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CORRELATIONAL STUDY OF BIOCHEMICAL MARKERS, IMMUNOHISTOCHEMISTRY AND RADIOLOGICAL IMAGING WITH STAGE AT PRESENTATION AND PROGNOSIS IN COLORECTAL CARCINOMA

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ABSTRACT

Background: The evaluation of specific biomarkers can offer valuable insights into the prognosis and treatment outcomes for colorectal carcinoma (CRC). This study investigates the correlation between carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) levels with CRC stage at presentation and clinical outcomes, alongside fecal occult blood test (FOBT) variations and tumor response rates over time.

Materials and Methods: A cohort of 38 CRC patients was prospectively followed. Biomarker levels (CEA, CA 19-9) were measured at baseline, three months, six months, and one-year post-treatment. Tumor response was assessed using RECIST criteria, and survival outcomes were analyzed using Kaplan-Meier survival analysis. FOBT positivity rates were also tracked to examine associations with disease progression and response to therapy.

Results: Most participants (63.2%) were male, with a median age of 61-70 years. CEA levels >5 ng/mL at baseline were significantly associated with advanced disease stage and poorer outcomes (P = 0.002), aligning with findings in prior literature. CA 19-9 levels were notably elevated in metastatic cases at six months but showed inconsistent correlation with survival outcomes. Complete tumor response rates increased significantly from 31.6% at three months to 47.4% at one year. Patients with elevated baseline CEA levels (>100 ng/mL) demonstrated a lower mean survival of 10.4 months compared to 12.0 months in those with ≤5 ng/mL (P = 0.0014).

Conclusion: CEA serves as a reliable prognostic marker for CRC progression and survival, while CA 19-9 may have value in combination with other indicators. Elevated FOBT rates at different time points correlated with changes in tumor burden, suggesting its utility in monitoring disease response. These findings support the integration of biomarker analysis in routine CRC prognosis and treatment monitoring.

Keywords: Colorectal carcinoma, carcinoembryonic antigen, carbohydrate antigen 19-9, fecal occult blood test, survival analysis.

INTRODUCTION

Colorectal carcinoma (CRC) remains a significant global health concern, ranking as the third most common cancer in males and the second in females.[1] By 2020, the global burden of CRC rose to approximately 1.9 million new cases and 935,000 deaths annually. High-incidence regions include Australia, New Zealand, Europe, and North

America, while lower rates are seen in regions like Africa and South-Central Asia.[2] CRC cases are generally higher in males compared to females, and incidence rates tend to rise with increasing levels of human development. In fact, countries with higher human development indices (HDI) experience incidence rates up to six times greater than low-HDI countries.[3]

In India, CRC is on the rise, although it remains less common compared to Western countries. Rapid urbanization, dietary shifts toward processed foods, and reduced physical activity contribute to this trend.[4] In 2020, India recorded about 65,000 new CRC cases. Despite relatively lower incidence, the mortality rate in India is concerning due to late-stage diagnosis and limited access to screening and treatment.[5] The increasing burden underscores the need for more robust screening programs and early detection strategies, given that survival rates are closely linked to the stage at diagnosis.[5]

Biomarkers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are commonly utilized in clinical practice.[6] While these markers are more specific for monitoring disease recurrence or metastasis—particularly in the liver—they lack sensitivity for detecting early-stage CRC, limiting their use as screening tools.[6] Elevated CEA levels are most indicative of tumor burden and are especially useful for detecting hepatic or retroperitoneal metastases. Postoperative rises in CEA levels can signal disease recurrence, making it an important marker for follow-up.[7]

Surgery is the cornerstone of treatment for localized CRC, often followed by adjuvant chemotherapy to minimize recurrence risk. Chemotherapeutic regimens like FOLFOX and FOLFIRI, which combine fluoropyrimidines with oxaliplatin or irinotecan, have significantly improved survival in metastatic cases, with median survival now approaching 30 months.[8] Despite these advances, survival beyond this point is uncommon after failure of first-line therapies, underscoring the need for better prognostic indicators and treatment strategies.[9]

Emerging research has highlighted the role of molecular markers such as KRAS, NRAS, BRAF, and PIK3CA mutations, along with immunohistochemical markers like TS, P21, and PTEN, in predicting treatment responses and disease outcomes.[10] These markers, coupled with radiological imaging techniques like computed tomography (CT) and magnetic resonance imaging (MRI), provide critical insights into tumor staging and guide therapeutic decision-making.[11] Early-stage diagnosis remains challenging due to the asymptomatic nature of CRC, emphasizing the need for reliable biomarkers and imaging techniques that can aid in early detection.[11]

The gastrointestinal microbiota has also been implicated in CRC pathogenesis, alongside established risk factors like poor diet, smoking, physical inactivity, and gastrointestinal disorders such as inflammatory bowel disease. Understanding these factors and their interactions with CRC progression is essential for improving preventive and therapeutic strategies.[12]

This study aims to correlate biochemical markers, immunohistochemistry, and radiological imaging findings with the stage at presentation and prognosis of CRC. Considering the rising trend in countries like India, understanding and correlating biochemical markers and imaging findings with disease stage and prognosis is increasingly important for improving outcomes and tailoring treatments.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted under the department of General Surgery at Northern Railway Central Hospital, New Delhi after obtaining the ethical approval. The study duration spanned 18 months, from January 2021 to June 2022, focusing on histopathologically proven cases of colorectal carcinoma (CRC) to investigate the correlation between biochemical markers, immunohistochemistry, radiological imaging, and stage at presentation, along with prognosis.

Study Population and Sample Size

The study included all patients with histopathologically confirmed CRC who presented at Northern Railway Central Hospital during the study period. Based on sample size calculations for detecting a moderate correlation ($r = 0.50$) between biomarkers and radiological imaging with disease stage, a minimum of 38 patients was required for 90% power to establish statistical significance at the 0.05 level. The inclusion criteria were all stages of CRC, while patients with a history of other malignancies were excluded.

Laboratory Investigations and Imaging

Routine blood work included complete blood counts, renal function tests, and random blood sugar, along with diagnostic investigations such as fecal occult blood testing (FOBT) and colonoscopy. For imaging, CT scans, MRI, and PET-CT were used in selected cases. Specific laboratory investigations were conducted for all patients. Serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were evaluated pre-operatively using chemiluminescence immunoassays (Elecsys 2010 Immunoassay Analyzer, Roche Diagnostics, Risch-Rotkreuz, Switzerland). Fasting venous blood (4 mL) was collected on the second day following admission, centrifuged within 1 hour, and serum not analyzed within 6 hours was stored at −20°C. Normal CEA levels are below 2.5 ng/mL, borderline at 2.5–5.0 ng/mL, and elevated above 5.0 ng/mL. For CA19-9, the upper limit for healthy adults is 37 U/mL [13].

Data Collection and Treatment

Data were collected through comprehensive clinical histories, physical examinations, and diagnostic procedures. A case record proforma captured relevant patient information, including clinical features, stage at presentation, results from laboratory investigations, and surgical procedures. Patients were evaluated for surgical fitness pre-operatively. Prior to each procedure, informed consent was obtained in the local language, explaining the illness, treatment options, potential risks (including morbidity and mortality), benefits, and the need for surgery and follow-up, including radiotherapy if required. After evaluation of the tumour by clinical examination and specific investigations, a surgical plan was formulated. The final decision of type of surgery was taken depending on the location of the tumour. The resected specimen was sent for Histopathological Examination (HPE) and staging was done. Appropriate antibiotics and analgesics were administered post operatively for all cases. Patients with malignancies in higher stage are subjected to adjuvant chemotherapy. Information was recorded systematically for subsequent analysis.

Follow-up

Patients were followed up postoperatively for a minimum of 6 months. Physical examinations, serum CEA and CA19-9 levels, and stool occult blood tests were performed at regular intervals. Colonoscopy was repeated one year after surgery, and flexible sigmoidoscopy was conducted every six months. Abdominal and chest CT scans were performed annually for stage 2 and stage 3 patients, and whenever postoperative CEA levels increased. In selected cases, PET-CT scans were conducted after one year and subsequently as needed. Responses to cancer treatment for the target and non-target lesions were assessed using the RECIST (Response Evaluation Criteria In Solid Tumors) guidelines.[14]

Data Analysis

Data were analyzed using SPSS version 17.0 and MedCalc software. Descriptive statistics were used to summarize continuous variables (mean ± SD) and categorical variables (frequencies and percentages). For correlation analysis between biomarkers (CEA and CA19-9), radiological imaging, and CRC stage at presentation, Spearman and Pearson correlation tests were applied. Kaplan-Meier survival curves were generated for survival analysis, and the Chi-square or Fisher's exact test was employed for categorical data analysis. A p-value < 0.05 was considered statistically significant.

RESULTS

In this study, a total of 38 patients were analyzed, with a mean age of 58.5 ± 12.2 years. The majority of patients (42.1%) were aged between 61-70 years, followed by those aged 41-50 years and 51-60 years (18.4% each). Males comprised 63.2% of the cohort, while females accounted for 36.8%. The most common primary tumor sites were the ascending colon (21.2%) and recto sigmoid (21.2%), with other locations including the rectum (18.4%) and transverse colon (13.1%). Histologically, adenocarcinoma was predominant, present in 92.1% of cases. Regarding treatment modalities, palliative chemotherapy was the most frequently administered (44.7%), followed by surgery combined with adjuvant chemotherapy (39.5%) and neoadjuvant chemotherapy (10.5%). [Table 1]

Fecal occult blood testing results revealed a significant increase in the detection of blood over time. At presentation, 50.0% of patients showed positive results, which increased to 84.2% at three months. However, at six months, the positive rate decreased to 71.1%, with a small rise in tests not done (2.6%). By one year, the percentage of positive tests was 63.2%, with 13.2% of tests not performed. [Table 2]

Biomarker levels were assessed at various time points for both metastatic and non-metastatic patients. At presentation, the overall mean CEA level was 45.0 ± 87.6 ng/mL, with non-metastatic patients showing significantly lower levels (5.4 ± 5.7) compared to metastatic patients (89.1 ± 112.9). After three months, overall CEA levels rose to 53.8 ± 106.1, with a notable increase in metastatic cases (110.3 ± 134.4). By six months, the overall mean was 78.2 ± 144.2 ng/mL, again reflecting higher levels in metastatic patients (163.4 ± 180.0). At one year, CEA levels decreased to 46.7 ± 94.2, with metastatic patients still higher at 106.9 ± 128.9. The P values for overall and metastatic groups were not statistically significant (0.533 and 0.124, respectively). In contrast, CA 19-9 levels showed an overall mean of 10.6 ± 19.9 U/mL at presentation, with metastatic patients at 18.7 ± 26.8 U/mL. Significant increases were observed at three months (14.5 ± 18.7) and six months (21.7 ± 38.1), particularly in the metastatic group (43.0 ± 48.6), with a P value of 0.012 indicating statistical significance for metastatic cases. [Table 3]

At six months, the tumor response based on RECIST criteria revealed that 31.6% of patients achieved a complete response, while stable disease was observed in 2.6% of cases. Progression of disease was noted in 44.8% of patients. At one year, the complete response rate increased to 47.4%, with no stable disease cases reported. Mortality rose from 5.3% at six months to 13.2% at one year, and partial response rates increased from 5.3% to 10.6%. Notably, surgery combined with adjuvant chemotherapy was only performed in 10.5% of patients at six months, with no instances recorded at one year. [Table 4]

The analysis of biomarkers revealed a significant correlation between CEA levels and disease progression. In the progressive disease (PD) group, 93.3% had high CEA levels (>5 ng/mL), compared to only 39.1% in the non-progressive disease (NPD) group (p=0.002). Conversely, normal CEA levels

(<5 ng/mL) were found in 60.9% of NPD patients, but only in 6.7% of PD patients. Regarding CA 19-9 levels, 20.0% of the PD group had high levels (>37 U/mL), while none in the NPD group showed elevated CA 19-9 (p=0.004). Normal CA 19-9 levels (<37 U/mL) were present in 73.3% of the PD group and all NPD patients, indicating a significant distinction between the two groups. [Table 5]

The results from the Kaplan-Meier survival analysis indicate significant differences in overall survival based on CEA levels among colorectal cancer (CRC) patients over a one-year follow-up. Patients with CEA levels of 0 to 5 ng/mL had a mean survival of 12.000 months, while those with CEA levels exceeding 5 ng/mL had a mean survival of 11.529 months, and those with levels over 100 ng/mL had a mean survival of 10.400 months. The statistical analysis, evidenced by a chi-squared value of 13.1705 and a p-value of 0.0014, confirms that CEA levels are a significant predictor of overall survival in this cohort ($P < 0.05$). [Figure 1 A]

In contrast, the analysis of CA 19-9 levels did not demonstrate a significant impact on survival. Patients with CA 19-9 levels below 37 ng/mL had a mean survival of 11.639 months, while those with levels exceeding 37 ng/mL had a mean survival of 10.500 months, with a median survival of 9.000 months. The log-rank test yielded a chi-squared value of 2.8987 and a p-value of 0.0886, indicating no significant correlation between CA 19-9 levels and overall survival ($P > 0.05$). These findings suggest that while CEA levels are a reliable prognostic factor for CRC outcomes, CA 19-9 levels do not provide additional predictive value within the one-year follow-up period. [Figure 1 B]

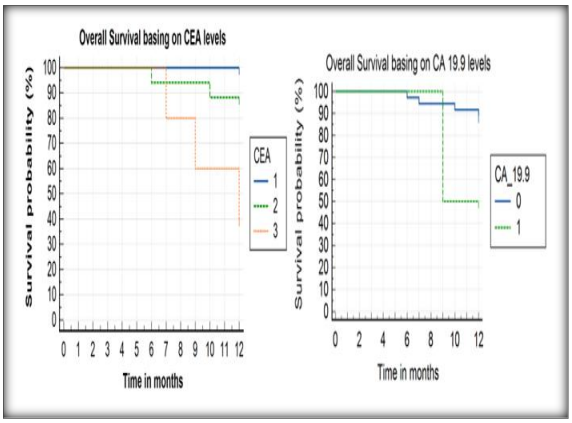

Figure 1: Kaplan Meier Survival analysis. A: Comparing Overall survival basing on CEA levels over median follow period of one year using Kaplan Meier Survival analysis. B: Comparing Overall survival basing on CA 19-9 levels over median follow period of one year using Kaplan Meier Survival analysis

Table 1: Baseline characteristics of the study participants (N=38)

Variables	Frequency	%
Age group (in years)		
≤40	3	7.9%
41-50	7	18.4%
51-60	7	18.4%
61-70	16	42.1%
>70	5	13.2%
Mean age (years)	58.5 ± 12.2	
Gender		
Female	14	36.8%
Male	24	63.2%
Primary site of tumour		
Ascending colon	8	21.2%
Caecum	3	7.8%
Descending colon	1	2.6%
Colorectal (Familial Adenomatous Polyposis)	1	2.6%
Rectum	7	18.4%
Recto sigmoid	8	21.2%
Sigmoid colon	5	13.1%
Transverse colon	5	13.1%
Histology of tumour		
Adeno Carcinoma	35	92.1%
Adeno-squamous Carcinoma	1	2.6%
Mucinous Adeno Carcinoma	2	5.3%
Type of treatment		
Neoadjuvant Chemotherapy	4	10.5%
Neoadjuvant Chemotherapy + Radiotherapy	2	5.3%
Palliative Chemotherapy	17	44.7%
Surgery + Adjuvant Chemotherapy	15	39.5%

Table 2: Follow-up Outcomes of Fecal Occult Blood Testing (FOBT) at Different Time Points (N=38)

Fecal occult blood testing	Absent	Not Done	Present
	Frequency		
At Presentation	19 (50.0%)	0 (0.0%)	19 (50.0%)
At 3 months	32 (84.2%)	0 (0.0%)	6 (15.8%)
At 6 months	27 (71.1%)	1 (2.6%)	10 (26.3%)
At 1 Year	24 (63.2%)	5 (13.2%)	9 (23.7%)

Table 3: Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 19-9 (CA 19-9) Levels Across Different Follow-Up Intervals (N=38)

Biomarkers	Overall	Non-Metastatic	Metastatic
	Mean ± SD		
CEA (ng/mL)			
At Presentation	45.0 ± 87.6	5.4 ± 5.7	89.1 ± 112.9
3 months	53.8 ± 106.1	3.0 ± 3.0	110.3 ± 134.4
6 months	78.2 ± 144.2	5.8 ± 10.9	163.4 ± 180.0
1 Year	46.7 ± 94.2	7.6 ± 18.6	106.9 ± 128.9
P value	0.533	0.361	0.124
CA 19-9 (U/mL)			
At Presentation	10.6 ± 19.9	3.3 ± 3.5	18.7 ± 26.8
3 months	14.5 ± 18.7	4.8 ± 4.3	25.3 ± 22.6
6 months	21.7 ± 38.1	3.6 ± 3.1	43.0 ± 48.6
1 Year	13.5 ± 20.5	6.8 ± 11.4	26.3 ± 26.9
P value	0.280	0.085	0.012

Table 4: Response of Tumors Based on RECIST Criteria at 6 Months and 1 Year Follow-Up (N=38)

Response of the Tumour Based on RECIST Criteria	At 6 Months	At 1 Year
	Frequency (%)	
Stable Disease	1 (2.6%)	0 (0.0%)
Complete Response	12 (31.6%)	18 (47.4%)
Mortality	2 (5.3%)	5 (13.2%)
Partial Response	2 (5.3%)	4 (10.6%)
Progression Of Disease Present	17 (44.8%)	11 (28.9%)
Surgery + Adjuvant Chemotherapy	4 (10.5%)	0 (0.0%)

Table 5: Comparison of Biomarker Levels in Patients with Progressive and Non-Progressive Disease (N=38)

Biomarkers	PD (n=15)	NPD (n=23)	P-value
	Frequency (%)		
CEA levels			
High CEA levels (>5) (n=23)	14 (93.3%)	9 (39.1%)	0.002
Normal CEA levels (<5) (n=15)	1 (6.7%)	14 (60.9%)	
CA 19-9 levels			
High CA 19-9 levels (>37 U/ml) (n=3)	3 (20.0%)	0 (0.0%)	0.004
Normal CA 19-9 levels (<37 U/ml) (n=35)	11 (73.3%)	24 (100.0%)	

PD: Progressive disease; NPD: Non-progressive disease

DISCUSSION

In this study, 38 colorectal cancer (CRC) patients were assessed for biomarker levels and their correlation with clinical outcomes, revealing important insights consistent with existing literature. The predominant demographic was males (63.2%), with the most common age group being 61-70 years. Adenocarcinoma histology, identified in 92.1% of cases, aligns with the global prevalence of this subtype in CRC patients. Numerous studies have reported similar findings.[15,16,17] For instance by Makmun et al., revealed a higher incidence of adenocarcinoma in males, particularly in older populations, supporting the demographic trends observed in this cohort.[15]

Two critical biomarkers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), were monitored longitudinally. The results showed that while CEA levels remained higher in metastatic patients compared to non-metastatic ones throughout the study, the differences were not statistically significant at every time point. However, high baseline CEA levels (>5 ng/mL) were significantly correlated with disease progression (P = 0.002), corroborating multiple studies. A study by Manojlovic et al., confirmed that elevated CEA is a strong predictor of advanced disease and poor survival outcomes.[18] Similarly, Kotzev et al., emphasized that monitoring CEA during follow-up after curative surgery improves detection of recurrent disease and provides prognostic insights.[19]

While CA 19-9 levels were only significantly elevated in metastatic patients at six months (P = 0.012), its role as a biomarker in CRC is less established. Previous studies have offered conflicting evidence.[20,21] Meira-Júnior et al., suggested that elevated CA 19-9 levels might indicate a worse prognosis in some patients, but its predictive value is less consistent compared to CEA.[3] A studies Lee et al., also noted that CA 19-9 could be useful when combined with other biomarkers but has limited standalone utility in CRC prognosis.[22]

FOBT positivity rates in this cohort fluctuated, with 50% of patients testing positive at presentation, increasing to 84.2% at three months, and decreasing to 63.2% at one year. This variation is likely reflective of changes in tumor burden and response to therapy. Kaur et al., have documented similar trends, showing that effective treatment reduces gastrointestinal bleeding, lowering FOBT positivity.[6] While FOBT is a cost-effective and widely available tool for CRC screening, it is less sensitive than more advanced diagnostic methods

like colonoscopy or fecal immunochemical testing (FIT) in detecting early-stage disease. However, FOBT remains relevant in resource-limited settings and for monitoring patients post-surgery.[23]

Tumor response, assessed using RECIST criteria, improved significantly over the course of the study, with complete response rates increasing from 31.6% at three months to 47.4% at one year. This reflects the benefits of combining surgery with adjuvant chemotherapy in CRC treatment. Findings from André et al., support these results, showing that adjuvant chemotherapy with FOLFOX (5-FU and oxaliplatin) significantly improves disease-free survival (DFS) and overall survival (OS) in stage III CRC patients.[24] The positive response rates seen in this study further echo results from Onishi et al., who found that multimodal approaches significantly reduce the risk of recurrence, particularly in patients with resected stage II and III disease.[25]

The Kaplan-Meier survival analysis in this study also showed a strong association between elevated CEA levels and poorer survival outcomes. Patients with baseline CEA levels >100 ng/mL had a mean survival of 10.4 months, compared to 12.0 months for those with CEA ≤5 ng/mL ($P = 0.0014$). These findings align with research by Song et al., which demonstrated that elevated CEA levels are associated with advanced-stage disease and worse OS [26]. In contrast, while elevated CA 19-9 was also observed in patients with metastatic disease, it was not as strongly correlated with survival outcomes ($P = 0.0886$). Other studies, such as Huang et al., similarly found that CA 19-9 might indicate advanced disease but does not consistently predict survival.[27]

Kim et al., reported that while both CEA and CA 19-9 levels increase with tumor progression, only CEA consistently correlated with survival and recurrence. This underscores the utility of CEA as a key biomarker for CRC monitoring, particularly in predicting recurrence and survival post-surgery.[28] Meanwhile, Taieb et al., showed that combined CEA and CA 19-9 analysis could improve detection of metastatic CRC, although CEA remains the primary marker for routine use in clinical practice.[29]

CONCLUSION

The findings from this study reaffirm the prognostic utility of CEA in monitoring disease progression and survival outcomes in CRC patients. The role of CA 19-9 remains less clear, although it may have value in specific contexts, such as in combination with other biomarkers. The survival benefit associated with adjuvant chemotherapy and surgery was evident, consistent with global standards of care for CRC management. Future research should continue to explore the combined use of multiple biomarkers for a more comprehensive assessment of CRC prognosis, particularly in varied patient populations across different treatment stages. Expanding cohort sizes and extending follow-up periods in future studies would provide more definitive insights into the long-term utility of these biomarkers in clinical practice.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.
2. Cho WK, Choi DH, Park HC, et al. Elevated CEA is associated with worse survival in recurrent rectal cancer. Oncotarget. 2017;8(62):105936-41.
3. Zhang LN, OuYang PY, Xiao WW et al. Elevated CA19-9 as the Most Significant Prognostic Factor in Locally Advanced Rectal Cancer Following Neoadjuvant Chemoradiotherapy. Medicine (Baltimore). 2015;94(45):e1793.
4. Meira-Júnior JD, Costa TN, Montagnini AL, Nahas SC, Jukemura J. Elevated CA 19-9 in an asymptomatic patient: what does it mean? Arq Bras Cir Dig. 2022;35:e1687.
5. Li Z, Zhu H, Pang X, et al. Preoperative serum CA19-9 should be routinely measured in the colorectal patients with preoperative normal serum CEA: a multicenter retrospective cohort study. BMC Cancer. 2022;22(1):962.
6. Kaur K, Zubair M, Adamski JJ. Fecal Occult Blood Test. Treasure Island (FL): StatPearls Publishing; 2024.
7. Chubak J, Bogart A, Fuller S, Laing SS, Green BB. Uptake and positive predictive value of fecal occult blood tests: A randomized controlled trial. Prev Med. 2013;57(5):671-8.
8. Soin S, Akanbi O, Ahmed A, et al. Use and abuse of fecal occult blood tests: a community hospital experience. BMC Gastroenterol. 2019;19(1):161.
9. Jang HJ, Cho JW, Park B, Choi HC, Kim HS, Kim JH. The assessment of tumor response by measuring the single largest lesion per organ in metastatic tumors: a pooled analysis of previously reported data. J Cancer. 2015;6(2):169-76.
10. Wu JB, Li XJ, Liu H, Liu YJ, Liu XP. Association of KRAS, NRAS, BRAF and PIK3CA gene mutations with clinicopathological features, prognosis and ring finger protein 215 expression in patients with colorectal cancer. Biomed Rep. 2023;19(6):104.
11. Hussain S, Mubeen I, Ullah N, et al. Modern Diagnostic Imaging Technique Applications and Risk Factors in the Medical Field: A Review. Biomed Res Int. 2022; 2022:5164970.
12. Adigun AO, Adebile TM, Okoye C, et al. Causes and Prevention of Early-Onset Colorectal Cancer. Cureus. 2023;15(9):e45095.
13. Laraib U, Sargazi S, Rahdar A, Khatami M, Pandey S. Nanotechnology-based approaches for effective detection of tumor markers: A comprehensive state-of-the-art review. Int J Biol Macromol. 2022; 195:356-83.
14. Nishino M, Jackman DM, Hatabu H, et al. New Response Evaluation Criteria in Solid Tumors (RECIST) guidelines for advanced non-small cell lung cancer: comparison with original RECIST and impact on assessment of tumor response to targeted therapy. AJR Am J Roentgenol. 2010;195(3):W221-8.
15. Makmun D, Simadibrata M, Abdullah M, et al. Colorectal cancer patients in a tertiary hospital in Indonesia: Prevalence of the younger population and associated factors. World J Clin Cases. 2021;9(32):9804-14.
16. Syed AR, Thakkar P, Horne ZD, et al. Old vs new: Risk factors predicting early onset colorectal cancer. World J Gastrointest Oncol. 2019;11(11):1011-20.
17. Chen FW, Sundaram V, Chew TA, Ladabaum U. Advanced-stage colorectal cancer in persons younger than 50 years not associated with longer duration of symptoms or time to diagnosis. Clin Gastroenterol Hepatol. 2017;15(5):728-37.e3.
18. Manojlovic N, Savic G, Nikolic B, Rancic N. Dynamic monitoring of carcinoembryonic antigen, CA19-9 and inflammation-based indices in patients with advanced

colorectal cancer undergoing chemotherapy. World J Clin Cases. 2022;10(3):899-918.
19. Kotzev AI, Draganov PV. Carbohydrate Antigen 19-9, Carcinoembryonic Antigen, and Carbohydrate Antigen 72-4 in Gastric Cancer: Is the Old Band Still Playing? Gastrointest Tumors. 2018;5(1-2):1-13.
20. Oh HH, Joo YE. Novel biomarkers for the diagnosis and prognosis of CRC. Intestinal research. 2020;18(2):168.
21. Lee T, Teng TZ, Shelat VG. Carbohydrate antigen 19-9—Tumor marker: Past, present, and future. World Journal of Gastrointestinal Surgery. 2020;12(12):468.
22. Jelski W, Mroczko B. Biochemical markers of CRC–present and future. Cancer Management and Research. 2020; 12:4789.
23. Gómez-Molina R, Suárez M, Martínez R, Chilet M, Bauça JM, Mateo J. Utility of Stool-Based Tests for Colorectal Cancer Detection: A Comprehensive Review. Healthcare (Basel). 2024;12(16):1645.
24. André T, de Gramont A, Vernerey D, et al. Adjuvant Fluorouracil, Leucovorin, and Oxaliplatin in Stage II to III Colon Cancer: Updated 10-Year Survival and Outcomes According to BRAF Mutation and Mismatch Repair Status of the MOSAIC Study. J Clin Oncol. 2015;33(35):4176-87.
25. Onishi T, Fukuda Y, Miyazaki S, et al. Practical guidance for echocardiography for cancer therapeutics-related cardiac dysfunction. J Echocardiogr. 2021;19(1):1-20.
26. Song J, Chen Z, Huang D, Xu B. Prognostic Impact of Pretreatment Elevated and Normalized Carcinoembryonic Antigen Levels After Neoadjuvant Chemoradiotherapy in Resected Locally Advanced Rectal Cancer Patients. Cancer Manag Res. 2021; 13:3713-21.
27. Huang D, Lin Q, Song J, Xu B. Prognostic Value of Pretreatment Serum CA199 in Patients with Locally Advanced Rectal Cancer Treated with CRT Followed by TME with Normal Pretreatment Carcinoembryonic Antigen Levels. Dig Surg. 2021;38(1):24-9.
28. Kim NH, Lee MY, Park JH, et al. Serum CEA and CA 19-9 Levels are Associated with the Presence and Severity of Colorectal Neoplasia. Yonsei Med J. 2017;58(5):918-24.
29. Taieb J, Gallois C. Adjuvant Chemotherapy for Stage III Colon Cancer. Cancers (Basel). 2020;12(9):2679.