Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i3.5121

HER2/NEU PROTEIN OVEREXPRESSION BY IMMUNOHISTOCHEMICAL ANALYSIS AND PROGNOSIS IN COLORECTAL CANCER CASES

Dr. Laxmidhara Padhy[1], Dr. Satyajeet Ray[2], Dr. Sujata Singh[3], Dr. Aparajita Mishra[4*]

[1]Associate Professor, Department of General Surgery, Government Medical College and Hospital, Sundargarh, Odisha, India.
[2]Associate Professor, Department of Orthopaedics, Government Medical College and Hospital, Sundargarh, Odisha, India.
[3]Professor, Department of Obstetrics and Gynaecology, Government Medical College and Hospital, Sundargarh, Odisha, India.
[4*]Associate Professor, Department of Pathology, Government Medical College and Hospital, Sundargarh, Odisha, India.

***Corresponding Author:** Dr. Aparajita Mishra
*Associate Professor, Department of Pathology, Government Medical College and Hospital, Sundargarh, Odisha, India.

Abstract

Background: HER2/neu is a member of the human epidermal growth factor receptors which includes HER1, HER3, and HER4, all of them are involved in the complex regulation of cell growth, proliferation and survival. HER2 is also known as proto-oncogene Neu. HER2 activation initiates signal cascades including the MAPK and P13K/AKT {3-kinase} pathways that are essential for cell proliferation and differentiation. In cancer cells dysregulation of these pathways and increased expression of HER2/neu promotes tumour cell growth and as a factor of poor prognosis. AIM: To determine overexpression of HER2/NEU protein by immunohistochemistry analysis in colorectal carcinoma cases.

Methods: A prospective study of 47 cases was carried out in the department of pathology in collaboration with department of surgery after consent of all cases. Clinical data like patient age, sex and other relevant details were noted from the pathological records. All cases of clinically diagnosed colorectal cancers prior to therapy were included. Cases diagnosed or suspected to be benign by clinically or colonoscopy were excluded.

Observations: Her2n/neu expression was correlated with age, gender, tumour location, lymph node status, Age more than 50 years shows 15 cases of her2 positive and three cases shows her2 negative. The correlation with age was statistically significant with p-value0.03. Seven cases were her2/neu positive and twenty-one (21) cases were her2/neu negative in females. Two (2) cases were her2/neu positive and 17 cases were her2/neu negative in males. Histopathological types of colorectal cancers were compared between the two groups and found insignificant with p-value 0.992. All the grades of the tumour significantly related to Her-2/neu expression of tumour. Death rate due to colorectal cancers were compared between Her2 positive and Her2negative groups were found insignificant with

p-value0.07 whereas two year disease free survival rate when compared found significantly related with p-value0.01. Grade-3 tumour was significantly associated with HER2/neu expression of the malignancy excluding others as confounding factors as proved by multivariate logistic regression with odds ratio1.34 and 95%CI (0.75-9.34) P-value0.007. Colorectal cancer with grade -3 growth was the only independent prognostic factor of the malignancy with irrespective of Her2/neu status. Thus Grade-3 of CRC had proved for the poor prognosis with HR 2.0, 95% CI (0.95-4.23), P=0.03.

Conclusion: HER2/neu overexpression indicates higher tumour grade, stage and prognosis of the patient while the patients undergone neoadjuvant chemo-radiotherapy were more risk in the Her2 negative groups than Her2/neu positive cases. A monoclonal antibody, Trastuzumab (marketed as Herceptin) directed against HER-2/neu has increased survival in many tumours. Two-year disease-free survival rate more in the her2/neu positive cases when compared to her2 neg patients. Hence targeted Therapy could be helpful in patients with high grade and lymph node or distant metastases. Grade-3 of CRC had proved for the poor prognosis. Because of many pitfalls in immunohistochemistry, further studies such as gene amplification studies involving large no of patients are needed to assess HER-2/neu expression in colorectal carcinomas and to develop new targeted therapy.

Keywords: Colorectal Cancer, HER2/NEU, Immunohistochemistry, Histopathology, oradiotherapy.

INTRODUCTION

Colorectal cancer includes all cancer originating from the caecum to the anus. Colorectal cancer can be divided into colon cancer, which ranges from the caecum to the sigmoid and rectal cancer that extends from recto-sigmoid to the anus.[1] CRC originates as a noncancerous growth called a polyp that grows on the inner lining of the colon or rectum and propagates slowly, over a period of 10 to 20 years.[2] About one third to one half of all individuals will develop one or more adenomas.[3] All adenomas have the potential to develop into malignancy over a time.[4] Ten percent of colorectal cancer progress to invasive cancer and third cancer prevalent in both sex.[5] Depth of invasion and lymphatic spread determines the proximal or distal extension of the tumour.[6] The likelihood that an adenoma will become more cancerous as it becomes larger.[7] Cancer originating from inner epithelium of colorectum is called adenocarcinoma and accounts for 96% of all CRCs.[8] Incidence and mortality rates are higher in males than in females.[9] Common symptoms consist of rectal bleeding, pain abdomen, altered bowel habits, and involuntary weight loss. Globally, incidence of colorectal cancer differs widely by over ten-fold, with the higher incidence rates in Australia and New Zealand, Europe and North America and the lowest rates in Africa and Asia.[10] These geographic differences appear to be attributable to difference in dietary and environmental exposures.[11] Several factors have been shown to put individuals at risk of CRC and these include increasing age, Polyp transformation, inflammatory bowel disease, Life style, genetic background, family and medical history. Other factors such as Obesity, physical inactivity, poor diet, smoking and alcohol consumption account for all colorectal cancer cases.[12] Genetic susceptibility is associated with familial adenomatous polyposis, Lynch Syndromes (hereditary non -polyposis colorectal cancer) which accounts for ten percent of all colorectal cancer cases.[13] Colorectal cancers develop locally and spreads by layers proximally and distally.[14] Metastasis to lymph nodes and distal organs primarily to liver are the characteristics of the tumour.[15] Colorectal cancer arises due to uncontrolled cell growth in rectum or colon, both part of large intestine.[16] Sporadic mutation of specific oncogenes and tumour suppressor genes cause progression of malignant transformation.[17] It involves either sporadically (85%}, or as a part of a hereditary cancer syndrome (<10%) or in the background of inflammatory bowel disease.[18] Accumulation of molecular alteration, including the mutation in K-RAS, P53, APC, contribution colorectal carcinogenesis.[19] Immunohistochemistry (IHC) is a method for localising specific antigens in tissues or cells placed on antigen-antibody recognition. HER2/neu is a member of the human epidermal growth factor receptors which includes HER1, HER3, and HER4, all of them are involved in the complex regulation of cell growth, proliferation and survival. HER2 is also known

as proto-oncogene Neu.[20] HER2 activation initiates signal cascades including the MAPK and P13K/AKT {3-kinase} pathways that are essential for cell proliferation and differentiation. In cancer cells dysregulation of these pathways and increased expression of HER2/neu promotes tumour cell growth and as a factor of poor prognosis.[21]

AIM

To determine overexpression of HER2/NEU protein by immunohistochemistry analysis in colorectal carcinoma cases.

MATERIALS AND METHODS

The study was conducted after approval from institutional ethical committee. A prospective study of 47 cases was carried out in the department of pathology in collaboration with department of surgery after consent of all cases. Clinical data like patient age, sex and other relevant details were noted from the pathological records. All cases of clinically diagnosed colorectal cancers prior to therapy were included. Cases diagnosed or suspected to be benign by clinically or colonoscopy were excluded. Gradation and staging of each case was done and confirmed by radiologically and cytologically as proved for invasion and distant metastasis. After obtaining approval from hospital ethical committee, the resected specimens for biopsy received in the department of pathology were taken for the study, after obtaining the proper informed written consent in each case, the following details were noted down.1-Serial number,2-name of the patient,3-Age of the patient,4-Sex,5-Registration number,6-Clinical history,7-investigation findings,8-Clinical and imaging diagnosis,9-treatment,10-survival status. This was followed by the histopathological examination of the cases after optimal processing. Clinical conditions of the patients with different therapeutic interventions were followed for 2years and the outcomes and events were noted.

Evaluation of HER2/neu was done paraffin blocks which were chosen for performing IHC. Expression of the marker was studied in each case, Panth Situ ready to use monoclonal antibody. IHC marker used in this study, brown membrane staining is considered as positive while absence of staining is considered as negative for Her2/neu. IHC stain in all cases were evaluated independently by two expert pathologists and in case of any discrepancy the slides were subjected concurrent review under penta-head microscope by both of them.

Grading of immunohistochemical staining for Her2/NEU Expression was done as mentioned in the Table- 1.

Scores	Staining Pattern	Interpretation
0	No staining at all or very slight partial membrane Staining in less than 10% of tumour cells.	Negative
1+	Faint barely perceptible membrane staining in more than 10% of tumour cells. Cells stained in only part of membrane (segmental)	Negative
2+	Weak to moderate complete membrane staining observed in more than 10% of tumour cells (circumferential, basolateral, or lateral).	Equivocal
3+	Strong complete membrane staining in more than 30% of tumour cells (circumferential, basolateral, or lateral)	Positive

Table 1: Scores of immunohistochemical staining to be positive Her2/NEU Expression

Statistical Analysis

Continuous variables measured with numerical data and categorical variables were measured with percentage. Two groups with Her2 positive and her2 negative were correlated after detection of type of data distribution. The significant correlation of the groups with different variables were noted by independent t test and chi square test with p value 0.05 or less. The risk ratio for CRC deaths between the two groups were calculated. The factors of manifestation of the disease(outcomes) and their

association with over expression of Her2/neu were analysed by multivariate logistic regression method. The independent prognostic factor for colorectal cancer and its association with HER2/Neu expression was determined by cox regression. The overall statistical analysis was done with the help of SPSS 22.

OBSERVATIONS

Clinic-Demographic Characters	Total no (47)	Her2/Neu (positive) (31) 65%	Her2/neu (negative) (16) (35%)	p-value
Age <30 y	9%	1	3	0.030
31-50 yrs	53%	15	10	0.030
>50 yrs	38%	15	3	0.030
Male	44%	2	17	0.646
Female	56%	7	21	0.646
Right colon	25%	2	10	0.791
Left colon	23,4%	2	9	0.791
Rectum	36.1%	1	16	0.791
Rectosigmoid	6.38%	0	3	0.791
lymph node involvement	31.91%	9	6	0.601
Intestinal obstruction	51%	14(29.78%)	10((21.27%	0.35
Metastatic	34.04%	1	15	0.601
Could not be assessed	25.5%	2	10	0.601
<5cm	55.31%	7	23	0.715
>5cm	36.17%	2	15	0.715
Neoadjuvant chemoradiotherapy	68%	12(25.53%)	26(55.31%)	0.001
Adjuvant chemoradiotherapy	100%	31(65.95%)	16((34%)	0.002

Table 2: Clinic-demographic characteristics of colorectal carcinoma for Her/Neu expression

Her2/neu expression was correlated with age, gender, tumour location, lymph node status, Age more than 50 years shows 15 cases of her2 positive and three cases shows her2 negative. The correlation with age was statistically significant with p-value0.03. Seven cases were her2/neu positive and twenty-one (21) cases were her2/neu negative in females. Two (2) cases were her2/neu positive and 17 cases were her2/neu negative in males. This was statistically insignificant (p value-0.646) when assessed by chi-square test.

Her 2 neu expression was correlated with tumour location of which 28% was in right hemi colon. 26% was left hemi colon, 40% in rectum and 7% in recto-sigmoid. The relationship between tumour location and her2 neu expression was statistically insignificant (P value – 0.791) when assessed by chi-square test.68% of patients undergone neoadjuvant chemoradiotherapy and was significant difference between two groups. All patients had undergone adjuvant chemoradiotherapy irrespective of HER2/neu status.

	Her2/neu(positive)	Her/neu(negative)	P- Value
Well differentiated adenocarcinoma	2	12	0.992
Moderately differentiated adenocarcinoma	3	18	0.992
Poorly differentiated adenocarcinoma	0	1	0.992
Mucinous adenocarcinoma	0	5	0.992
Signet adenocarcinoma	0	1	0.992
Invasive squamous cell carcinoma	0	1	0.992
GRADE 1	9%	13%	0.040
GRADE 2	17%	32%	0.040
GRADE 3	21.27%	7.8%	0.040
2yr disease free survival	10(21.27)	2(4.25%)	0.01
CRCs Deaths	21(44.6%)	14(29.78%)	0.07

Table 3: Histopathological types and survival of colorectal cancer for HER2/Neu expression

Histopathological types of colorectal cancers were compared between the two groups and found insignificant with p-value 0.992. All the grades of the tumour significantly related to Her-2/neu expression of tumour. Death rate due to colorectal cancers were compared between Her2 positive and Her2negative groups were found insignificant with p-value0.07 But 2 yr disease free survival rate more in the her2/neu positive cases as compared found significantly related with p-value 0.01.

	2Yr. disease free survival	Died of colorectal cancer	Her2/neu positive	Her-2/neu negatives	RR For CRC Deaths	95% CI	P-Value
Survival status	12	35	21	14	0.77	(0.57-1.05)	0.05
Age <30 Y	2	2	1	1	1.0	(0.14-7.09)	0.5
31-50 Y	7	20	9	11	0.97	(0.54-1.8)	0.47
>50 Y	5	13	11	2	1.03	(0.43-2.41)	0.47
GRADE 1	4	7	4	3	2.80	(0.80-9.79)	0.05
GRADE 2	8	15	8	7	1.90	(1.0-3.43)	0.01
GRADE 3	1	13	9	4	1.12	(0.693-1.826)	0.04
Neoadjuvant chemoradiotherapy	15	17	10	7	3.24	(1.56-6.12)	0.0005
Adjuvant chemoradiotherapy	25	22	16	6	1.37	(0.67-2.82)	0.19

Table 4: Risk ratio for colorectal cancer deaths between Her2+ and Her 2- cases

Colorectal cancer deaths with Her2/neu over expression as cases whereas patients without Her2/neu expression were classified as control group for analysing risk factors. The factors strongly related for colorectal cancer deaths were Grade1, Grade2, Grade-3 growth and patients undergone neoadjuvant chemoradiotherapy were significant risk factors .and Cancer deaths with her2 positive cases were less risk than negative cases as RR were 0.77(0.57-1.05) and p=0.05. The patients undergone neoadjuvant chemo-radiotherapy were more risk in the Her2 negative groups than Her2/neu positive cases.

Outcomes	Odds Ratio	95% CI	significance
Grade -1	0.75	0.08-0.90	0.93
Grade-2	0.9	0.75-1.01	0.93
Grade-3	1.34	0.75-9.34	0.007
Metastases	1.08	0.9-1.2	O.24
Intestinal obstruction	2.3	0.2-23.0	0.43

Table 5: Multivariate logistic regression of prognostic factors for HER-2/neu positive

Grade-3 tumour was significantly associated with HER2/neu expression of the malignancy excluding others as confounding factors as proved by multivariate logistic regression with odds ratio 1.34 and 95% CI(0.75-9.34) P-value 0.007 as in (Tab-5).

Variables	HR	95% CI	P-Value	Prognosis
Her2/neu positive	0.72	(0.359-1.476)	0.35	Non- significant
Neo adjuvant chemoradiotherapy	1.00	(0.447-2.247)	0.99	Non- significant
Grade-1	0.58	(0.258-1.212)	0.20	NS
Grade-2	0.84	(0.437-1.649)	0.63	NS
Grade-3	2.00	(0.954-4.232)	0.03	Significant

Table 6: Cox regression for independent prognostic factor for poor prognosis of colorectal cancers

Colorectal cancer with grade -3 growth was the only independent prognostic factor of the malignancy with irrespective of Her2/neu status. Thus Grade-3 of CRC had proved for the poor prognosis with HR 2.0, 95% CI (0.95-4.23), P=0.03.

DISCUSSION

Immunohistochemistry is relatively inexpensive, widely available, easy to preserve and less time consuming and it requires a routine microscope. Her-2/neu is a useful marker, to predict the outcome of colorectal cancers such as lymph node involvement, Metastases, intestinal obstruction, Bleeding and the prognosis. Its overexpression correlates with poor prognosis. It is used to predict the patient response to adjuvant chemotherapy and endocrine therapy and select patients for immunotherapy with a targeted monoclonal antibody therapy. The patients who overexpress Her-2/neu should respond to trastuzumab therapy, independent of tissue origin of the cancer.A study by Stahler A et al biomarker expression was correlated with the outcome of patients. NRG1 (low: 192 vs. high: 16), HER2/neu (low: 201 vs. high: 7) and HER3 (low: 69 vs. high: 139) expressions were assessed in 208 patients. High versus low NRG1 expression significantly affected progression-free survival (PFS) [4.7 vs. 8.2 months, hazard ratio (HR): 2.45; 95% confidence interval (CI): 1.45-4.13; P=0.001], but not overall survival (OS) (15.5 vs. 20.7 months, HR: 1.33; 95% CI: 0.76-2.35; P=0.32). High versus low HER3 expression (PFS: 7.1 vs. 8.8 months, HR: 1.11; 95% CI: 0.82-1.50; P=0.50; OS: 19.8 vs. 21.1 months, HR: 0.95; 95% CI: 0.70-1.30; P=0.75) and high compared with low HER2/neu expression (PFS: 7.7 vs. 8.0 months, HR: 1.07; 95% CI: 0.71-1.60; P=0.75; OS: 16.6 vs. 21.1 months, HR: 1.13; 95% CI: 0.75-1.71; P=0.57) did not influence outcome.[22] Paired primary tumours and lymph node metastases from 79 patients with colorectal cancer were retrospectively collected and analyzed for EGFR, HER2, and HER3 expression. High EGFR, HER2, and HER3 expression (2+ and 3+) was found in 64.2%, 66.0%, and 85.0% of primary tumors, and 56.8%, 46.0%, and 76.0% of lymph node metastases, respectively. Correlation rates between primary and metastatic lesions were 67.1%, 63.3%, and 74.7% for EGFR, HER2, and HER3, respectively. Stage IV tumors (with distant metastasis) had higher correlation rates of HER2 expression compared to stage III tumors (without distant metastasis) (P = 0.050). Moderate correlation rates in EGFR, HER2, and HER3 expression were observed between primary and metastatic lesions of colorectal cancer. Tumour stage or existence of distant metastasis could serve as potential predictive markers for the correlation of HER2 expression between primary tumours and lymph node metastases of colorectal cancer as studied by Ye P et al.[23] From the total of 95 cases, 75 (78.9 %) cases showed Her-2/neu expression. Pattern of Her-2/neu staining was significantly associated with the grade of colorectal cancer depicting cytoplasmic Her-2/neu expression higher in low grade (50 %) while membranous Her-2/neu expression more in high grade colorectal cancer (45 %) (P-value = 0.030). Pattern of Her-2/neu staining was also significantly associated with the type of colorectal cancer representing membranous Her-2/neu expression to be more common in mucinous type (38.5 %) while cytoplasmic Her-2/neu expression to be more frequent in non-mucinous type (42.7 %) of colorectal cancer (p-value = 0.024) as observed by Shabbir A et al.[24] HER2/neu status was unrelated to age, sex, location and positive-nodal percentage. Intramucosal carcinomas had earlier HER2/neu protein upregulation than regional stromal invasion within Dukes' A CRCs as studied by Li JW et al.[25]

Our study included 47 patients of colorectal adenocarcinoma. Mean age was 53 years with range 17-76 years among them 44%were male patients and 56% were female patients (Table-2].

In our study, histological types of colorectal carcinoma, 84% cases were adenocarcinoma, 12% were mucinous adenocarcinoma and signet ring for 4% of cases. Majority of cases were grade 2 tumours (49%) followed by grade 1 tumours (33%). Regarding the immunohistochemical staining for her2 neu, in our study 88% cases were HER2 Neu positive. In our study, no statistically significant relationship was detected between HER2 expression and histological types. No statistically significant relationship was detected between Her2/neu expression and histological types. Simillarly there was no significant relationship between tumour site and HER2/neu expression. HER2 over expression was not associated with tumour size, sex of patients but with patient age. In our study out of 47 cases, 40% of cases were located in the rectum, 28% in the right hemi colon, 26% in the left hemi colon. Tumours of right colon outnumber those of left colon. There was no significant relationship between lymph node status and Her2 expression in our study.

CONCLUSION

HER2/neu overexpression indicates higher tumour grade, stage and prognosis of the patient while The patients undergone neoadjuvant chemo-radiotherapy were more risk in the Her2 negative groups than Her2/neu positive cases.A monoclonal antibody, Trastuzumab (marketed as Herceptin) directed against HER-2/neu has increased survival in many tumours. Two year disease free survival rate more in the her2/neu positive cases when compared to her2 neg patients Colorectal carcinomas are one among the most common human malignancies. Most of the tumours are diagnosed, classified and graded with H&E stained sections. Various immunological markers are expressed in colorectal malignancies and these are studied to identify targeted therapy and to increase the survival of patients. Hence targeted Therapy could be helpful in patients with high grade and lymph node or distant metastases. Because of many pitfalls in immunohistochemistry, further studies such as gene amplification studies involving large no of patients are needed to assess HER-2/neu expression in colorectal carcinomas and to develop new targeted therapy.

REFERENCES

1. Winawer SJ, Zauber AG. The advanced adenoma as the primary target of screening. Gastrointest Endosc Clin N Am 2002;12:1-9.
2. Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL. Natural history of untreated colonic polyps. Gastroenterology 1987;93:1009-13.
3. Andrea M, Jepsen RK, Klein MF, Gögenur I, Kuhlmann TP. Colorectal serrated lesions and polyps in the Danish population: A large nationwide register-based cohort study. Endosc Int Open 2023;11(12):E1116-22.
4. Myint A, Corona E, Yang L, Nguyen BS, Lin C, Huang MZ, et al. Gastroenterology visitation and reminders predict surveillance uptake for patients with adenomas with high-risk features. Sci Rep 2021;11(1):8764.
5. Abdelrahman DI, Elhasadi I, Anbaig A, Bakry A, Mandour D, Wasefy T, et al. Immunohistochemical Expression of Immune Checkpoints; CTLA-4, LAG3, and TIM-3 in Cancer Cells and Tumor-infiltrating Lymphocytes (TILs) in Colorectal Carcinoma. Appl Immunohistochem Mol Morphol. 2023 Dec 18. doi: 10.1097/PAI.0000000000001181. Epub ahead of print. PMID: 38108390.
6. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural invasion is a strong prognostic factor in colorectal cancer: a systematic review. Am J Surg Pathol 2016;40(1):103-12.
7. Good NM, Suresh K, Young GP, Lockett TJ, Macrae FA, Taylor JM. A prediction model for colon cancer surveillance data. Stat Med 2015;34(18):2662-75.
8. Qin ZF, Xu GH, Zhou SQ, Zheng PW, Zhu YP, Ju HX, Li DC, Ma DN. [Analysis of clinicopathological features and prognosis of sporadic synchronous multiple primary colorectal cancers]. Zhonghua Wei Chang Wai Ke Za Zhi. 2023;26(12):1171-8.
9. Zhai G, Wang Y. Disease burden of colorectal cancer in China from 1990 to 2019: Age and sex-specific time trends and 10-year forecast. Oncol Res Treat. 2023 Dec 11. doi: 10.1159/000535664. Epub ahead of print. PMID: 38081158.
10. Waldenstedt S, Haglind E, Angenete E. Symptoms and diagnosis of local recurrence after rectal cancer treatment. Acta Oncol 2022;61(9):1043-1049.
11. Barot S, Rantanen P, Nordenvall C, Lindforss U, Hallqvist Everhov Å, Larsson SC, Lindblom A, Liljegren A. Combined associations of a healthy lifestyle and body mass index with colorectal cancer recurrence and survival: a cohort study. Cancer Causes Control. 2023 Oct 2. doi: 10.1007/s10552-023-01802-y. Epub ahead of print. PMID: 37782382.
12. Corrêa Lima MP, Gomes-da-Silva MH. Colorectal cancer: lifestyle and dietary factors. Nutr Hosp 2005;20(4):235-41.
13. Semenova AB, Byakhova MM, Makarova MV, Galkin VN, Nemtsova MV, Chernevskiy DK, et al. Struktura patogennykh germinal'nykh variantov pri kolorektal'nom rake v vyborke patsientov Moskvy [The structure of pathogenic germline variants in colorectal cancer in Moscow patients]. Arkh Patol 2023;85(6):16-25.

14. Prade M, Bognel C, Duvillard P, Charpentier P, Lasser P. Classification histologique d'extension des cancers du colon et du rectum [Histological classification of the spread of colorectal cancers]. Bull Cancer 1982;69(5):483-4.
15. Abdelrahman DI, Elhasadi I, Anbaig A, Bakry A, Mandour D, Wasefy T, et al. Immunohistochemical Expression of Immune Checkpoints; CTLA-4, LAG3, and TIM-3 in Cancer Cells and Tumor-infiltrating Lymphocytes (TILs) in Colorectal Carcinoma. Appl Immunohistochem Mol Morphol. 2023 Dec 18. doi: 10.1097/PAI.0000000000001181. Epub ahead of print. PMID: 38108390.
16. Mzoughi S, Schwarz M, Wang X, Demircioglu D, Ulukaya G, Mohammed K, et al. A Mutation-driven oncofetal regression fuels phenotypic plasticity in colorectal cancer. bioRxiv [Preprint]. 2023 Dec 10:2023.12.10.570854. doi: 10.1101/2023.12.10.570854. PMID: 38106050; PMCID: PMC10723414.
17. Johnstone MS, McSorley ST, McMillan DC, Horgan PG, Mansouri D. The relationship between systemic inflammatory response, screen detection and outcome in colorectal cancer. Colorectal Dis. 2023 Dec 14. doi: 10.1111/codi.16824. Epub ahead of print. PMID: 38095280.
18. Srivastava P, Mishra S, Shukla S, Sharma P, Husain N. Concomitant Non-V600E BRAF and KRAS Mutations in Colorectal Carcinoma by Next-Generation Sequencing: A Distinct Subtype. Int J Surg Pathol. 2023 Dec 12:10668969231215425. doi: 10.1177/10668969231215425. Epub ahead of print. PMID: 38086758.
19. Zheng-Lin B, Graham RP, Bekaii-Saab TS. Targeting ERBB2/HER2 genetic alterations: an expanding therapeutic opportunity in gastrointestinal cancers. Chin Clin Oncol. 2023 Oct;12(5):55. doi: 10.21037/cco-23-72. PMID: 37964543.
20. Kılıçarslan A, Dogan HT, Süngü N, Dogan M, Yalcin A, Dede DŞ. Association between Her2/neu status in colorectal carcinoma and clinicopathological features: a retrospective study using whole - tissue sections. Pol J Pathol. 2018;69(2):143-149.
21. Miura A, Yamada D, Nakamura M, Tomida S, Shimizu D, Jiang Y, et al. Oncogenic potential of human pluripotent stem cell-derived lung organoids with HER2 overexpression. Int J Cancer 2021;149(8):1593-1604.
22. Stahler A, Heinemann V, Neumann J, Crispin A, Schalhorn A, Stintzing S, et al. Prevalence and influence on outcome of HER2/neu, HER3 and NRG1 expression in patients with metastatic colorectal cancer. Anticancer Drugs 2017;28(7):717-22.
23. Ye P, Li F, Wei Y, Zhang Y, Cui J, Dai R, Chen H, Xie J, Cai P. EGFR, HER2, and HER3 protein expression in paired primary tumor and lymph node metastasis of colorectal cancer. Sci Rep 2022;12(1):12894.
24. Shabbir A, Mirza T, Khalid AB, Qureshi MA, Asim SA. Frequency of Her2/neu expression in colorectal adenocarcinoma: a study from developing South Asian Country. BMC Cancer 2016;16(1):855.
25. Li JW, Chuang TC, Yang AH, Hsu CK, Kao MC. Clinicopathological relevance of HER2/neu and a related gene-protein cubic regression correlation in colorectal adenocarcinomas in Taiwan. Int J Oncol 2005;26(4):933-43.