

HER2/neu Expression in Gastrointestinal Carcinoma Among South Indians

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ABSTRACT

Background: HER2/neu oncogene overexpression and amplification in breast cancer is well known. Studies have proved its role in gastric cancer and its correlation with the prognosis. Very few studies are there in literature regarding HER2/neu expression in entire gastrointestinal carcinoma. Our study was aimed at HER2/neu expression in gastrointestinal tumors in South Indian Population.

Methods: We included all patients with gastrointestinal carcinoma who either underwent biopsy or surgical excision over the past five years. Slides were reviewed for confirmation of the diagnosis and immunohistochemistry was done using SP3 monoclonal anti-HER2 antibodies. Three independent observers did the scoring for HER2 positivity.

Results: Among 35 cases of gastric cancer, only 2 (5.7%) females showed positivity for HER2 scoring and one (2.9%) female showed an equivocal result. All positive and equivocal cases in gastric cancer were intestinal type. Both the cases with HER2 positivity were poorly differentiated tumors. The one with equivocal was a moderately differentiated carcinoma. In colorectal cancer out of 19 cases, only one (5.3%) showed positivity for HER2 whereas in one there was an unequivocal response. All the seven cases of small intestinal carcinoma showed negative results for HER2 expression.

Conclusion: Overall, HER2/neu expression in gastrointestinal cancer was 4.9%. Female gender, intestinal-type and poorly differentiated cancer showed positivity in gastric cancer. Female gender, left side and low-grade tumor showed positivity in colorectal cancer. Further studies are required with a large sample size to correlate HER2 expression with clinicopathological parameters and its role in prognosis in gastrointestinal carcinomas in the Indian population.

Keywords: HER2, Gastric Carcinoma, Colorectal Cancers, Small Intestinal Carcinoma

Introduction

Gastrointestinal carcinoma is one of the leading causes of cancer-related deaths worldwide, especially in gastric and colorectal carcinomas.^[1,2] Small intestinal carcinoma is rare.^[3] Recently the search for newer therapeutic regimens has focused on HER2/neu (Human Epidermal Growth factor receptor) expression and gene amplification. This has been studied as a therapeutic and prognostic target using a monoclonal antibody against HER2/neu in a number of human cancers including breast, gastric, colorectal and lung carcinoma.^[4] An evidence-based clinical practice guideline on HER2 testing for patients with gastroesophageal cancers was recently released by American society of clinical oncology (ASCO), College of American Pathologists (CAP) and the American Society for Clinical Pathology (ASCP) and recommends that HER2 status should be established in all patients with advanced gastroesophageal adenocarcinoma who are eligible for systemic therapy.^[5] Also, recent trials like HERACLES and MyPathway suggested the possibility of HER2 blockade as a targeted therapy in advanced colorectal carcinoma (CRC) patients.^[6,7] Together, the trials supported preclinical results that targeting HER2 with trastuzumab plus either lapatinib

or pertuzumab is more effective than standard combination chemotherapy in HER2-positive CRC patients. Very few studies were done in the Indian population regarding HER2 expression in gastric and colorectal cancers alone. Hence, we decided to study the HER2 expression in carcinoma of the entire gastrointestinal tract including gastric, colorectum and small intestine in the South Indian population. Though we have not done mutation testing, the current study could serve as a basis for searching for HER2 as a common pathogenetic event(s) in all these neoplasms.

Materials and methods

The present study was carried out in our Department after obtaining approval from the Institute Ethics Committee. A total number of sixty-one gastrointestinal carcinoma cases including both biopsies and surgical specimens from Jan 2011 to Jun 2015 were collected. Patients with biopsy-proven gastrointestinal carcinoma irrespective of age, sex, grade, histological type and extent of disease were included in this study. Patients who have received Trastuzumab for any cancer were excluded.

Histopathological grading and Immunohistochemistry (IHC) for HER2/neu expression were carried out on

all gastrointestinal biopsies and surgical specimens from archival material available in our department. The procedure for IHC was done as per the instructions on the manufacturer's kit. IHC was done by using Primary antibody, HER2-neu SP3 Clone (GENNOVA EUROPE), Secondary antibody, Polymer kit (QUARTETT GERMANY) and positive control was HER2-neu positive breast carcinoma.

Histopathological grading was done. Scoring of IHC was done as per the updated criteria given by Hoffmann et al.^[8] for gastric cancer. For small intestinal carcinomas and colorectal carcinomas, scoring was done by Ghaffarzadegan et al.^[9] criteria. Three pathologists independently carried out the grading and scoring. If two pathologists agree on a value that was taken as the final score. Interobserver variability was noted.

Results

Out of 61 cases, gastric carcinoma accounted for 35(57%) cases followed by 19(31%) cases of colorectal carcinoma and 7(12%) cases of small intestinal carcinoma. The mean age of patients with gastric, colorectal and small intestinal carcinoma was 61.46, 59.63 and 55.71 years respectively.

Gastric Carcinoma: Among 35 cases, only 2(13.3%) females showed positivity for HER2 scoring and one female(6.7%) showed equivocal result. Out of 7 cases located in the body, one (14.3%) showed HER2 positivity. Out of 4 cases located in the fundus, one (25%) showed positivity. Among 23 cases in the antrum, one (4.3%) showed an equivocal response in HER2 expression. Both the cases with HER2 positivity (5.7%) were poorly differentiated tumor. The one(2.9%) with equivocal

scoring was moderately differentiated. Out of 25 intestinal type gastric carcinoma, two(8%) were positive and the one (4%)was equivocal. None were positive in diffuse-type

Colorectal Carcinoma: Out of 19 cases of colorectal carcinoma, only one(5.3%) patient showed positivity with HER2 scoring while one other patient (5.3%) showed an equivocal response. Both were located in the left colon. Among the 10 cases of low-grade colorectal carcinoma, one(10%) showed 3+ positivity. Among the 9 high-grade tumors, one(11.1%) patient showed an equivocal response.

Small Intestinal Carcinoma: All the seven cases of small intestinal carcinoma showed a negative response for HER2 expression. 4 of them had a score of 0 (57.1%) and the rest 3 had 1+ (42.9%) scoring.

The histological and immunohistochemical profile of gastrointestinal tumors is shown in table 1.

HER 2 scoring in Metastasis: Out of 61 cases, the three cases (2 gastric and one colon) with 3+ positivity had no metastasis. Out of two cases with equivocal response, the patient with colonic carcinoma alone had metastasis.

Among 61 cases of gastrointestinal cancer, 56(91.8%) cases showed negative, 2(3.3%) showed equivocal and 3(4.9%) showed strong positivity for HER2 expression (Table 2).

Based on Fleiss kappa for 3 raters, gastric(0.605) had a moderate agreement and colorectal carcinoma (0.366) had a fair agreement. Small intestinal carcinoma had excellent concordance (1.000). Overall, there was moderate agreement (0.509).

Table 1: Histologic and immunohistochemical profile of gastrointestinal carcinomas.

Group		Gastric	Colorectal	Small intestinal
Histologic type	Intestinal	27	NA	NA
	Diffuse	8	NA	NA
Total		35	19	7
Histologic grade (differentiation)	Well differentiated	4	6	2
	Moderately differentiated	13	4	3
	Poorly differentiated	18	9	2
Total		35	19	7
HER 2 / neu	Positive	2	1	0
	Negative	32	17	7
	Equivocal	1	1	0
Total		35	19	7

Table 2: HER 2 / neu scoring of gastrointestinal carcinomas.

Sl. No	Tumor site	Tumor type	Tumor grade	HER 2 score	Metastasis
1	Gastric	Intestinal	Poorly diff	3 +	No
2	Gastric	Intestinal	Poorly diff	3 +	No
3	Colon	NA	Well diff	3 +	No

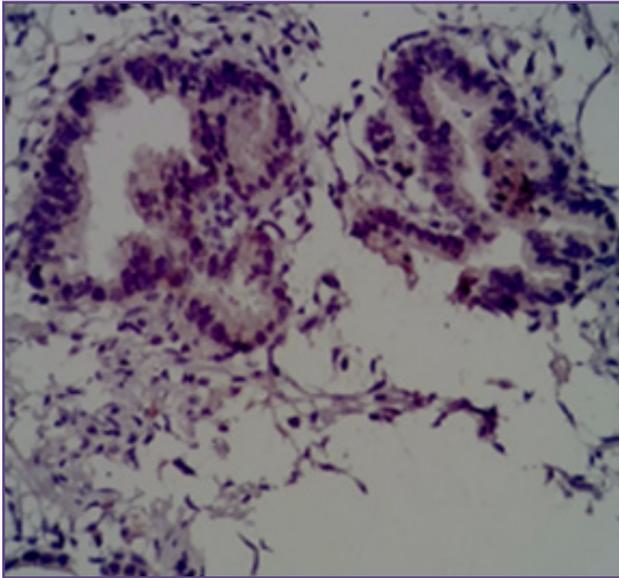


Fig. 1: Equivocal HER 2 positivity 2+in gastric adenocarcinoma x400.

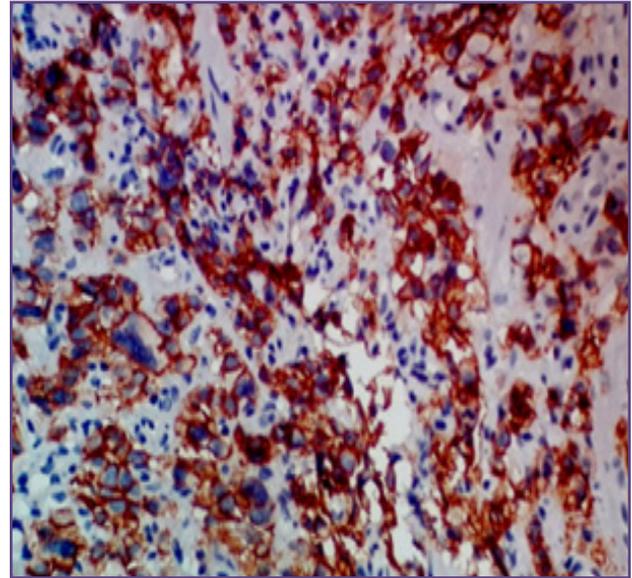


Fig. 2: Poorly differentiated gastric adenocarcinoma showing strong membranous HER 2 positivity 3+ (x400 IHC).

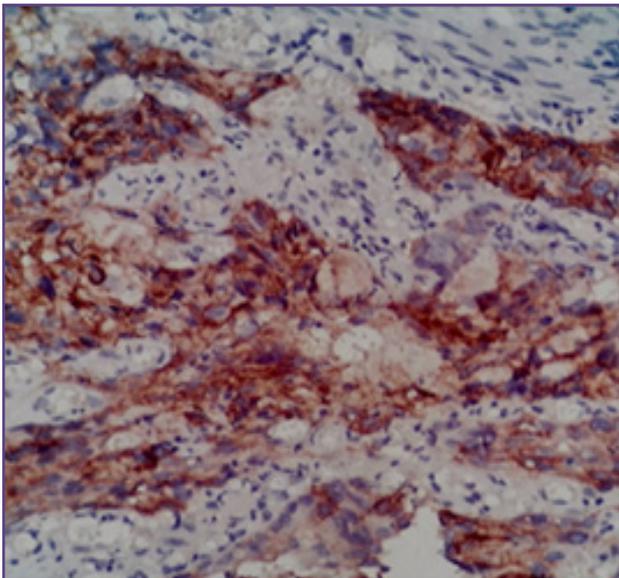


Fig. 3: Moderately differentiated colonic adenocarcinoma showing intense cytoplasmic and membranous HER 2 positivity 3+ (x400 IHC).

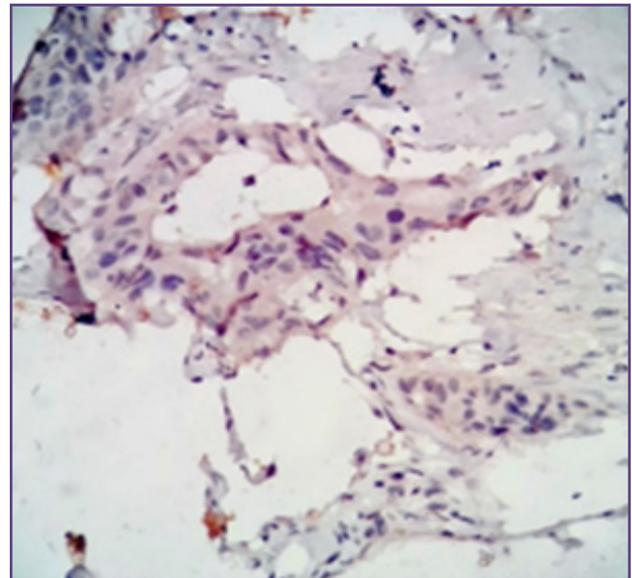


Fig. 4: Equivocal HER 2 positivity 2+in colonic adenocarcinomax400.

Discussion

Gastric and colonic cancer are the second and third most common cause of death worldwide.^[10] Despite the recent advances in the treatment of gastrointestinal carcinoma, the prognosis is poor because most of them present at an advanced stage of the disease. Targeted therapy is gaining momentum in the last decade to improve survival of these patients. HER2/neu gene is one such potential target and research on its ability to show overexpression and amplification in breast tumours is well established.^[4,11] Recently, the role of HER2 expression in gastrointestinal tumour is also under study. The range of HER2 positivity by American and European studies was between 10% to 22.8%.^[8,12-14] Asian studies have reported a range between 11.7% to 15.74%.^[15,16] Very few studies have been published in India.^[17-20]

In the present study, out of 35 gastric carcinoma cases, 24 (68.6%) cases were negative for HER2 expression (score 0) and 8(22.9%) cases had a score of 1+ (incomplete membranous staining in less than 10% of tumor cells. One (2.9%) case had equivocal (2+) response (moderate, basolateral, membranous staining) (Fig.1) and 2(5.7%) cases showed strong membranous positivity for HER2 expression(3+)(Fig.2).

In our study, both the positive cases and one equivocal case were in the intestinal type of cancer like other studies. Gravlos et al.^[15] in 2008 observed a higher rate of HER2 overexpression in intestinal than in diffuse-type (16%). In the ToGA trial, HER2 positivity differed significantly by histological subtype. It was found to be 34% in intestinal, 6% in diffuse, 20% in mixed gastric carcinomas.^[10] In India, a study done by Rajagopal et al.^[19] showed a correlation between HER2 expression and intestinal type of tumor. The association of HER2 with a specific histologic tumour type suggests that intestinal and diffuse types develop along different molecular pathways.

Our study shows that both the cases with HER2 positivity (5.7%) were poorly differentiated tumor. One (2.9%) with equivocal was moderately differentiated. However, there was no statistical significance. Yan et al.^[1] from China and Tewari et al.^[20] from India also showed similar findings in their studies. In contrast, a statistically significant correlation was observed between grading and HER2 expression in many studies having a well and moderately differentiated tumor.^[15,16,19]

HER2 Scoring in Colorectal Cancers: In our present study, out of 61 cases of gastrointestinal cancers, 19(31%) were colorectal in origin which was next to gastric cancer(57%).

Several studies evaluating HER2 in colorectal cancer resulted in a large debate at the end of the last century because overexpression rates varied between 0 and 83%.^[21] The majority of carcinomas showed membranous overexpression rates between zero and 15%. Some studies reported both membranous and cytoplasmic overexpression with much higher rates of up to 60%.^[22] Until now, there is no consistency in the proportion of HER2 overexpression in colorectal tumors.

In our study, we considered both membranous and cytoplasmic staining as positive. We did HER2 scoring as recommended by Ghaffarzagdegan et al.^[9] in 2006 which showed that there were 41 (59.4%) HER2 positive and 28 (40.6%) HER2 negative cases. Twenty-seven (65.9%) cases had cytoplasmic and 14 (34.1%) cases had membranous (predominant) and cytoplasmic staining. There was no case with pure membranous staining. In our study, among 19 cases of colorectal carcinoma 17 cases were negative (89.4%), one was equivocal (5.3%) and one was positive (5.3%) which both cytoplasmic and membranous positive (Fig.3,4).

Both equivocal and positive cases were in the age group between 41-80 years and both were female patients. Left-sided colon had both equivocal and positive cases. Regarding grading one of the low-grade tumors had 3+ positivity and high-grade tumor had 2+. One of the case had evidence of metastasis showing 2+ positivity. 3+ positive cases had no evidence of metastasis clinically and radiologically. However, there was no statistically significant correlation between HER2 positivity and clinicopathological parameters.

With respect to the grade of differentiation, we did not observe any significant relationship between HER2/neu expression and grade of differentiation. This result was consistent with studies by Half et al.^[23] and Gruenberger et al.^[24] On the other hand, a significant correlation between histological grade and HER2/neu expression was reported in the studies done by Ghaffarzagdegan et al.,^[9] McKay et al.^[25] and Deng et al.^[26] Like our study, Ghaffarzagdegan et al.^[9] found positivity in low-grade tumor. However, these observations were not statistically significant.

Small Intestinal Carcinoma: Although the small intestine has approximately 75% of the length and 90% of the mucosal surface of the gastrointestinal tract, adenocarcinoma occurs 50 times less frequently than colorectal adenocarcinoma.

In the present study, all seven cases were considered as negative having 0+ and 1+ score on IHC. Similarly, Chan et al.^[3] found that none of their 49 cases showed greater

than 1+ positive on both IHC and FISH. Overman et al.^[27] used monoclonal antibody clone neu Ab8 and reported HER2 immunohistochemical expression in only 1 (2%) of 54 small intestinal (nonampullar) adenocarcinomas. In contrast, Zhu et al.^[28] examined HER2 protein expression in duodenal (nonampullar) adenocarcinoma and found that 9 (60%) of 15 cases exhibited positive expression by immunohistochemical analysis. Chan et al.^[3] described that the disparity of findings in his study compared to that of Zhu et al.^[28] may be related to differences in immunohistochemical reagents and interpretation criteria used in different studies. These findings indicate that HER2 overexpression or amplification is not a primary event in the pathogenesis of small intestinal cancers.

Interobserver Variability: Interobserver variability is an accepted caveat in reporting HER2/neu by IHC and the area of focus is more problematic when it comes to 2+ and 3+. In general, human perception varies among individuals and can be affected by training, experience, physical differences and fatigue.^[29]

Our study showed that based on Fleiss kappa for 3 raters, Her2 score of gastric (0.605) had a moderate agreement and colorectal carcinoma (0.366) had a fair agreement. Overall there was moderate agreement (0.509). There was an excellent concordance when scoring was 3+. We have faced problems in scoring between 1+ and 2+ and in scoring between 0 and 1+. Haddabi et al.^[29] also found frequent discrepancies between the scores of 0 and 1+ and the major disagreement to score 2+. However, the differences between the scores of 0 and 1+ had no clinical significance. 2+ cases reported as equivocal should be confirmed by FISH analysis.

Our limitation was the non-use of the FISH technique to confirm the immunohistochemical expression of HER2 in cases classified as 2+ (equivocal).

Conclusion

To conclude, 4.9% of cases showed strong HER2 protein expression and 3.3% of equivocal cases were observed among gastrointestinal cancers. All seven cases of small intestinal cancer were negative for HER2 expression. HER2/neu expression can be evaluated satisfactorily using IHC in Gastrointestinal carcinomas. But equivocal cases need confirmation with FISH. However, studies with larger sample sizes are needed to understand the utility of HER2 expression, especially in small intestinal cancers and to clarify the relationship between the HER2 protein and clinicopathological parameters.

References

1. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X et al. Clinicopathologic significance of HER2/neu protein expression and gene amplification in gastric carcinoma. *World J Gastroenterol* 2011; 17: 1501-06.
2. Elwy DA, Ahmed M, El Aziz A, Samar A, El-Shekh, Ebrahim HA. Immunohistochemical Expression of HER2/neu in Colorectal Carcinoma. *Med. J. Cairo Univ* 2012; 80: 467-77.
3. Chan OT, Chen ZM, Chung F, Kawachi K, Phan DC, Himmelfarb E et al. Lack of HER2 overexpression and amplification in small intestinal adenocarcinoma. *Am J Clin Pathol* 2010;134:880-85.
4. Liu L, Wu N, Li J. Novel targeted agents for gastric cancer. *J Hematol Oncol* 2012; 5: 31-38.
5. Bartley AN, Washington MK, Ventura CB, Ismaila N, Colasacco C, Benson III AB et al. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *American journal of clinical pathology* 2016;146(6):647-69.
6. Siena S, Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S et al. Therapeutic dual inhibition of HER2 pathway for metastatic colorectal cancer (mCRC): The HERACLES trial. *J Clin Oncol* 33, 2015 (suppl 3; abstr 565).
7. Hainsworth JD, Meric-Bernstam F, Swanton C, Hurwitz H, Spigel DR, Sweeney C et al. Targeted therapy for advanced solid tumors on the basis of molecular profiles: results from MyPathway, an open-label, phase IIa multiple basket study. *Journal of Clinical Oncology*. 2018;36(6):536-44.
8. Hofmann M, Stoss O, Shi D, Buttner R., Van De Vijver M, Kim W et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; 52: 797-805.
9. Ghaffarzadegan K, Sharifi N, Vosooghynia H, Shakeri T, Ghiasi Moghadam T, Kafi SG et al. HER2/neu expression in colon adenocarcinoma and its correlation with clinicopathologic variables. *IJBMS* 2006;9:64-9.
10. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *The Lancet*. 2010 Aug 28;376:687-97.
11. Ross JS, Mulcahy M. HER2 Testing in Gastric/Gastroesophageal Junction Adenocarcinomas: Unique Features of a Familiar Test. *Gastrointest Cancer Res* 2012; 4:62-66.
12. Tafe LJ, Janjigian YY, Zaidinski M, Hedvat CV, Hameed MR, Tang LH, et al. Human epidermal growth factor receptor 2 testing in gastroesophageal cancer: correlation between immunohistochemistry and fluorescence in situ

- hybridization. *Archives of pathology & laboratory medicine* 2011;135:1460-65.
13. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Annals of Oncology* 2008; 19: 1523-29.
 14. Lordick F, Bang YJ, Kang YK, Reyes DO, Manikhas GM, Shen L, et al. HER2-positive advanced gastric cancer: similar HER2-positivity levels to breast cancer. *EJC Supplements*. 2007;5:272.
 15. Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and gastroesophageal junction adenocarcinoma in a Chinese population. *Diagn Pathol* 2013;8:76.
 16. He C, Bian XY, Ni XZ, Shen DP, Shen YY, Liu H et al. Correlation of human epidermal growth factor receptor 2 expression with clinicopathological characteristics and prognosis in gastric cancer. *World J Gastroenterol* 2013;19: 2171-78.
 17. Sekaran A, Kandagaddala RS, Darisetty S, Lakhtakia S, Ayyagari S, Rao GV et al. HER2 expression in gastric cancer in Indian population—an immunohistochemistry and fluorescence in situ hybridization study. *Indian Journal of Gastroenterology* 2012; 31: 106-10.
 18. Patil PS, Mehta SA, Mohandas KM. Over-expression of HER2 in Indian patients with gastric cancer. *Indian Journal of Gastroenterology* 2013; 32: 350.
 19. Rajagopal I, Niveditha SR, Sahadev R, Nagappa PK, Rajendra SG. HER 2 Expression in Gastric and Gastroesophageal Junction (GEJ) Adenocarcinomas. *Journal of clinical and diagnostic research* 2015; 9: 6-10.
 20. Tewari M, Kumar A, Mishra RR, Kumar M, Shukla HS. HER2 expression in gastric and gastroesophageal cancer: report from a tertiary care hospital in North India. *Indian Journal of Surgery* 2013; 1-5.
 21. Schuell B, Gruenberger T, Scheithauer W, Zielinski C, Wrba F. HER 2/neu protein expression in colorectal cancer. *BMC Cancer* 2006; 6:123-27.
 22. Blok EJ, Kuppen PJK, Leeuwen JEM, Sier CFM. Cytoplasmic Overexpression of HER2: a Key Factor in Colorectal Cancer. *Oncology* 2013;7: 41–51.
 23. Half EE, Bresalier RS. Clinical management of hereditary colorectal cancer syndromes. *Current opinion in gastroenterology* 2004; 20: 32-42.
 24. Gruenberger T, Scheithauer W, Zielinski CH, Wrba F. Expression of HER-2/neu in CRC. *BMC Cancer* 2006; 6: 123-35.
 25. McKay JA, Murray LJ, Curran S, Ross VG, Clark C, Murray GI et al. Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumours and lymph node metastases. *European journal of cancer* 2002; 38: 2258-64.
 26. Deng W, Dong WG, Zhan N, Zhan F, Wu HX. Human epidermal growth factor receptor (HER 2)/neu expression and gene amplification in colorectal cancer. *African Journal of Biotechnology* 2013; 10: 16732-39.
 27. Overman MJ, Pozadzides J, Kopetz S, Wen S, Abbruzzese J L, Wolff RA et al. Immunophenotype and molecular characterisation of adenocarcinoma of the small intestine. *British journal of cancer* 2010; 102: 144-50.
 28. Zhu L, Kim K, Domenico DR, Don R, Hubert E, Appert HE et al. Adenocarcinoma of duodenum and ampulla of Vater: Clinicopathology study and expression of p53, c-neu, TGF- α , CEA, and EMA. *Journal of surgical oncology* 1996; 61: 100-05.
 29. Al Haddabi I, Qureshi A, Saparamadu A, Al Hamdani A, Al Riyami M, Ganguly S. Inter-observer agreement in reporting HER 2 Neu protein over expression by immunohistochemistry. *Indian Journal of Pathology and Microbiology* 2014; 57: 201-04.

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