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A preliminary investigation of the association between KRAS, NRAS, and BRAF mutations and colorectal cancer in Turkish patients.

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Keywords: NRAS; BRAF; KRAS; colorectal cancer.

Abstract. Somatic mutations in the GTPase RAS protein family and the downstream serine-threonine kinase BRAF are predicted to be key driver mutations in colorectal carcinogenesis by disrupting critical control points in cell cycle regulation. In our study, we aimed to investigate the relationship between KRAS, NRAS, and BRAF mutations in colorectal cancer (CRC) samples and corresponding clinicopathological data. This retrospective study included 64 CRC patients who were evaluated for KRAS, NRAS, and BRAF mutations in our department between 2022 and 2024. The findings were evaluated according to the age, gender, tumor localization in the colon, and histopathological subtype of the patients in whom the mutation was detected, and the relationships between these variables were analyzed using the chi-square test. KRAS mutations were detected at 29.6%, NRAS mutations at 3.1% and BRAF mutations at 1.6%. No significant relationship was found between mutation rates and the patients' age, gender and colon localization. Our study demonstrated that mutations in KRAS, NRAS, and BRAF were not associated with the age, sex, and tumor location of CRC patients. The data presented are preliminary findings, and more research is needed to evaluate the clinical and pathological impact of these mutations on colorectal cancer progression and outcomes.

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Una investigación preliminar sobre la asociación entre mutaciones KRAS, NRAS y BRAF en cáncer colorrectal en pacientes Turcos.

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Palabras clave: NRAS; BRAF; KRAS; cáncer colorrectal.

Resumen. Se predice que las mutaciones somáticas en la familia de las proteínas GTPasa RAS y la serina-treonina quinasa BRAF son mutaciones clave en la carcinogénesis colorrectal al interrumpir puntos de control críticos en la regulación del ciclo celular. En este estudio, el objetivo fue evaluar la relación entre las mutaciones KRAS, NRAS y BRAF en muestras de cáncer colorrectal (CCR) y datos clinicopatológicos. Este estudio retrospectivo incluyó a 64 pacientes con CCR que fueron evaluados para mutaciones en KRAS, NRAS y BRAF en nuestro servicio entre 2022-2024. Los hallazgos se evaluaron según la edad, el sexo, la localización del tumor en el colon y el subtipo histopatológico de los pacientes en los que se detectó la mutación, y se analizaron las relaciones entre ellos mediante la prueba de chi-cuadrado. Las mutaciones en KRAS se detectaron en un 29,6%, en NRAS en un 3,1% y en BRAF en un 1,6%. No se encontró una relación significativa entre las tasas de mutación y la edad, el sexo y la ubicación del colon de los pacientes. Nuestro estudio demostró que las mutaciones en KRAS, NRAS y BRAF no se asociaron con la edad, el sexo y la localización del tumor de los pacientes con CCR. Los datos presentados son nuestros hallazgos preliminares y se necesita más investigación para evaluar el impacto clínico y patológico de estas mutaciones en la progresión y los resultados del cáncer colorrectal.

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INTRODUCTION

The incidence rates of colorectal cancer (CRC) in adults (under 55 years of age) have been increasing by 1–2% annually. In the late 1990s, CRC was the fourth leading cause of cancer death in both men and women under 50. However, it has since escalated to become the leading cause of cancer death in men and the second leading cause in women within this age group [1]. CRCs are the second leading cause of death from malignancy, and more than half of CRCs become metastatic [2]. Despite the substantial impact this disease has on both quality of life and the healthcare system, data regarding the molecular analysis of biomarkers in patients diagnosed with CRC is limited [3].

Several oncogenes, particularly mutations in RAS and BRAF, have a significant influence on colorectal carcinogenesis. These mutations lead to the activation of the mitogen-activated protein kinase signalling pathway, which is crucial for cell proliferation, differentiation, and survival. Activation of this pathway promotes tumorigenesis by driving uncontrolled cell growth and facilitating processes such as angiogenesis and metastasis. Understanding the role of these oncogenes provides insight into the mecha-

nisms of colorectal cancer and identifies potential therapeutic targets [4].

Identifying abnormalities in the KRAS, NRAS, and BRAF genes is crucial for accurately qualifying CRC patients for treatment with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies [5]. Oncogenic RAS mutations are found in approximately 50–55% of metastatic colorectal cancer (CRC) cases, with regional variations in prevalence. Importantly, these mutations serve as negative predictive markers for response to monoclonal antibodies that target the epidermal growth factor receptor (EGFR). This means that patients with RAS mutations are less likely to benefit from EGFR-targeted therapies, making it crucial to screen for these mutations when determining treatment options for metastatic colorectal cancer (mCRC). Identifying RAS status can help guide more effective and personalized treatment strategies [4]. BRAF mutations occur in approximately 5–17% of CRC cases, with the BRAFV600E mutation being the most prevalent. In mCRC, the existence of the BRAFV600E mutation not only serves as a negative predictive marker for response to EGFR-targeted MoAbs but is also associated with a markedly poor prognosis. This mutation often indicates a more aggressive disease course and resistance to conventional therapies, highlighting the need for alternative treatment strategies and close monitoring of affected patients. Understanding the implications of BRAF status is crucial for optimizing management and enhancing outcomes in mCRC. The status of tumor localization and CC laterality in determining prognosis and guiding treatment remains controversial. It is thought to be due to differences in histological, genetic and immunological features between the left and right colon [6,7]. In the present study, we hypothesized that somatic mutations in the GTPase RAS protein family and the downstream serine-threonine kinase BRAF could interfere with essential checkpoints in cell cycle regulation, acting as significant driving mutations in colorectal carcinogenesis. Our goal was to assess the presence of KRAS, NRAS, and BRAF mutations in clinicopathological features of CRC samples in our hospital.

PATIENTS AND METHODS

Study population and design

For this retrospective study, KRAS, NRAS, and BRAF mutations were analyzed in 64 CRC biospecimens at the Bakırçay University Faculty of Medicine Laboratory. Patients were characterized by age, gender, and tumor location, with rectal and sigmoid cancers being the most prevalent. The histological classification of tumors by the WHO was used as a guide to assign the histological subtypes (classical adenocarcinoma and mucinous adenocarcinoma) and tumor location (colon segment other than rectum, and rectum) [8].

To conduct the study, ethics committee approval was obtained from the İzmir Bakırçay University Non-Interventional Clinical Research Ethics Committee on 03.04.2024, with decision number 1681 and research number 1661.

DNA extraction was conducted using the QIAamp formalin-fixed paraffin-embedded (FFPE) kit (Qiagen, USA). Tissue samples were collected at the time of diagnosis for colon and rectal cancer. FFPE primary tumor samples from 64 CRC patients between January 2022 and December 2024 were retrospectively reviewed. Mutation analysis was performed with the KRAS/BRAF, NRAS, and BRAF Mutation Analysis Kit for Real-Time PCR (Diatech Easy, Italy). The tests targeted the most common mutations in exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), exon 4 (codons 117 and 146) of the KRAS and NRAS genes, and exon 15 (V600E) of the BRAF gene.

Statistical Analysis

For the statistical analysis, a chi-square test was used to examine the associations

between mutated genes (KRAS, NRAS, and BRAF) and various clinicopathological variables, including age, gender, and the specific localization of the tumor in the colon. The analysis was conducted using SPSS version 23.0, and p-values of ≤ 0.05 were deemed statistically significant, contributing to a better understanding of their implications in colorectal cancer.

RESULTS

The study included patients aged 33 to 79 years, with a mean age of 62.54 and a median age of 64. Among them, 41 (64%) were male and 23 (36%) were female (Table 1). In our study, no mutations were found in 20 patients (31.2%). KRAS exon 2 mutation was observed in 29.7% of cases, while other RAS mutations were found in 3.1% of cases, and the BRAFV600E mutation was seen in 1.6%. One patient had KRAS mutations at positions 12 and 13, and the other had KRAS mutations at position 61 and NRAS mutations at position 12.

Table 1. Mutation profile of colorectal cancer patients stratified by age and gender.

	Wild type *n = 44*	Mutant *n = 20*	*p*
Age <64	26 (76.5%)	8 (23.5%)	
Age ≥64	18 (60%)	12 (40%)	0.125
Female	18 (78.3%)	5 (21.7%)	
Male	26 (63.4%)	15 (36.6%)	0.172

No associations were found between the age and gender of CRC patients and the frequency of KRAS, NRAS, and BRAF gene mutations. Notably, these mutations were more frequently diagnosed in men (64%) than in women (36%). Table 1 shows the distribution of mutation presence in patients with colorectal cancer according to age and gender.

A total of 47 cases had tumor localization identified. In 14 cases, the tumors were located in the left colon, while 33 cases were localized in the right colon. In the case of the BRAFV600 mutation, tumor localization could not be determined. The distribution of RAS and BRAF mutation values in colorectal cancer patients according to age, sex and localization is shown in Tables 2, 3, and 4.

DISCUSSION

Colorectal cancer is the third most common type of cancer in both sexes in Turkey. According to the 2024 official cancer statistics, 152,810 (7.6%) of the 2,001,140 cancer cases diagnosed in 2019 were CRC. Reports indicate a significantly higher incidence in men (53.3%) compared to women [1,4]. In our study, most of the patients were male (64.1%), consistent with national cancer statistics [1].

Our study aimed to evaluate the presence of somatic mutations in the GTPase RAS family of proteins (KRAS and NRAS) and the downstream serine-threonine kinase BRAF in CRC samples. It is hypothesized

Table 2. Distribution of KRAS, NRAS and BRAF mutations by age in colorectal cancer patients.

	KRAS			NRAS			BRAF		
	Wild type *n=45*	Mutant *n=18*	Total *n=63*	Wild type *n=62*	Mutant *n=2*	Total *n=64*	Wild type *n=63*	Mutant *n=1*	Total *n=64*
Age <64	24 (38%)	8 (12.7%)	32 (50.7%)	33 (51.6%)	1 (1.6%)	34 (53.2%)	34 (53.1%)	0 (0%)	34 (53.1%)
Age ≥64	21 (33.3%)	10 (15.9%)	31 (49.2%)	29 (45.3%)	1 (1.6%)	30 (46.9%)	29 (45.3%)	1 (1.6%)	30 (46.9%)
p		0.360			0.722			0.469	

Table 3. Sex-based frequency of BRAF, KRAS, and NRAS mutations in colorectal cancer patients.

	BRAF			KRAS			NRAS		
	Wild type *n=63*	Mutant *n=1*	TOTAL *n=64*	Wild type *n=46*	Mutant *n=18*	Total *n=64*	Wild type *n=62*	Mutant *n=2*	Total *n=64*
Female	23 (36%)	0 (0%)	23 (36%)	33 (51.5%)	4 (6.2%)	23 (35.9%)	22 (34.2%)	1 (1.7%)	23 (35.9%)
Male	40 (62.5%)	1 (1.5%)	41 (64%)	27 (42.2%)	14 (21.9%)	41 (64%)	40 (62.5%)	1 (1.5%)	41 (64%)
p		0.414			0.126			0.641	

Table 4. Distribution of KRAS and NRAS mutation frequencies according to tumor location in colorectal cancer.

	KRAS		NRAS		
Tumor Location	Wild type *n=31*	Mutant *n=16*	Wild type *n=45*	Mutant *n=2*	Total *n=47*
Colon	11 (23.4%)	3 (6.4%)	13 (19.4%)	1 (1.5%)	14 (20.9%)
Rectum	20 (29.9%)	13 (19.4%)	32 (47.8%)	1 (1.5%)	33 (49.3%)

that these mutations might disrupt critical checkpoints in cell cycle regulation and serve as key driving factors in colorectal carcinogenesis. The detection rates of KRAS, NRAS, and BRAF mutations in our cohort of 64 CRC patients were 29.7%, 3.1%, and 1.6%, respectively. Consistent with the findings of Mosaferi et al. [8], our study also observed that the majority of KRAS mutations were located in exon 2, while most NRAS mutations were found in exon 3 [8]. In line with the findings of Khoshnoudi et al. [2] and Mosaferi et al. [8], our study found no significant association between KRAS mutation status and age or gender.

The prevalence of KRAS mutations in our study aligns with findings from the literature, which consistently reports that KRAS mutations occur in approximately 40% of CRC cases [9]. These mutations are critical in terms of poor prognosis and resistance to targeted therapies, such as anti-EGFR agents. [10,11] The relatively lower frequency of NRAS (3.1%) and BRAF mutations (1.6%) observed in our study is consistent with their reported rates in the literature, where BRAF mutations are often associated with more advanced disease stages and poorer outcomes [12,13]. Our findings showed that mutation rates were not significantly associated with the patients' age, gender, or colon localization.

A study by Yamauchi et al. [14] showed that the frequency of BRAF mutations increased from the rectum to the ascending colon and decreased towards the cecum. Ekmekciu et al. [15] reported that KRAS mutations were predominantly located in the right colon, but no significant differences were observed in age, stage, or histopathological subtype. Bylsma et al. [16] found BRAF and RAS mutations more frequently in right-sided colon cancers, consistent with Ekmekciu et al. [15]. In addition, Bylsma et al. reported that BRAF and RAS mutations were more common in young patients [16]. Our findings showed that mutation rates were not significantly associated with patients' age, gender, or colon localization.

We would like to emphasize that the present study has limitations related to the cohort size. The limitations of the study included that some cases in the study group were obtained from consultations with external centers, while others were obtained from colonoscopic biopsy materials. For these reasons, the relationship between prognostic markers for tumor staging and mutation results could not be compared in our study. This study is essentially an exploratory, preliminary study, which requires definitive, confirmatory values. Our findings highlight the complex interaction between molecular subtypes, clinical and histopathological features in CRC and the need for further investigation of the underlying mechanisms driving these relationships. In the future, stratifying patients according to molecular subtypes may provide advantages in personalized treatment approaches.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this article.

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Author contributions

TD and SST designed the study. TD analyzed and interpreted data and searched the literature. TD and GE drafted the manuscript. All authors reviewed the manuscript and approved the final version.

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