromosome 17 and genomic heterogeneity which can lead to discrepant non-correlating cases.<sup>22</sup>

A number of publications have analyzed HER2 overexpression in CRCs with FISH and reverse transcription polymerase chain reaction and concluded that for colorectal cancers, there was a strong correlation between gene amplification and membranous overexpression. However, in these studies cases with cytoplasmic HER2 expression gene amplification was not observed.<sup>17,23,24</sup>

On performing histological analysis, we found that 20.5% cases were well differentiated, 53.8% moderately differentiated and 25.6% poorly differentiated tumours. Similar to our findings other studies also observed majority of their cases were moderately differentiated.<sup>25</sup> Another Indian study also showed predominance of well differentiated tumours.<sup>15</sup> This difference can be explained by the randomized selection and the relatively small sample size.

The histological grades were compared to HER2/neu status and a statistically significant association was found between grade and HER2/ neu status (P=0.001). In our study Score 3 was commonly expressed by grade II tumors and score 0 by grade III tumors. Our results were in concordance with an Egyptian study where positive expression was more commonly seen in moderately differentiated tumours.<sup>8</sup> Studies conducted by Goldstein and Armin also supported our findings.<sup>26</sup> A study done in Iraq also showed expression of Her2/neu in well and moderately differentiated tumours although it was not statistically significant.<sup>27</sup> In contrast to the above findings, Steel et.al studied HER-2/neu receptor expression in CRC

showed more expression in poorly differentiated tumours.<sup>28</sup>

Amongst the various histological types, we found that conventional/NOS were the most frequently occurring 65.4% cases followed by mucin secreting type 9.6% and signet cell type 3.8% cases. These histological types were significantly associated with Her2/neu expression ( $p=0.008$ ) in the present study. It was noticed that score 3 was most common in the conventional NOS subtype and least common in mucinous and signet ring cell types. Dalal et al observed similar results in their study.<sup>8</sup> A study by Foda et al observed that signet ring cell type did not express HER2/neu which was again concordant to our findings.<sup>19</sup>

Neutrophilic infiltration was noticed in 32% cases. Lymphocytic infiltration was also observed in all the cases studied. Necrosis was noted in 82.6% cases and desmoplastic reaction was seen in 50% cases. This could be explained as, oxidative stress and its associated cellular damage, is thought to play a key role in the pathogenesis of the colitis and colon carcinogenesis.<sup>29</sup> T B Halvorsen and E Seim quoted in their study that a tumour with a poorly defined border, lack of inflammatory reaction, and a desmoplasia at the tumour edge predicts unfavourable stage which again had an effect on prognosis of the patient.<sup>30</sup>

In our study we noticed 40.4% of the cases showed involvement of adjacent vessels and nerves; of which 28.8% showed perineural invasion and 11.5% showed lymphovascular invasion. Pappas et.al stated 10% of their cases were showing vascular invasion, and 8% perineural invasion which was in agreement with our study.<sup>25</sup> In current study 73.3% cases showed metastasis whereas Gill et.al found only 50% cases showed evidence of metastasis.<sup>15</sup> Previous studies also have observed perineural, lymphovascular invasion and lymph node metastasis.<sup>151</sup>

In the present study most commonly affected age group was 40-50years (32.7%) and least common was >80years (1.9%). The youngest patient was 22 years and oldest was 85years old. Nearly similar results were obtained by Gill et al who observed the most common age group affected was 5th to 7th decades of life and the mean age of the patients being 53.9 years ( $SD \pm 16.7$ ).<sup>15</sup> However Western studies by Terzi et al,<sup>31</sup> and Office for National Statistics,<sup>32</sup> noticed 86% of cases were detected in people who were 60 years or older.

Majority of the patients in our study group consumed non vegetarian diet 75%. A study done in London showed similar results.<sup>33</sup> Previous studies have suggested that consuming more meat would increase fecal nitrosaminoamines and carcinogenic tryptophan which could increase the risk of CRC in nonvegetarian subjects.<sup>34</sup> There is very less evidence quoting that foods containing high amounts of fiber are rich in poly phenols which can alter molecular processes that can lead to colorectal carcinogenesis which explains a lower CRC in vegetarian subjects.<sup>33</sup>

Our study showed male predilection (59.6%). The same was reported by another Indian study (60%),<sup>15</sup> and Ghaffarzadegan et al also.<sup>4</sup> This however differs from what was reported by Cressey et al.,<sup>35</sup> where higher incidence was detected in females. Overweight, may increase estrogen exposure, particularly after menopause. Estrogen has been associated with decreased risk for both proximal and distal CRC.<sup>36</sup> The differences between the current study and other studies may be also due to random collection of cases.

In the present study we observed that left sided lesions were commonly encountered 59.6% compared to right sided 40.4%. Which was concordant with an Iranian study.<sup>37</sup> A study done at Egypt showed tumors of the right colon outnumbered those of the left colon unlike our study.<sup>8</sup> This was a similar observation brought out by Smyrk, who detected a shift towards right-sided cancers occurring during the second half of the twentieth century.<sup>38</sup> Fenoglio-Preiser et al stated that in carcinomas of caecum and ascending colon occur frequently in low-risk countries than carcinomas of the colon and rectum which is seen mostly in high-risk countries, which could explain the rising trend of colorectal carcinomas in our country.<sup>39</sup>

In the current study we commonly observed a polypoid/cauliflower growth pattern grossly in 48.7% cases followed by ulcerative 32.7% and annular 19.0% which was in agreement with a study by Poincloux et al.<sup>40</sup> Whereas another study group showed that most cancers of the colon and rectum were ulcerating tumors.<sup>8</sup> Compton et al explained the variability in reporting the gross tumor configuration due to variable interpretation of complex configuration.<sup>41</sup>

Schuell et al who also proved that there was no significant association with gender, localization of the primary tumour with Her2/neu expression which was in agreement with our study.<sup>13</sup> However, in our study there was no statistically significant correlation between age, gender, site, and Her2/neu expression.

There was no statistically significant correlation between HER2/neu scores and AJCC stage of patient ( $P=0.076$ ). Score 3 was seen expressed by Stage II and III in our study. This is again similar to another German study,<sup>42</sup> an Iraqi study<sup>27</sup> and a study by K. Ghaffarzadegan et al. Staining in higher stages, drugs that targeting HER2 may be helpful in these cases.<sup>4</sup>

McKay et al conducted a similar study in a large cohort of colorectal tumors and lymph node metastases. They did not find any correlation between staining and lymph node metastases similar to our study.<sup>43</sup> However other studies have shown associations between lymph node metastasis with Her2/neu overexpression. The reason might be the difference in interpretation of IHC and difference in the sample size.<sup>27</sup>

## 5. Conclusion

Colorectal adenocarcinoma overexpresses HER2/neu and using IHC one can effectively determine its frequency. Histological grade and histological type and stage of colorectal carcinomas play a role in determining the level of overexpression of Her2/neu and hence can be used as a predictive tool for determining the prognosis of the patients diagnosed with CRC. However, a subset of these cases having intermediate results that might be subjected for FISH analysis to confirm HER2/ neu amplification/overexpression. Patients who overexpress HER2/neu could become potential candidates for this monoclonal antibody based targeted immunotherapy which could exponentially reduce the morbidity and mortality of CRC worldwide.

## 6. Limitations

Although IHC is used as a useful tool in determining the potential candidates for Anti HER2/neu therapy in breast and gastric carcinomas as per standard protocols however in CRC till date there is no standard protocol and hence there is high variation in different studies across the world due to different methodology followed regarding the HER2/ neu status. Few of our retrospective cases showed expression in normal looking glands which were considered false positive as it is one of the fixation artefact.

A long term study in a larger cohort of cases along with treatment and follow-up history and FISH analysis for amplification of HER2/neu would have been beneficial in studying the exact behavior of the cases expressing HER2/ neu.

## 7. Source of funding

None.

## 8. Conflict of interest

None.

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### Author biography

**Neha Ratan B** Post Graduate

**Vijayalaxmi V Suranagi** Professor

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