e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2024; 16(1); 1668-1672

Original Research Article

HER 2 NEU and KI67 Expression as Immunolocalization in Colorectal Carcinoma

T Shruthi[1], S. P. Smitha Vadana[2]

[1]Assistant Professor, Department of Pathology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, Telangana State

[2]Assistant Professor, Department of Pathology, Government Medical College, Nizamabad, Telangana State

Received: 25-10-2023 / Revised: 23-11-2023 / Accepted: 26-12-2023

Corresponding Author: Dr. T. Shruthi

Conflict of interest: Nil

Abstract:

Introduction: Colorectal adenocarcinoma is the fourth most common malignant disease and it is the leading cause of morbidity and mortality. Both HER-2 neu and Ki-67 in immunohistochemical studies can be used for predicting the prognosis and treatment in colorectal carcinomas.

Aim and Objectives: To study the expression pattern of HER2 neu and Ki67 expression of different grades in colorectal carcinomas.

Materials and Method: The present study was conducted in 65 cases of histopathologically proven colorectal carcinoma cases and evaluated the clinicopathological patterns of colorectal carcinoma and the relationship of with the clinicopathological variables.

Results: HER 2 neu was positive in 87.7% of the cases. It was seen in 32.6% cases of well differentiated, 48.8% of moderately differentiated, 2.3% of poorly differentiated adenocarcinomas. Mucinous carcinomas showed 11.6% positivity for HER 2 neu. HER 2 neu positivity was more in grade II tumors compared to that in other grades.

Conclusion: Colorectal carcinomas which express HER2 neu showed more positive cases but there was not significant association with clinicopathologic parameters. Immunohistochemical technique for detection of KI-67 proliferative index is simple and applicable to surgical specimens. However, it is not enough to monitor KI-67 proliferation index alone for prognosis in colorectal cancer as it was not significantly related to variable clinicopathologic parameters.

Keywords: Colorectal adenocarcinoma, Immunohistochemical, HER2 neu, KI67.

Introduction

Cancer of the large bowel, commonly referred to as colorectal cancer, is a type of cancer that starts in the colon or rectum. The colon and rectum are parts of the large intestine, which is the final portion of the digestive system. Colorectal cancer is more common in older adults. Individuals with a family history of colorectal cancer are at a higher risk, also with a history of colorectal polyps or inflammatory bowel disease (such as Crohn's disease or ulcerative colitis) have an increased risk. Lack of physical activity, a diet high in red and processed meats, smoking, and heavy alcohol consumption may contribute to the risk.

About one-third to one-half of all individuals will develop one or more adenomas [1, 2]. All adenomas have the potential to become cancerous, fewer than 10% progresses to invasive cancer [3, 4]. The likelihood that an adenoma will become more cancerous as it becomes larger [5]. Cancer originating from the inner lining of the colorectum is called adenocarcinoma and accounts for approximately 96% of all CRCs [6]. Incidence and mortality rates are higher in males than in females [7]. Common symptoms consist of abdominal pain, rectal bleeding, altered bowel habits, and involuntary weight loss.

Globally, the incidence of colorectal cancer differs widely by over 10-fold, with the highest incidence rates in Australia and New Zealand, Europe and North America, and the lowest rates in Africa and Asia [8]. These geographic differences appear to be attributable to differences in dietary and environmental exposures [9] several factors have been shown to put individuals at risk to CRC and these include age, the presence of polyps, inflammatory bowel disease, lifestyle, genetic background, and family medical history. Environmental factors such as obesity, physical

inactivity, poor diet, smoking and heavy alcohol consumption account for approximately 80% of all colorectal cancer cases [10].

Genetic susceptibility is associated with familial adenomatous polyposis (FAP) and Lynch Syndrome (hereditary non-polyposis colorectal cancer (HNPCC) which accounts for 10% of all colorectal cancer cases. Individuals who have these diseases have an increased lifetime risk of CRC of up to 80% [10]. In this study we are going to study the expression pattern of HER2 neu and Ki67 expression of different grades in colorectal carcinomas.

Materials and Method

This was Retrospective study was conducted in the Department of Pathology, Chalmeda Anand Rao Institute of medical Sciences, Karimnagar for the duration of Jan 2022 to May 2023.

In the study total of 65 cases were included in this study. Clinical data like patient age, sex, and other relevant details were noted from the pathology records, after getting permission from institutional ethical committee and following inclusion and exclusion criteria given bellow.

Inclusion Criteria:

- Adenocarcinoma , NOS
- Mucinous adenocarcinoma
- Signet ring carcinoma
- Micropapillary carcinoma
- Squamous cell carcinoma
- Adenosquamous carcinoma
- Medullary carcinoma
- Undifferentiated carcinoma

Exclusion Criteria:

- Benign lesions of colon and rectum

Materials

1. Donor blocks which contain formalin fixed paraffin embedded tissue obtained from all the cases of colorectal adenocarcinoma
2. Hematoxylin and eosin stained tissue sections made from the donor blocks.
3. Black glass marking pen for marking area of interest.
4. Microtome and incubator for obtaining tissue sections and to dewax the sections
5. Positively charged slides for holding tissue sections for IHC
6. Chemicals for preparing antigen retrieval solutions and for wash buffers.
7. Pressure cookers for antigen retrieval
8. Kit for performing immunohistochemistry which includes primary antibody
9. (HER 2 neu & ki-67) and universal kit . Microscope, used for interpretation and grading of IHC

Methodology

The method of performing immunohistochemistry over the paraffin tissue includes the following steps.

1. Collection of the donor blocks
2. Preparation of the recipient paraffin blocks
3. Immunohistochemistry and analysis

Statistical Analysis: Data was collected and entered in the Microsoft Excel 2016, for further statistical analysis. Categorical data was expressed in the form of frequency and percentage. Chi-square test was used to see an association between the variables. P-value<0.05 considered as statistically significant at 5% level of significance.

Observation and Results: In the study, we have included 65 samples after following inclusion and exclusion criteria and demographics profile of the study sample given below table 1.

Table 1: Distribution of demographic profile

Parameter	Frequency	Percentage
Age		
≤ 30 Years	5	7.7
31 - 50 Years	18	27.7
> 50 Years	42	64.6
Gender		
Male	29	44.2
Female	36	55.8
HPE		
Well differentiated adenocarcinoma	21	32.6
Moderately differentiated adenocarcinoma	32	48.8
Poorly differentiated adenocarcinoma	2	2.3
Mucinous carcinoma	8	11.6
Signet ring cell carcinoma	2	2.3
Invasive squamous cell carcinoma	2	2.3
Histologic Grade		
Grade I	21	32.3

Grade II	32	49.2
Grade III	12	18.5

Of the 65 patients included in the study majority of the patients were more than 50 years, who constituted 65% of the group. Patient's age range from 21-69 years with a mean age of 49 years. 48.8 % were moderately differentiated adenocarcinoma, 32.6% were well differentiated adenocarcinoma and Mucinous carcinoma constitutes 11.6 % of the cases. 32 cases (49.2%) were grade II, followed by 21 cases (32.3%) were grade I, and 12 cases (18.5%) were under grade III.

Table 2: Distribution of HER 2 neu and KI-67

Parameter	Frequency	Percentage
HER 2 neu		
Positive	57	87.7
Negative	8	12.3
KI-67		
High	20	30.8
Intermediate	1	1.5
Low	44	67.7

Table 3: Association between Histopathologic type and HER 2 neu, KI-67

HPE	HER 2 neu		P-value	KI-67			P-value
	Negative	Positive		Low	Intermediate	High	
Well differentiated adenocarcinoma	3	18	0.737	12	0	9	0.208
Moderately differentiated adenocarcinoma	5	25		19	1	10	
Poorly differentiated adenocarcinoma	0	2		1	0	1	
Mucinous carcinoma	0	8		8	0	0	
Signet ring cell carcinoma	0	2		2	0	0	
Invasive squamous cell carcinoma	0	2		2	0	0	

5 cases of moderately differentiated adenocarcinoma were HER2 negative and 25 cases were HER2 Positive.

3 cases of well differentiated adenocarcinoma were HER2 Negative and 18 cases were HER2 positive. 8 cases of mucinous adenocarcinoma were HER2 positive. The relationship between HER2 neu and histologic type were statistically not significant (p value – 0.737). Also, 10 cases of moderately differentiated adenocarcinoma show high proliferative activity, 19 cases shows low proliferative activity. In well differentiated adenocarcinoma, 9 cases shows high proliferative activity and 12 cases shows low proliferative activity.

In mucinous carcinoma, 8 cases shows low proliferative activity. The relationship between histologic type and KI-67 was statistically not significant (p value – 0.208).

Table 4: Association between Histopathologic Grade and HER 2 neu, KI-67

HPE Grade	HER 2 neu		P-value	KI-67			P-value
	Negative	Positive		Low	Intermediate	High	
Grade I	3	18	0.735	12	0	9	0.108
Grade II	5	27		20	1	11	
Grade III	0	12		11	0	1	

Above table showed that, I tumors shows 3 cases of HER2 Negative and 18 cases were HER2 positive. Grade II tumors shows 5 cases of HER2 Negative and 27 cases were HER2 positive. Grade III tumors shows 12 cases of HER2 positive. The relationship between histologic grade and HER2neu were statistically not significant

Grade I tumors shows 9 cases of high proliferative index and 12 cases of low proliferative index. Grade II tumors shows 11 cases of high proliferative index and 20 cases of low proliferative index, 1 case shows intermediate proliferative index. Grade III tumors shows 1 case of high proliferative index and 11 cases of low proliferative index. The relationship between histologic grade and KI-67 was statistically not significant.

Discussion

Colorectal cancer (CRC) is the fourth most common malignant disease with over one million novel cases and over 5,00,000 deaths each year worldwide [11].

Early diagnosis of CRC, successful surgical treatment, better knowledge of its clinicopathological prognostic factors and response to adjuvant therapy have contributed to improved outcome in affected patients. 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.

Although the tumor is diagnosed histopathologically on light microscopy, various immunological markers are expressed by colorectal carcinomas and depending on them, the treatment and the prognosis differ.

Her2 neu is a useful marker, to predict the outcome of colorectal cancers. Its over-expression correlates with poor prognosis. It is used to predict the patient response to adjuvant chemotherapy and endocrine therapy and to select patients for immunotherapy with a targeted monoclonal antibody therapy. The patients who overexpress Her2 neu should respond to transtuzumab (Herceptin) therapy, independent of the tissue origin of the cancer.

In the present study, histologic types of colorectal carcinoma, 83.7% cases were adenocarcinoma, 11.6% were mucinous adenocarcinoma. Lanza et al, [12] reported 85% were adenocarcinomas, 10-15% were mucinous adenocarcinomas. Usual type adenocarcinoma was the most common histologic type reported by Bhagyalakshmi et al and also in other studies [13-15]. In one study mucinous carcinomas accounted for 11.6% cases and signet ring for 4% of cases. Sen et al observed moderately differentiated adenocarcinoma (69.1%) constitutes the most common type, followed by well differentiated and poorly differentiated adenocarcinomas (11.8%)

In the present study, majority was grade II tumors (49.2%), followed by grade I tumors (32.3%). Bhagyalakshmi et al reported majority were grade I tumors. In several other studies [16-20], CRCs were mostly of grade II tumors (moderately differentiated). Dalal A. Elwy et al reported 72% cases of grade II tumors (moderately differentiated) in accordance with the results obtained by Triest et al [21]. Sharifi et al [22] reported majority of the cases were grade I tumors (well differentiated).

Present study showed no statistically significant relationship was detected between HER2 neu expression and histologic types. The same was reported by Kavanagh et al [23]. KI67 expression with histologic types shows no statistically significant relationship between them. Ahmed et al observed the proliferative activity was higher in non-mucinous tumors than the mucinous ans signet ring carcinoma.

Lanza et al observed higher levels of KI67 reactivity in mucinous tumors than non-mucinoid adenocarcinomas. In our study, no statistically significant relationship was observed between histologic grades and HER2 neu expression. This result was consistent with Goldstein and Armin and Gruenberger et al. In another study a significant correlation was observed between histologic grades and HER2 neu expression done by Steel et al, Mckay et al, Ghaffarzadegan et al and Deng et al. In our study, KI67 expression with histologic grade was not statistically insignificant. A study conducted in Japan concluded KI67 positivity was lower in poorly differentiated and mucinous carcinoma compared with well differentiated and moderately differentiated adenocarcinoma, suggesting that proliferative activity is lower in cancers with poor differentiation.

Conclusion

From above observation in our study we can conclude that, colorectal carcinomas which expresses HER2 neu showed more positive cases but there was no significant association with clinicopathologic parameters, its because of low sample size so require more sample size and a different therapeutic approach, as these cases could respond to transtuzumab (Herceptin) therapy as well as other new modalities. Immunohistochemical technique for detection of KI-67 proliferative index is simple and applicable to surgical specimens. However, it is not enough to monitor KI-67 proliferation index alone for prognosis in colorectal cancer as it was not significantly related to variable clinicopathologic parameters.

Limitation : Study showed limitation of small sample size, though other studies showed that biomarkers can be used as useful predictors in primary screening and identifying of CRC, but in our study it showed non-significant association with clinopathologic parameters so further studies requires with more sample size.

References

1. Bond JH. Polyp guideline: diagnosis, treatment, and surveillance for patients with colorectal polyps. Practice Parameters Committee of the American College of Gastroenterology. Am J Gastroenterol. 2000; 95:3053-3063.
2. Schatzkin A, Freedman LS, Dawsey SM, Lanza E. Interpreting precursor studies: what polyp trials tell us about large-bowel cancer. J Natl Cancer Inst. 1994; 86: 1053-1057.
3. Levine JS, Ahnen DJ. Clinical practice. Adenomatous polyps of the colon. N Engl J Med. 2006; 355: 2551-2557.
4. Risio M. The natural history of adenomas. Best Pract Res Clin Gastroenterol. 2010; 24: 271-280.
5. Pickhardt PJ, Kim DH, Pooler BD, et al. Assessment of volumetric growth rates of small colorectal polyps with CT colonography: a longitudinal study of natural history. Lancet Oncol. 2013; 14: 711-720.
6. Stewart SL, Wike JM, Kato I, Lewis DR, Michaud F. A populationbased study of colo-

rectal cancer histology in the United States, 19982001. Cancer. 2006; 107: 1128-1141.
7. Siegel R., Ward E., Brawley O. and Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA: a cancer journal for clinicians. 2010; 61(4): 212-236.
8. Jemal A., Bray F., Center M.M., Ferlay J., Ward E. and Forman D. Global cancer statistics. CA: a cancer journal for clinicians. 2011; 61(2): 69-90.
9. Center M.M. and ME J.F. Global cancer statistics. CA: a cancer journal for clinicians. 2011; 61(2): 69.
10. Haggar F.A. and Boushey R.P. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clinics in colon and rectal surgery. 2009; 22(4): 191.
11. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 2011; 61:69–90.
12. Lanza G., Messerini L., Gafà R. And Risio M.: Colorectal tumors: The histology report. Digestive and Liver Disease, 2011; 43S: 344-S355.
13. Chalya PL, Mchembe MD, Mabula JB, Rambau PF, Jaka H, Koy M, et al. Clinicopathological patterns and challenges of management of colorectal cancer in a resource-limited setting: a Tanzanian experience. World J Surgical Oncol. 2013; 11:88.
14. Song Wu, Sujiing Wu, Yu-long HE, Shirong C, Chang-hua Z, Xinhua et al Clinicopathologic features and survival of patients with colorectal mucinous signet-ring cell or non-mucinous adenocarcinoma: experience at an institution in southern China. Chin Med J. 2012; 125:3171-4.
15. Halder SK, Bhattacharjee PK, Bhar P, Pachaury A, Biswas RR, Majhi T, et al. Epidemiological, clinico-pathological profile and management of colorectal carcinoma in a Tertiary Referral Center of Eastern India. JKIMSU. 2013; 2:45-50.
16. Missaouia N, Jaidaine L, Abdelkader AB,Beizig N, Anjorin A, Yaa.coubi MT, et al. Clinicopathological patterns of colorectal cancer in Tunisia. Asian Pacific J Cancer Prev. 2010; 11:1719-22.
17. Aljebreen AM. Clinico-pathological patterns of colorectal cancer in Saudi Arabia: younger with an advanced stage presentation. Saudi J Gastroenterol 2007; 13:84-7.
18. Fazeli MS, Adel MG, Lebaschi AH. Colorectal carcinoma: a retrospective, descriptive study of age, gender, subsite, stage, and differentiation in Iran from 1995 to 2001 as observed in Tehran University. Dis Colon Rectum. 2007; 50: 990-5.
19. Triest B., Pinedo H., Blaauwgeers J., et al.: Prognostic Role of Thymidylate Synthesis, Thymidin Phosphorylas/Platlet-drived Endothelial Cell Growth Factor, and proliferation markers in CRC. Clin. Cancer Res., 2000; 6: 1063-1072.
20. Sharifi N., Ghaffarzadegan K., Ayatollahi H., et al.: Evaluation of angiogenesis in colorectal carcinoma by CD34 IHC and its correlation with clinicopathologic parameters. Acta. Medica Iranica., 2009; 47: 161-1644.
21. Mckay J., Murray S., Curran V., Ross C., et al.: Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumors and lymph node metastases. Eur. J. Cancer, 2002;38: 2258-2264.
22. Valera V, Yokoyama N, Walter B, Okamoto H, Suda T, Hatakeyama K. Clinical significance of Ki-67 proliferation index in disease progression and prognosis of patients with resected colorectal carcinoma. Br J Surg. 2005; 92: 1002-1027.
23. Koenders P.G., Peters W.H., Wobbes T., Beex L.V., Nagengast F.M. And Benraad T.J.: Epidermal growth factor receptor levels are lower in carcinomatous than in normal colorectal tissue. Br. J. Cancer, 1992;65: 189- 192.