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A STUDY ON SELECTED CLINICAL EPIDEMIOLOGICAL CHARACTERISTICS AND KRAS, NRAS, AND BRAF GENE MUTATIONS IN PATIENTS WITH COLORECTAL CANCER TREATED AT NGHE AN ONCOLOGY HOSPITAL (2024 – 2026)

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignancies worldwide. According to GLOBOCAN data released in 2024, CRC ranked third in incidence and second in cancer-related mortality globally, with 2,041,007 new cases and 917,895 deaths. In Vietnam, CRC ranked third in incidence among all cancers in both sexes, with 22,584 new cases and 10,990 deaths [1]. CRC is a multifactorial disease involving interactions among genetic, environmental, and lifestyle factors. Advanced age, a family history of cancer, a history of colorectal diseases, high red meat consumption, smoking, overweight, and obesity have been associated with CRC [7], [8], [9]. However, the distribution and strength of these associations may vary across populations. Investigating the clinical epidemiological characteristics of CRC and its associated factors is important for informing prevention, screening, and early detection strategies.

Advances in molecular biology have transformed the management of CRC, particularly in the metastatic setting. Mutations in the *KRAS*, *NRAS*, and *BRAF* genes play important roles in treatment selection and prognostic assessment. *RAS* mutations are predictive biomarkers of resistance to anti-epidermal growth factor receptor (anti-EGFR) therapy, whereas *BRAF* mutations are associated with an unfavorable prognosis [12], [13]. In addition, concurrent mutations and their associations with progression-free survival (PFS) have attracted increasing research interest [14], [15]. In this context, we conducted the study entitled **"A Study on Selected Clinical Epidemiological Characteristics and KRAS, NRAS, and BRAF Gene Mutations in Patients with Colorectal Cancer Treated at Nghe An Oncology Hospital (2024–2026)"**, with the following two objectives:

1. To describe selected clinical epidemiological characteristics of patients with colorectal cancer treated at Nghe An Oncology Hospital during 2024–2026.
2. To determine the characteristics of *KRAS*, *NRAS*, and *BRAF* gene mutations, their associations with selected factors, and their prognostic value in patients with stage IV colorectal cancer.

2. New Contributions of the Thesis

This thesis provides data on the clinical epidemiological characteristics of CRC and factors associated with CRC among patients at Nghe An Oncology Hospital. The simultaneous analysis of demographic characteristics, medical history, dietary habits, and lifestyle factors contributes to a better understanding of CRC in the study population and provides a scientific basis for identifying high-risk groups and guiding prevention, screening, and early detection strategies at the local level.

The thesis also provides additional data on *KRAS*, *NRAS*, and *BRAF* gene mutations in patients with stage IV CRC, particularly specific mutation variants and concurrent mutations. The analysis of associations between these mutations and clinical characteristics, paraclinical findings, and progression-free survival (PFS) contributes to a better understanding of the clinical significance and prognostic value of these molecular biomarkers, providing further evidence to inform treatment selection and patient monitoring.

3. Structure of the Thesis

The thesis comprises 124 pages of main text, excluding references and appendices. The Introduction consists of 2 pages, the Literature Review 38 pages, Subjects and Methods 19 pages, Results 25 pages, Discussion 37 pages, Conclusions 2 pages, and Recommendations 1 page. The thesis includes 41 tables, 12 figures, and 1 flowchart.

CHAPTER 1
LITERATURE REVIEW

1.1. Overview of Colorectal Cancer

1.1.1. Definition of Colorectal Cancer

Colorectal cancer (CRC) is a malignant disease arising from the colorectal mucosa, predominantly in the form of adenocarcinoma. It develops through the accumulation of genetic and epigenetic alterations and has the potential for invasion and metastasis. Tumor location, histopathological characteristics, and disease stage are important determinants of treatment selection and prognosis.

1.1.2. Pathogenesis

CRC develops through the accumulation of genetic and epigenetic alterations, primarily involving three molecular mechanisms: chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island methylator phenotype (CIMP). These alterations occur through the adenoma–carcinoma sequence or the serrated neoplasia pathway and involve genes such as *APC*, *KRAS*, *BRAF*, and *TP53*, as well as DNA repair systems. These molecular processes influence disease progression, treatment response, and prognosis.

1.1.3. Risk Factors for Colorectal Cancer

CRC is a multifactorial disease resulting from interactions among genetic factors, individual characteristics, environmental exposures, and lifestyle factors. Non-modifiable risk factors include advanced age, a family history of CRC, and hereditary syndromes such as Lynch syndrome and familial adenomatous polyposis (FAP). A history of adenomatous polyps and chronic inflammatory bowel diseases is also associated with an increased risk of CRC.

Modifiable risk factors include high consumption of red and processed meat, smoking, alcohol consumption, overweight, obesity, and physical inactivity. Identifying these factors is important for developing prevention strategies, providing lifestyle modification counseling, and implementing CRC screening among high-risk populations.

1.2. Clinical Epidemiological Characteristics of Colorectal Cancer

1.2.1. Epidemiological Characteristics

CRC is one of the most common malignancies worldwide, with variations in incidence and mortality across geographical regions.

Incidence rates are generally higher in countries with greater socioeconomic development but are increasing in many developing countries, particularly in Asia.

CRC predominantly affects middle-aged and older adults, with incidence increasing markedly after the age of 50. However, an increasing incidence of early-onset CRC among individuals younger than 50 years has been reported in many countries. In general, incidence rates are higher in men than in women. These epidemiological characteristics are important for identifying priority populations and developing appropriate screening strategies.

1.2.2. Clinical Characteristics

The clinical manifestations of CRC are diverse and depend on tumor location, extent of invasion, and disease stage. Common symptoms include abdominal pain, changes in bowel habits, rectal bleeding, constipation, diarrhea, weight loss, and anemia. In the early stages, CRC may be asymptomatic or present with nonspecific symptoms, potentially leading to delayed detection.

Clinical manifestations vary according to tumor location. Right-sided colon cancer commonly presents with anemia, dull abdominal pain, and systemic symptoms. Left-sided colon cancer frequently causes changes in stool caliber, altered bowel habits, or symptoms of partial bowel obstruction. Rectal cancer is commonly associated with rectal bleeding, tenesmus, and changes in bowel habits.

1.2.3. Paraclinical Characteristics of Colorectal Cancer

Colonoscopy combined with histopathological examination of biopsy specimens is the principal method for confirming the diagnosis of CRC, allowing assessment of tumor location, gross morphology, and histological characteristics. Diagnostic imaging is used to evaluate the extent of tumor invasion, regional lymph node involvement, and distant metastases for TNM staging. Carcinoembryonic antigen (CEA) is useful for prognostic assessment, monitoring treatment response, and detecting recurrence; however, it should not be used alone to establish a definitive diagnosis of CRC.

1.3. Molecular Characteristics of Colorectal Cancer

1.3.1. Molecular Basis of Colorectal Cancer

CRC develops and progresses through the accumulation of genetic and epigenetic alterations that affect cellular proliferation, differentiation, invasion, and metastasis. These molecular alterations contribute to tumor heterogeneity and have important implications for prognosis, prediction of treatment response, and therapeutic decision-making. Among them, mutations in the *KRAS*, *NRAS*, and *BRAF* genes play significant roles in patients with advanced and metastatic CRC.

1.3.2. Gene Groups Involved in Colorectal Cancer

Colorectal carcinogenesis involves several groups of genes, including oncogenes (*KRAS*, *NRAS*, and *BRAF*), tumor suppressor genes (*APC* and *TP53*), and DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Dysregulation of these genes leads to uncontrolled cell proliferation, impaired DNA repair, and malignant progression.

1.3.3. EGFR–RAS–RAF–MEK–ERK Signaling Pathway

The EGFR–RAS–RAF–MEK–ERK signaling pathway regulates cell proliferation, differentiation, and survival by transmitting signals from the epidermal growth factor receptor (EGFR) to the cell nucleus. Activating mutations in *KRAS*, *NRAS*, or *BRAF* can sustain proliferative signaling even in the absence of EGFR stimulation. This provides the molecular basis for the predictive role of these mutations in response to anti-EGFR therapy and treatment selection in metastatic CRC.

1.3.4. Characteristics of *KRAS, NRAS,* and *BRAF* Gene Mutations

***KRAS*:** The *KRAS* gene belongs to the RAS oncogene family and is involved in intracellular signal transduction. *KRAS* mutations occur in approximately 40% of CRC cases, predominantly at codons 12 and 13 of exon 2. Common variants include G12D, G12V, G13D, and G12C.

***NRAS*:** The *NRAS* gene also belongs to the RAS family and plays a role in regulating cell proliferation. *NRAS* mutations are less frequent than *KRAS* mutations and mainly occur at codons 12, 13, and 61. Activating *NRAS* mutations predict resistance to anti-EGFR therapy.

***BRAF*:** The *BRAF* gene encodes a protein kinase involved in the MAPK signaling pathway. The *BRAF* V600E mutation, located in exon 15, is associated with unfavorable biological characteristics, poor prognosis, and therapeutic decision-making in patients with metastatic CRC.

Mutations in *KRAS*, *NRAS*, and *BRAF* are generally mutually exclusive but may occasionally coexist, reflecting the molecular heterogeneity of CRC.

1.3.5. Techniques for Detecting Gene Mutations in Colorectal Cancer

Techniques used to detect gene mutations include polymerase chain reaction (PCR), Sanger sequencing, next-generation sequencing (NGS), and molecular hybridization. In this study, the StripAssay technique was used to detect *KRAS*, *NRAS*, and *BRAF* mutations. This technique is based on multiplex PCR combined with reverse hybridization on membrane strips, enabling the simultaneous detection of multiple known mutation variants with high specificity. The method is suitable for routine molecular testing and supports treatment selection in patients with metastatic CRC.

1.4. Diagnosis and Treatment of Colorectal Cancer

1.4.1. Disease Staging

Colorectal cancer (CRC) staging is based on the eighth edition of the American Joint Committee on Cancer (AJCC) TNM classification system, which assesses the extent of primary tumor invasion (T), regional lymph node involvement (N), and distant metastasis (M). CRC is classified into four stages, from stage I to stage IV, with stage IV defined by the presence of distant metastasis. Accurate staging is essential for treatment selection and prognostic assessment.

1.4.2. Treatment of Colorectal Cancer

Treatment of CRC depends on disease stage, tumor location, the patient's general condition, and molecular characteristics. The main treatment modalities include surgery, chemotherapy, radiotherapy, and targeted therapy. Surgery plays a central role in early-stage disease and may be combined with adjuvant treatment. In stage IV CRC, systemic treatment primarily consists of chemotherapy regimens containing fluoropyrimidines, oxaliplatin, or irinotecan, which may be combined with targeted agents.

1.4.3. Role of Targeted Therapy in Colorectal Cancer

Targeted therapy for metastatic CRC includes anti-vascular endothelial growth factor (anti-*VEGF*) agents, such as bevacizumab, and anti-epidermal growth factor receptor (anti-*EGFR*) agents, such as cetuximab and panitumumab, which are commonly combined with chemotherapy. *KRAS* or *NRAS* mutations predict resistance to anti-*EGFR* therapy, whereas the *BRAF* V600E mutation is associated with an unfavorable prognosis and has implications for treatment selection.

1.5. Research on Colorectal Cancer

1.5.1. Research on the Clinical Epidemiological Characteristics of Patients with Colorectal Cancer in Vietnam

In Vietnam, numerous studies have investigated the epidemiological characteristics, tumor locations, and clinical manifestations of patients with colorectal cancer (CRC). Overall, CRC predominantly affects middle-aged and older adults, with a higher proportion of cases occurring in men than in women. Clinical manifestations vary according to tumor location and disease stage. Pham Xuan Dung et al. (2022) analyzed 12,938 CRC cases in Ho Chi Minh City during 1996–2015. Men accounted for 53.9% of cases, and the mean age was 60.0 ± 15.0 years. Colon cancer accounted for 63.6% of cases, while rectal cancer accounted for 36.4%. CRC incidence showed an increasing trend over time, particularly among men and older adults.

Studies conducted in Vietnam have demonstrated differences in clinical manifestations according to tumor location. Rectal bleeding is more common in rectal cancer, whereas abdominal pain is more frequently observed in colon cancer. However, comprehensive data simultaneously assessing epidemiological characteristics, associated factors, clinical manifestations, and pathological features in the North Central region of Vietnam remain limited.

1.5.2. Research on KRAS, NRAS, and BRAF Mutations in Colorectal Cancer

International studies have reported that *KRAS* mutations are the most common molecular alterations among these three genes in CRC, occurring in approximately 40–50% of cases, whereas *NRAS* and *BRAF* mutations are less frequent. Peeters et al. investigated 3,196 patients with metastatic CRC across 36 countries and reported a *RAS* mutation rate of 55.9%. El Zaitouni et al. reported mutation rates of 46.6%, 5.6%, and 2.4% for *KRAS*, *NRAS*, and *BRAF*, respectively.

In Vietnam, previous studies have primarily focused on mutation frequencies and their associations with clinical and histopathological characteristics. Data on concurrent mutations and their prognostic value for progression-free survival (PFS) remain limited, particularly in the North Central region of Vietnam.

1.6. Colorectal Cancer in Nghe An and Clinical Practice at Nghe An Oncology Hospital

Nghe An is a geographically extensive province with a population distributed across diverse areas, including urban, lowland, coastal, and

mountainous regions. These geographical characteristics contribute to disparities in access to specialized healthcare services, particularly for patients with cancer who require long-term diagnosis, treatment, and follow-up. Nghe An Oncology Hospital is a provincial specialized oncology hospital that provides care for patients from Nghe An and neighboring provinces. In recent years, the hospital has progressively developed its diagnostic, therapeutic, and molecular testing capabilities, including testing for *KRAS*, *NRAS*, and *BRAF* gene mutations to guide the treatment of CRC.

However, research data on the clinical epidemiological characteristics, associated factors, and gene mutation profiles of patients with CRC in Nghe An remain limited. Conducting this study at Nghe An Oncology Hospital contributes additional scientific evidence to support CRC prevention, early detection, treatment selection, and prognostic assessment.

CHAPTER 2
STUDY SUBJECTS AND METHODS

2.1. Study of the Clinical Epidemiological Characteristics of Colorectal Cancer

2.1.1. Study Population

The study included patients presenting for examination or treatment at Nghe An Oncology Hospital between January 2024 and May 2026 who had symptoms or signs suggestive of colorectal disease.

2.1.1.1. Inclusion Criteria

- Presence of at least one symptom or sign suggestive of colorectal disease, including abdominal pain, changes in bowel habits (diarrhea or constipation), passage of blood or blood-stained mucus in the stool, or weight loss.
- Underwent colonoscopy and histopathological examination when a suspicious lesion was identified.
- Availability of sufficient information as required by the study case report form.

2.1.1.2. Criteria for Classification into the Colorectal Cancer Group

Patients were classified into the CRC group if they were diagnosed with CRC for the first time based on histopathological examination of endoscopic biopsy specimens or surgical specimens.

2.1.1.3. Exclusion Criteria

- History of or concurrent diagnosis of another primary malignancy.

- Medical records containing insufficient information to determine the key study variables.

2.1.2. Research Methods

2.1.2.1. Study Design

A descriptive cross-sectional study.

2.1.2.2. Sample Size and Sampling Method

The sample size was calculated using the formula for estimating a single population proportion:

$$n = Z^2_{1-\alpha/2} \frac{p(1-p)}{d^2}$$

Where: α = 0.05; Z = 1.96; p = 0.24 (Ngo Ngoc Tho, 2023); and d = 0.025.

The minimum required sample size was 1,121 patients. Consecutive sampling was applied, enrolling all eligible patients during the study period. The actual sample size was 1,126 patients.

2.1.2.3. Study Procedures

Data were collected using a standardized case report form, including demographic characteristics, body mass index (BMI), medical history, risk factors, and clinical symptoms. Patients underwent colonoscopy and histopathological examination of biopsy specimens and were classified into two groups: CRC and non-CRC, for analysis of associated factors.

2.2. Study of KRAS, NRAS, and BRAF Gene Mutations in Patients with Stage IV Colorectal Cancer

2.2.1. Study Population

Patients with stage IV colorectal adenocarcinoma treated at Nghe An Oncology Hospital between January 2024 and May 2026.

2.2.1.1. Inclusion Criteria

- Histopathologically confirmed colorectal adenocarcinoma, classified as stage IV according to the eighth edition of the American Joint Committee on Cancer (AJCC) staging system.

- Availability of complete *KRAS*, *NRAS*, and *BRAF* mutation testing results.

- Received chemotherapy, with or without targeted therapy, for at least three months.

- Availability of sufficient clinical, paraclinical, and follow-up data.

2.2.1.2. Exclusion Criteria

Patients with a history of or concurrent diagnosis of another malignancy.

2.2.2. Research Methods

2.2.2.1. Study Design

A descriptive study with analytical components, incorporating longitudinal follow-up.

2.2.2.2. Sample Size and Sampling Method

Consecutive sampling was applied. During the study period, 154 patients with stage IV colorectal cancer were identified, of whom 110 met the inclusion criteria and were enrolled in the study.

2.2.2.3. Study Procedures

Gene mutation testing: Tumor tissue samples were obtained through biopsy or surgery. Testing for *KRAS, NRAS,* and *BRAF* mutations was performed in the Department of Pathology using the StripAssay technique. *Treatment and follow-up:* Patients received first-line chemotherapy with mFOLFOX6, XELOX, or FOLFIRI, with or without bevacizumab or cetuximab. Treatment response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. *Assessment of progression-free survival (PFS):* PFS was defined as the time from initiation of first-line treatment for metastatic disease to disease progression or death. Patients without an event were censored at their last assessment.

2.3. Bias and Methods of Bias Control

Potential biases were minimized through consecutive sampling, standardized inclusion criteria, the use of standardized case report forms, adherence to established laboratory testing procedures, and data

verification before analysis. Patients included in Objective 2 underwent regular follow-up to minimize bias in the assessment of progression-free survival (PFS).

2.4. Data Processing and Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 27.0. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation or median and interquartile range. Appropriate statistical tests and univariable and multivariable logistic regression analyses were used to identify associated factors, with results reported as odds ratios (ORs), adjusted odds ratios (aORs), and 95% confidence intervals (95% CIs).

Progression-free survival (PFS) was analyzed using the Kaplan–Meier method, log-rank test, and Cox regression, with results reported as hazard ratios (HRs) and 95% CIs. A p-value of less than 0.05 was considered statistically significant.

2.5. Research Ethics

Participation in the study was voluntary. Patients had the right to refuse participation or withdraw from the study at any time without affecting their treatment. Genetic testing was performed based on clinical indications and with patients' consent. All study data were coded, kept confidential, and used exclusively for scientific research purposes.

CHAPTER 3
RESULTS

Between January 2024 and May 2026, a total of 1,126 patients presenting with symptoms or signs suggestive of colorectal disease were enrolled in the study. Of these, 511 patients were confirmed to have CRC, accounting for 45.4% of the study population.

3.1. Epidemiological Characteristics of the Study Population

Among the 1,126 study participants, 60.1% were male, 95.7% belonged to the Kinh ethnic group, 84.6% resided in rural areas, 77.3% were manual workers, and 71.0% had an educational level below upper secondary school. Participants aged 60 years or older accounted for 64.2%.

Regarding nutritional and lifestyle characteristics, 9.4% had a body mass index (BMI) ≥ 23 kg/m², 44.0% consumed ≥ 100 g of red meat per day, 29.6% were smokers, and 53.1% reported alcohol consumption.

3.2. Demographic Characteristics According to Colorectal Cancer Status

Characteristics		Patients examined (n)	CRC cases (n)	Rate %	p-value
Sex	Male	677	318	47.0	0.19
	Female	449	193	43.0	
Age group	< 60	403	163	40.4	0.013
	≥ 60	723	348	48.1	
Ethnicity	Kinh	1078	495	45.9	0.086
	Other	48	16	33.3	
Area of residence	Rural	953	447	46.9	0.015
	Urban	173	64	37.0	
Occupation	Manual Labor	870	417	47.9	0.002
	Non-manual Labor	256	94	36.7	
Educational level	Below upper secondary school	800	394	49.3	< 0.001
	Upper secondary school or higher	326	117	35.9	

The CRC detection rate was significantly higher among participants aged 60 years or older (48.1%), those residing in rural areas (46.9%), manual workers (47.9%), and those with an educational level below upper secondary school (49.3%), compared with their respective counterparts ($p < 0.05$).

3.3. Factors Associated with Colorectal Cancer

Table 3.16. Multivariable Logistic Regression Analysis of Factors Associated with Colorectal Cancer

Factors	aOR	95% CI	p-value
BMI ≥ 23	2.31	1.50–3.56	< 0.001
Red meat consumption ≥100 g/day	3.42	2.65–4.42	< 0.001
Smoking	1.31	1.02–1.68	0.035

Alcohol consumption	0.98	0.77–1.25	0.87
History of colorectal disease	2.39	0.85–6.72	0.098
Family history of cancer	3.32	1.83–6.02	< 0.001

After adjustment, four factors were independently associated with CRC: red meat consumption ≥ 100 g/day, family history of cancer, BMI ≥ 23 kg/m², and smoking ($p < 0.05$). Among these factors, red meat consumption showed the strongest association (aOR = 3.42). Alcohol consumption and a history of colorectal disease were no longer statistically significant in the multivariable analysis ($p > 0.05$).

3.4. Clinical and Paraclinical Characteristics of Patients with Colorectal Cancer

Among the 511 patients with CRC, the most common primary tumor location was the rectum (41.7%), followed by the left colon (37.2%) and right colon (21.1%). Common symptoms included abdominal pain (78.5%), bloody stools (48.9%), constipation (48.1%), and weight loss (40.5%).

Table 3.18. Clinical Symptoms According to Primary Tumor Location

Clinical symptoms	Right colon (n=108) n (%)	Left colon (n=190) n (%)	Rectum (n=213) n (%)	p-value
Abdominal pain	92 (85.2)	154 (81.1)	155 (72.8)	0.021
Bloody stools	45 (41.7)	77 (40.5)	128 (60.1)	< 0.001
Diarrhea	42 (38.9)	55 (28.9)	62 (29.1)	0.145
Weight loss	36 (33.3)	92 (48.4)	79 (37.1)	0.016
Constipation	48 (44.4)	88 (46.3)	110 (51.6)	0.388

Abdominal pain, bloody stools, and weight loss differed significantly according to primary tumor location ($p < 0.05$). Abdominal pain was most frequent in right-sided colon cancer (85.2%), bloody stools in rectal cancer (60.1%), and weight loss in left-sided colon cancer (48.4%).

On colonoscopy, lesions involving more than three-quarters of the bowel circumference accounted for 38.4%. Ulceroproliferative and proliferative lesions accounted for 36.6% and 31.5%, respectively. Median carcinoembryonic antigen (CEA) levels were 3.61, 3.10, and 3.69 ng/mL in right-sided colon, left-sided colon, and rectal cancer, respectively, with no statistically significant difference (p = 0.22).

Table 3.26. Distribution of Metastatic Sites According to Primary Tumor Location

Metastatic site	**Right colon (n=31) n (%)**	**Left colon (n=64) n (%)**	**Rectum (n=59) n (%)**	**p-value**
Liver	21 (67.7)	40 (62.5)	37 (62.7)	0.868
Lung	5 (16.1)	24 (37.5)	21 (35.6)	0.092
Ovary	0	2 (3.1)	1 (1.7)	0.577
Bone	2 (6.5)	3 (4.7)	4 (6.8)	0.874
Peritoneum	8 (25.8)	15 (23.4)	6 (10.2)	0.092
Other sites	6 (19.4)	20 (31.3)	7 (11.9)	0.031

The liver was the most common metastatic site (62.5–67.7%). The proportion of metastases to other sites differed significantly according to primary tumor location (p = 0.031).

Table 3.28. Distribution of AJCC 8th Edition Stages According to Primary Tumor Location

Stage	**Right colon n (%)**	**Left colon n (%)**	**Rectum n (%)**	**p-value**
I	5 (4.6)	14 (7.4)	34 (16.0)	< 0.001
II	47 (43.5)	64 (33.7)	46 (21.6)	
III	25 (23.1)	48 (25.3)	74 (34.7)	
IV	31 (28.7)	64 (33.7)	59 (27.7)	
Total	108 (100)	190 (100)	213 (100)	

The distribution of disease stages differed significantly according to primary tumor location (p < 0.001).

3.5. *KRAS*, *NRAS*, and *BRAF* Mutation Characteristics, Associated Factors, and Prognostic Significance in Patients with Stage IV Colorectal Cancer

3.5.1. KRAS, NRAS, and BRAF Mutation Characteristics

Table 3.29. Frequency and Characteristics of KRAS, NRAS, and BRAF Mutations (n = 110)

Mutation status	**Patients tested (n)**	**Patients with mutations (n)**	**Percentage (%)**
Any mutation	110	73	66.4
Single mutation	110	62	56.4
Concurrent mutations	110	11	10.0

Overall, 66.4% of patients harbored at least one mutation in KRAS, NRAS, or BRAF. Single mutations were identified in 56.4% of patients, while concurrent mutations were detected in 10.0%.

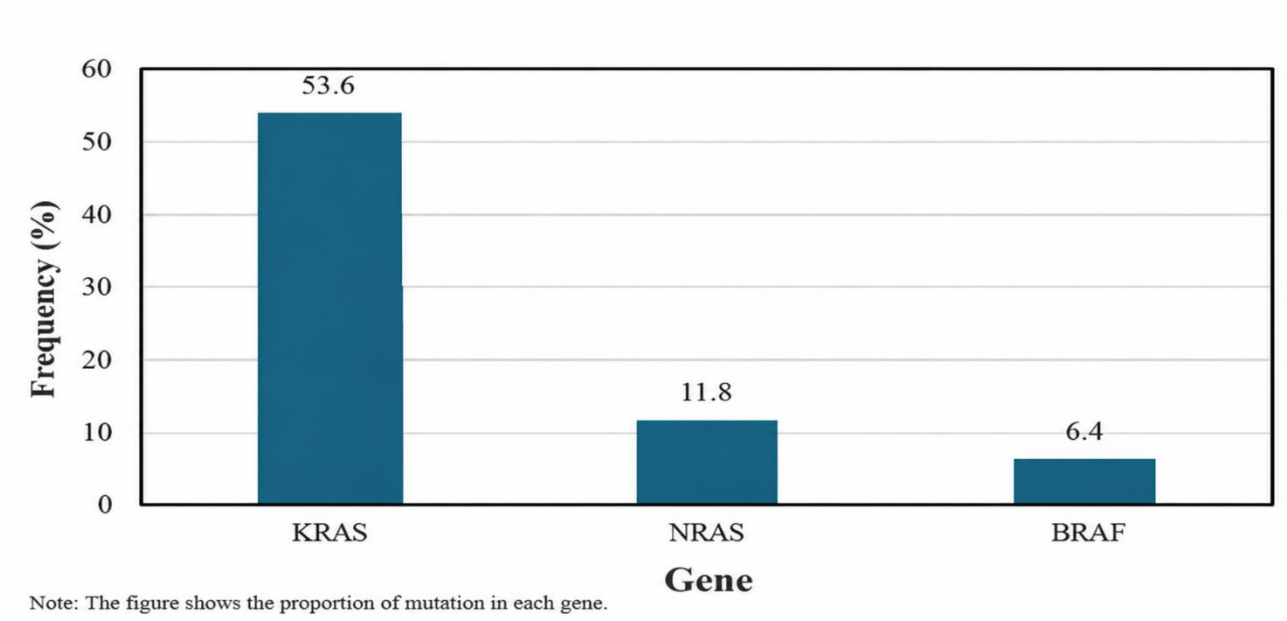

Figure 3.5. Distribution of KRAS, NRAS, and BRAF Mutation Frequencies

KRAS mutations were the most frequent (53.6%), followed by NRAS (11.8%) and BRAF (6.4%).

KRAS mutations occurred predominantly in exon 2 (49.1%), with codon 12 accounting for the highest proportion (39.1%). G12D was the most common variant (22.7%). NRAS mutations were mainly located at codon 61, with Q61L being the predominant variant (7.3%). BRAF mutations were detected in 6.4% of patients, all of which were V600E.

3.5.2. Associations Between KRAS, NRAS, and BRAF Mutation Status and Clinical and Paraclinical Characteristics

No statistically significant associations were observed between

overall KRAS, NRAS, or BRAF mutation status and the clinical or paraclinical characteristics examined ($p > 0.05$).

Table 3.32. Associations of Specific Gene Mutations with Selected Clinical Characteristics

Gene mutation	Clinical Characteristic	OR	95% CI	p-value
BRAF V600E	Poor differentiation	12.40	2.42–63.47	0.005
NRAS Q61L	Liver metastasis	11.47	1.37–1498.15	0.024
NRAS (any mutation)	Liver metastasis	8.42	1.05–67.39	0.029

The BRAF V600E mutation was significantly associated with poor tumor differentiation (OR = 12.40; 95% CI: 2.42–63.47; $p = 0.005$). Both *NRAS* mutations overall and the NRAS Q61L variant were significantly associated with liver metastasis ($p < 0.05$).

Table 3.33. Associations Between Single or Concurrent Mutations and Selected Clinical Characteristics

Characteristic	Concurrent mutations (n)	Single mutation (n)	OR (95% CI)	p-value
Age ≥ 60 years	7	46	0.609 (0.16–2.36)	0.48
Male sex	5	36	0.60 (0.17–2.19)	0.52
Right-sided colon cancer	1	13	0.377 (0.04–3.22)	0.68
Tumor ≥3/4 of the bowel circumference	9	21	**8.786 (1.73–44.39)**	**0.006**

Tumors involving at least three-quarters of the colorectal circumference were significantly associated with concurrent mutations (OR = 8.786; 95% CI: 1.73–44.39; $p = 0.006$).

3.5.3. Prognostic Significance of KRAS, NRAS, and BRAF Mutations in Stage IV Colorectal Cancer

Table 3.34. First-Line Treatment and Treatment Response According to KRAS, NRAS, and BRAF Mutation Status

Gene mutation	First-line treatment		Response to first-line treatment		p-value
	Chemotherapy alone	Chemotherapy + targeted therapy	CR, PR or SD	PD	
KRAS	19	40	43	16	0.18
NRAS	7	6	7	6	0.35
BRAF	2	5	3	4	0.21

No statistically significant differences in first-line treatment response were observed between patients with and without KRAS, NRAS, or BRAF mutations ($p > 0.05$).

Table 3.35. Progression-Free Survival According to the Number of Mutations

Mutation status	**Mean PFS (months)**	**p-value**
No mutation	10.2	0.6
Single mutation	9.27	
Concurrent mutations	8.72	

Mean PFS tended to decrease from patients without mutations (10.20 months) to those with a single mutation (9.27 months) and those with concurrent mutations (8.72 months). However, the difference was not statistically significant ($p = 0.60$).

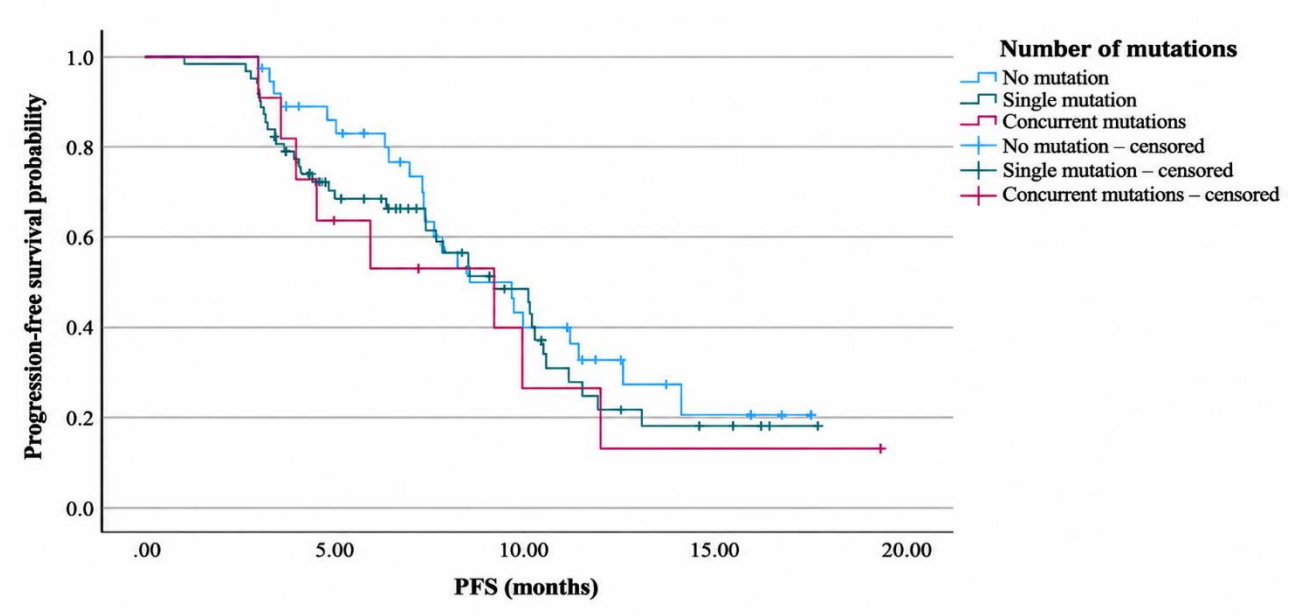

Figure 3.7. Kaplan–Meier Curves for Progression-Free Survival

According to the Number of Mutations

Kaplan–Meier analysis showed a tendency toward higher progression-free survival probabilities in patients without mutations, whereas patients with concurrent mutations tended to experience earlier declines. However, the differences among the three groups were not statistically significant (log-rank test, p = 0.60).

Table 3.36. Progression-Free Survival According to Specific Mutation Variants

Mutation variant	Median PFS (months)	p-value
KRAS G12D	8.6	0.19
KRAS G13D	9.2	0.85
KRAS G12C	3.0	0.76
KRAS G12S	6.0	0.33
NRAS Q61L	6.4	0.45
BRAF V600E	3.7	**0.02**

Median PFS varied across mutation variants. Notably, patients harboring the BRAF V600E mutation had a median PFS of 3.7 months, which was significantly shorter than that of patients without this mutation (p = 0.02).

Table 3.37. Multivariable Cox Regression Analysis of Factors Associated with Progression-Free Survival

Factors	HR	95% CI	p-value
Age ≥ 60 years	1.29	0.67 – 2.48	0.444
Male sex	1.44	0.87 – 2.37	0.154
Tumor location (right/left colon +rectum)	1.59	0.79 – 3.20	0.194
≥ 2 metastatic organs	1.05	0.61 – 1.79	0.870
KRAS mutation (present vs absent)	0.92	0.55 – 1.53	0.745
BRAF V600E mutation	2.57	0.94 – 6.99	0.065

Multivariable Cox regression analysis showed that the BRAF V600E mutation was associated with a trend toward an increased hazard of disease progression or death (HR = 2.57; 95% CI: 0.94–6.99), although this association did not reach statistical significance (p = 0.065).

The remaining factors, including age, sex, primary tumor location,

number of metastatic organs, and KRAS mutation status, were not significantly associated with PFS ($p > 0.05$).

CHAPTER 4
DISCUSSION

4.1. Epidemiological Characteristics of the Study Population

The study enrolled 1,126 patients presenting with symptoms or signs suggestive of colorectal disease at Nghe An Oncology Hospital. The study population predominantly comprised individuals of Kinh ethnicity (95.7%), rural residents (84.6%), manual workers (77.3%), and those with an educational level below upper secondary school (71.0%).

Males accounted for 60.1% of the study population. The mean age was 62.95 ± 11.8 years, with 64.2% of participants aged 60 years or older. These findings were consistent with those reported in a study conducted at Soc Trang Provincial General Hospital and with the observations of Siegel et al. (2026), indicating that CRC continues to be predominantly detected among middle-aged and older adults.

Regarding nutritional and lifestyle characteristics, 9.4% of participants had a BMI ≥ 23 kg/m², 44.0% consumed ≥ 100 g of red meat per day, 29.6% were smokers, and 53.1% reported alcohol consumption.

4.2. Selected Clinical Epidemiological Characteristics of Patients with Colorectal Cancer

4.2.1. Demographic Characteristics

The CRC detection rate was higher among males than females (47.0% vs. 43.0%), although the difference was not statistically significant ($p = 0.19$), consistent with the findings of La Ngoc Quang at Bach Mai Hospital [116]. In contrast, participants aged 60 years or older had a significantly higher CRC detection rate than those younger than 60 years (48.1% vs. 40.4%; $p = 0.013$). This finding was consistent with domestic studies and SEER data, indicating that CRC remains predominantly diagnosed among middle-aged and older adults [112], [117].

No statistically significant difference was observed according to ethnicity ($p = 0.086$). The CRC detection rate was higher among rural residents than urban residents (46.9% vs. 37.0%; $p = 0.015$). This difference may be associated with socioeconomic conditions and access to healthcare services [118].

Manual workers had a higher CRC detection rate than non-manual

workers (47.9% vs. 36.7%; p = 0.002), consistent with the findings of La Ngoc Quang [116]. Similarly, the detection rate was 49.3% among participants with an educational level below upper secondary school, compared with 35.9% among those with higher educational attainment (p < 0.001). These differences may reflect disparities in living conditions, health awareness, and access to screening services.

4.2.2. Selected Risk Factors for Colorectal Cancer

Multivariable logistic regression identified four factors independently associated with CRC: red meat consumption, family history of cancer, BMI, and smoking. Red meat consumption ≥ 100 g/day showed the strongest association (aOR = 3.42; 95% CI: 2.65–4.42; p < 0.001). This finding was consistent with international epidemiological evidence linking high red meat consumption to CRC [48], [50]. A family history of cancer was also significantly associated with CRC (aOR = 3.32; 95% CI: 1.83–6.02; p < 0.001), consistent with previous studies highlighting the role of familial factors in CRC [121]. This association may reflect the combined influence of genetic susceptibility, shared environmental exposures, and lifestyle habits among family members.

BMI ≥ 23 kg/m² remained significantly associated with CRC after adjustment (aOR = 2.31; 95% CI: 1.50–3.56; p < 0.001). This finding was consistent with studies examining the relationship between overweight, obesity, and CRC. Smoking showed a weaker but statistically significant association (aOR = 1.31; 95% CI: 1.02–1.68; p = 0.035). This finding was consistent with meta-analyses reporting higher CRC occurrence among smokers than nonsmokers [51]. Prolonged exposure to carcinogenic substances in tobacco smoke may contribute to DNA damage and carcinogenesis.

In contrast, alcohol consumption was not significantly associated with CRC (aOR = 0.98; p = 0.87). A history of colorectal disease was associated with CRC in univariable analysis (OR = 2.65), but the association was no longer statistically significant after adjustment (aOR = 2.39; p = 0.098). This may be attributable to the influence of other variables in the model and the limited number of participants with a history of colorectal disease.

4.2.3. Clinical Presentation and Disease Distribution

Among the 511 patients with CRC, rectal tumors were the most common (41.7%), followed by left-sided colon tumors (37.2%) and right-sided colon tumors (21.1%). This distribution was consistent with the

study by Nguyen Kien Du, which reported corresponding proportions of 40.0%, 34.5%, and 25.5% [108]. Abdominal pain was the most frequent symptom (78.5%), followed by bloody stools (48.9%) and weight loss (40.5%). These three symptoms differed significantly according to primary tumor location ($p < 0.05$). Abdominal pain was most common in right-sided colon cancer (85.2%), bloody stools in rectal cancer (60.1%), and weight loss in left-sided colon cancer (48.4%).

These findings were consistent with studies by Nguyen Kien Du, Tran Thang, and Luy et al. Anatomical and physiological differences among colorectal segments may partly explain variations in clinical presentation. However, the interval from symptom onset to diagnosis did not differ significantly among the three tumor locations ($p = 0.149$). On colonoscopy, lesions involving more than three-quarters of the colorectal circumference were the most frequent (38.4%), consistent with the findings of Nguyen Kien Du and Le Quang Minh [108], [124]. The predominant macroscopic appearances were ulceroproliferative (36.6%) and proliferative lesions (31.5%). Histopathologically, moderately differentiated adenocarcinoma was predominant (86.3%). No statistically significant differences according to primary tumor location were observed in circumferential involvement, endoscopic morphology, CEA levels, or histological differentiation.

At diagnosis, stages III and IV accounted for 58.9% of cases, whereas stage I accounted for only 10.4%, indicating that most patients were diagnosed at an advanced stage. Stage IV alone accounted for 30.1%, consistent with national and international reports [1], [2]. Disease stage distribution differed significantly according to primary tumor location ($p < 0.001$). Stage II was most frequent in right-sided colon cancer (43.5%), whereas stage III was particularly common in rectal cancer (34.7%). The liver was the most common metastatic site, followed by the lungs and peritoneum, consistent with the findings of Nguyen Kien Du and Bokemeyer et al. [108], [99]. This pattern may be related to portal venous drainage and the metastatic dissemination pathways of colorectal cancer cells.

4.3. KRAS, NRAS, and BRAF Mutation Characteristics, Associated Factors, and Prognostic Significance in Patients with Stage IV Colorectal Cancer

4.3.1. Frequency and Patterns of KRAS, NRAS, and BRAF Mutations

Among 110 patients with stage IV colorectal cancer, 66.4% harbored

at least one mutation in KRAS, NRAS, or BRAF, including 56.4% with single mutations and 10.0% with concurrent mutations. KRAS mutations were the most frequent (53.6%), followed by NRAS (11.8%) and BRAF (6.4%). These findings were broadly consistent with those of Peeters et al., who reported a RAS mutation frequency of 55.9%, whereas El Zaitouni reported mutation frequencies of 46.6%, 5.6%, and 2.4% for KRAS, NRAS, and BRAF, respectively [91], [106]. KRAS mutations were predominantly located in exon 2 (49.1%), particularly at codon 12 (39.1%), with G12D being the most common variant (22.7%). Among NRAS mutations, Q61L was the predominant variant (7.3%), while all BRAF mutations were V600E. Differences in variant distribution across studies may be attributable to population characteristics and genetic testing methods. Notably, 11 patients harbored concurrent mutations, including six with two KRAS variants, four with concurrent KRAS/NRAS mutations, and one with concurrent NRAS/BRAF mutations. The observed frequency of 10.0% was higher than that reported in several previous studies [102], [106], potentially reflecting tumor molecular heterogeneity.

4.3.2. Factors Associated with Single and Concurrent KRAS, NRAS, and BRAF Mutations in Patients with Stage IV Colorectal Cancer

No statistically significant associations were identified between overall KRAS, NRAS, or BRAF mutation status and the clinical or paraclinical characteristics examined ($p > 0.05$). The findings regarding sex were consistent with those reported by Nguyen Trong Hoa and Ottaiano et al. [138], [13]. When individual genes were analyzed, NRAS mutations were significantly associated with liver metastasis (OR = 8.42; 95% CI: 1.05–67.39; $p = 0.029$), particularly the Q61L variant (OR = 11.47; $p = 0.024$). In addition, the BRAF V600E mutation was significantly associated with poor histological differentiation (OR = 12.40; 95% CI: 2.42–63.47; $p = 0.005$), consistent with the adverse biological characteristics of this mutation reported in the literature. Notably, concurrent mutations were significantly associated with tumors involving at least three-quarters of the colorectal circumference compared with single mutations (OR = 8.79; 95% CI: 1.73–44.39; $p = 0.006$). This finding may reflect molecular heterogeneity and the accumulation of genetic alterations during tumor development [15], [149].

4.3.3. Prognostic Significance of Single and Concurrent KRAS, NRAS, and BRAF Mutations in Patients with Stage IV Colorectal Cancer

No statistically significant differences in treatment response were

observed between patients with and without KRAS, NRAS, or BRAF mutations ($p > 0.05$). However, this finding does not negate the established predictive value of RAS mutation status for response to anti-EGFR therapy demonstrated in previous studies [91].

Mean progression-free survival (PFS) was 10.20, 9.27, and 8.72 months in patients without mutations, those with single mutations, and those with concurrent mutations, respectively. Although PFS tended to decrease with increasing numbers of mutations, the difference was not statistically significant ($p = 0.60$). In contrast, Pretta et al. reported significantly shorter PFS in patients with concurrent mutations [150]. This discrepancy may be related to the limited sample size and the small number of patients harboring concurrent mutations in the present study. Notably, patients harboring the BRAF V600E mutation had a median PFS of 3.7 months, significantly shorter than that of patients without this mutation ($p = 0.02$). This finding was consistent with the unfavorable prognostic trend reported by Tang et al. [142]. However, multivariable Cox regression analysis did not establish BRAF V600E as an independent prognostic factor (HR = 2.57; 95% CI: 0.94–6.99; $p = 0.065$).

4.4. *Study Limitations*

This study was conducted at a single specialized oncology hospital, which may limit the generalizability of the findings to the broader population. For the second study objective, the sample size was limited to 110 patients, with particularly small numbers of patients harboring NRAS, BRAF, or concurrent mutations, thereby reducing the precision of the estimates.

Furthermore, differences in treatment regimens and follow-up duration may have influenced the progression-free survival (PFS) results. Therefore, larger multicenter studies with longer follow-up periods are needed to validate and strengthen these findings.

CONCLUSIONS

1. Clinical Epidemiological Characteristics and Associated Factors

Among 1,126 study participants, 511 patients were diagnosed with colorectal cancer (CRC), corresponding to a detection rate of 45.38%. The CRC detection rate differed significantly according to age, area of residence, occupation, and educational level ($p < 0.05$).

Multivariable analysis identified four factors independently associated with CRC: red meat consumption ≥ 100 g/day (aOR = 3.42), family history of cancer (aOR = 3.32), BMI ≥ 23 kg/m² (aOR = 2.31), and smoking (aOR = 1.31; $p < 0.05$). Alcohol consumption and a history of colorectal disease were not statistically significant after adjustment.

Rectal tumors accounted for 41.7% of cases, followed by left-sided colon tumors (37.2%). Abdominal pain, bloody stools, and weight loss differed significantly according to tumor location. Stages III–IV accounted for 58.9% of cases, and disease stage distribution differed significantly by primary tumor location ($p < 0.001$).

2. KRAS, NRAS, and BRAF Mutation Characteristics and Prognostic Significance in Stage IV Colorectal Cancer

Among 110 patients with stage IV CRC, 66.4% harbored at least one mutation, including 56.4% with single mutations and 10.0% with concurrent mutations. The frequencies of KRAS, NRAS, and BRAF mutations were 53.6%, 11.8%, and 6.4%, respectively. NRAS mutations were significantly associated with liver metastasis (OR = 8.42; $p = 0.029$), particularly the Q61L variant (OR = 11.47; $p = 0.024$). The BRAF V600E mutation was associated with poor histological differentiation ($p = 0.005$).

Concurrent mutations were significantly associated with tumors involving at least three-quarters of the colorectal circumference (OR = 8.79; 95% CI: 1.73–44.39; $p = 0.006$). Patients harboring the BRAF V600E mutation had a median progression-free survival (PFS) of 3.7 months ($p = 0.02$). However, its independent prognostic significance was not established in multivariable Cox regression analysis ($p = 0.065$).

RECOMMENDATIONS

Strengthen health education, counseling, and colorectal cancer screening, prioritizing individuals with risk factors to facilitate early detection. Perform KRAS, NRAS, and BRAF mutation testing in patients with stage IV colorectal cancer to support treatment selection. Particular attention should be given to patients harboring BRAF V600E, NRAS mutations, or concurrent mutations during follow-up.

Conduct larger multicenter studies with longer follow-up periods to further clarify the clinical significance and prognostic value of these mutations.

LIST OF PUBLICATIONS RELATED TO THE THESIS

1. Duong, D. P., Phan, D. V., Nguyen, T. N., Nguyen, T. T., Nguyen, T. T. L., Hoang, D. C., & Nguyen, T. T. (2026). Characteristics of Selected Factors Associated with Colorectal Cancer Among Patients with Suspected Symptoms at Nghe An Oncology Hospital. *Vietnam Medical Journal*, 563(3). https://doi.org/10.51298/vmj.v563i3.19152
2. Duong, D. P., Phan, D. V., Nguyen, T. T. L., Nguyen, T. N., Nguyen, T. T., Hoang, D. C., & Nguyen, T. T. (2026). Clinical and Paraclinical Characteristics and Differences According to Tumor Location in Patients with Colorectal Cancer at Nghe An Oncology Hospital. *Vietnam Medical Journal*, 563(3). https://doi.org/10.51298/vmj.v563i3.191652