THE PREVALENCE OF KRAS, NRAS, AND BRAF MUTATIONS IN METASTATIC COLORECTAL CANCER PATIENTS IN JORDAN

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ABSTRACT

Background: Metastatic Colorectal Cancer (mCRC) contributes significantly to cancer-related fatalities worldwide. Mutations within the Kirsten rat sarcoma viral oncogene homolog (KRAS), neuroblastoma RAS viral oncogene homolog (NRAS), and v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) genes have been identified as pivotal drivers in the development and progression of colon cancer. Their presence is closely linked to a dismal prognosis and resistance to therapy. The prevalence of these mutations among mCRC patients varies among different populations and geographical regions, with limited documentation regarding their prevalence in Jordan.

Aim: This study aimed to assess the prevalence of KRAS, NRAS, and BRAF mutations among mCRC patients in Jordan.

Materials and Methods: In this retrospective study, we examined the medical records of 129 mCRC patients treated at the Military Cancer Center (MCAC) from 2017 to 2022. The tumors were subjected to analysis for KRAS, NRAS, and BRAF mutations using quantitative polymerase chain reaction (PCR).

Results: The analysis encompassed a total of 129 patients. Among them, 52 (40.3%) exhibited KRAS mutations, while 5 (3.9%) displayed NRAS mutations, and another 5 (3.9%) carried BRAF mutations.

Conclusion: Our findings indicate that the prevalence of KRAS mutations in Jordan surpasses that observed in other Middle Eastern countries, such as Saudi Arabia, and is comparable to rates seen in several North African countries like Egypt and Algeria, as well as certain European countries.

Keywords: Colon cancer, KRAS, NRAS, BRAF, prevalence, Jordan
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INTRODUCTION

Colorectal cancer (CRC) ranks as the third most common malignancy globally, and its incidence has witnessed an upward trend in Jordan during recent decades [1, 2]. In Jordan, CRC is the most frequently diagnosed cancer among men and the second most prevalent among women [2]. KRAS, NRAS, and BRAF mutations have emerged as pivotal contributors to the pathogenesis of CRC, prompting their incorporation into routine diagnostic assessments in numerous countries [3]. This is primarily because these biomarkers serve as the primary indicators for predicting the response of metastatic CRC (mCRC) patients to targeted therapy involving anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (MAbs) [4].

Nonetheless, there exists a paucity of data regarding the prevalence of these mutations in Jordanian patients with colon cancer.The family of oncogenes known as RAS includes three key members: KRAS, NRAS, and HRAS genes, whose protein products play pivotal roles in essential cellular processes like cell division, differentiation, and apoptosis [5]. Among RAS mutations identified in human tumors, activating mutations are observed in these genes in approximately 85%, 15%, and less than 1% of the KRAS, NRAS, and HRAS genes, respectively. In roughly 30–40% of cases involving CRC, a mutated KRAS gene is recognized as a critical genetic alteration that propels the transition from adenoma to CRC [5].

BRAF-activating mutations are detected in 5–15% of CRC cases and are associated with poor prognoses. The predominant BRAF mutation, responsible for nearly 95% of reported mutations, entails a substitution of valine with glutamic acid at codon 600 (BRAF V600E). This genetic alteration results in a persistently active protein akin to tumors with KRAS mutations. It is worth noting that simultaneous mutations involving both RAF and RAS are rarely encountered [6]; RAS and RAF mutations are typically considered mutually exclusive, occurring only 1% of the time [7].

MATERIALS & METHODS

Study Design

This retrospective study examined the medical records of 129 patients diagnosed with mCRC between 2017 and 2022 at the Military Cancer Center (MCAC). Tumor samples were subjected to analysis for KRAS, NRAS, and BRAF mutations through quantitative PCR conducted at an accredited diagnostic laboratory located at Jordan University Hospital. Additionally, the study documented clinical and pathological patient characteristics, such as age, gender, primary tumor location, and metastatic sites.

The primary tumor location was determined using the following criteria: (1) Right-sided tumors were defined as those located in the cecum, ascending colon, hepatic flexure, and transverse colon; (2) Left-sided tumors were categorized based on the primary tumor's location in the splenic flexure, descending colon, sigmoid colon, and rectum.

Tumor Molecular Analysis

Quantitative polymerase chain reaction (PCR) and reverse hybridization techniques were employed to determine the mutation status of KRAS and NRAS in tumor tissue biopsies obtained from all patients. These biopsies were analyzed at an accredited diagnostic laboratory at Jordan University Hospital. For KRAS testing, the focus was on detecting mutations in the following codons: 12 and 13 of exon 2, codons 59, 60, and 61 of exon 3, as well as codons 117 and 146 of exon 4. Similarly, NRAS testing aimed to identify mutations in codons 12 and 13 of exon 2, 59, 60, and 61 of exon 3, and codon 146 of exon 4.

Statistical Analysis

Descriptive statistics were employed to summarize the patient characteristics. Categorical data were presented in terms of frequencies and percentages, while numerical data were described using either the mean and standard deviation or the median and range, depending on the nature of the data. Chi-square tests or Fisher's Exact tests were utilized to assess the association between KRAS, NRAS, and BRAF mutations and the characteristics of patients' diseases.

Ethical Considerations

This study is an observational retrospective investigation, and all patients received standard clinical care within routine clinical practice. The Institutional Review Board (IRB) at the Jordanian Royal Medical

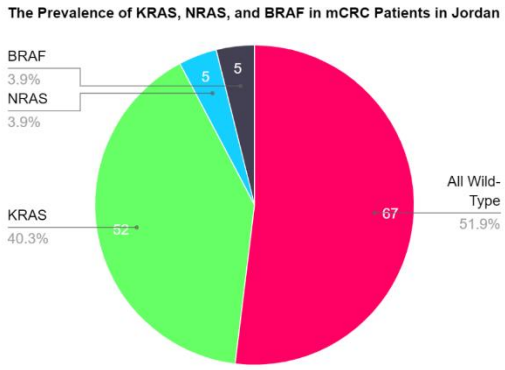
Services in Amman, Jordan, approved the study.

RESULTS

A total of 129 patients were included in the analysis. Among these, 52 (40.3%) had a KRAS mutation, five (3.9%) had an NRAS mutation, and five (3.9%) had a BRAF mutation (Figure 1). Most patients fell within the age range of 40 to 60 years (57.4%), followed by those aged 61 or older (27.9%) and those under 40 (14.7%). There were slightly more male patients (60.5%) than female patients (39.5%). The primary tumor location was predominantly on the left side of the colon (84.5%), with only 15.5% located on the right side. The most common metastatic site was the liver (48.1%), followed by the lung (17.8%) and peritoneal involvement (12.4%). Metastasis involving two or more organs was present in 14.7% of patients, and 7% had metastasis in other sites. Descriptive statistics can be found in Table 1.

Nine different codons were identified within the mutant KRAS gene. The most prevalent mutation was Gly12Asp, observed in 19 patients (36.5%), followed by Gly12Val in 12 patients (23.1%) and Gly13Asp in seven patients (13.5%). The remaining KRAS mutations were less frequently encountered.

Figure 1: The Prevalence of KRAS, NRAS, and BRAF in CRC Patients in Jordan



Three different codons were identified within the mutant NRAS gene: three patients (60%) had a Gln61Lys mutation, one patient (20%) had a Gln61Arg mutation, and one patient (20%) had a Gly12Val mutation. All five patients with a BRAF mutation exhibited the V600E mutation.

Table 1: Descriptive statistics of patient's demographics and tumor characteristics

Variable	Category	Number of Patients	Percentage
Age in groups	< 40	19	14.7%
	40-60	74	57.4%
	≥ 61	36	27.9%

Variable	Category	Number of Patients	Percentage
Gender	Female	51	39.5%
	Male	78	60.5%
KRAS Status	Mutant	52	40.3%
	Wild-type	77	59.7%
NRAS Status	Mutant	5	3.9%
	Wild-type	124	96.1%
BRAF Status	Mutant	5	3.9%
	Wild-type	124	92.2%
Primary Tumor Location	Left	109	84.5%
	Right	20	15.5%
Metastatic Site	Liver	62	48.1%
	Lung	23	17.8%
	Peritoneal	16	12.4%
	≥2 organs involved	19	14.7%
	Other	9	7%

These findings suggest that KRAS mutations are the most prevalent genetic alterations within the studied population, with Gly12Asp and Gly12Val being the most frequently observed mutations. The presence of NRAS and BRAF mutations was less common. Notably, the V600E mutation was the sole BRAF mutation identified in this cohort. Descriptive statistics detailing specific mutations in the KRAS, NRAS, and BRAF genes can be found in Table 2.

Table 2: Specific mutations in KRAS, NRAS, and BRAF genes.

Gene	Codon	Frequency (%)	Total Mutations
KRAS	Gly12Asp	19 (36.5%)	52
	Gly12Val	12 (23.1%)	
	Gly13Asp	7 (13.5%)	
	Lys117Asp	3 (5.8%)	
	Gly12Cys	3 (5.8%)	
	Gly12Ser	4 (7.7%)	
	Gly12Ala	2 (3.8%)	
	Ala59Glu	1 (1.9%)	
	Ala59Thr	1 (1.9%)	
NRAS	Gln61Lys	3 (60%)	5
	Gln61Arg	1 (20%)	
	Gly12Val	1 (20%)	
BRAF	V600E	5 (100%)	5

We conducted a Chi-square analysis to investigate potential associations between KRAS mutations and the clinicopathological characteristics of patients, including age, gender, primary tumor location, and metastatic site. Conversely, for NRAS and BRAF mutations, we employed Fisher's Exact test.

KRAS

There is a statistically significant association between KRAS mutation and primary tumor location, with KRAS mutation being most observed in right-sided tumors ($p = 0.014$). No significant associations were found between KRAS mutation and age ($p = 0.549$), gender ($p = 0.567$), or metastatic site ($p = 0.765$) (Table 3).

Table 3: Chi-square analysis between KRAS mutation and the patient's clinicopathological characteristics.

Characteristic	Mutant KRAS	Wild-Type KRAS	Total	p-value
Age				0.549
<40	9 (47.4%)	10 (52.6%)	19	
40-60	31 (41.9%)	43 (58.1%)	74	
≥61	12 (33.3%)	24 (66.7%)	36	
Gender				0.567
Male	33 (42.3%)	45 (57.7%)	78	
Female	19 (37.3%)	32 (62.7%)	51	
Primary Tumor Location				
Left	39 (35.8%)	70 (64.2%)	109	0.014
Right	13 (65%)	7 (35%)	20	
Metastatic Site				0.765
Liver	25 (40.3%)	37 (59.7%)	62	
Lung	11 (47.8%)	12 (52.2%)	23	

Characteristic	Mutant KRAS	Wild-Type KRAS	Total	p-value
≥2 Organs	8 (42.1%)	11 (57.9%)	19	
Other	2 (22.2%)	7 (77.8%)	9	
Peritoneal	6 (37.5%)	10 (62.5%)	16	

NRAS

The analysis did not reveal significant associations between NRAS mutation and the clinicopathological characteristics studied. The p-values for all characteristics were greater than 0.05 (Table 4).

Table 4: Fisher's Exact test between NRAS mutation and the patient's clinicopathological characteristics.

Clinicopathological Characteristic	Mutant NRAS	Wild-type NRAS	Total	p-value
Age				0.688
< 40	0 (0%)	19 (100%)	19	
40-60	3 (4.1%)	71 (95.9%)	74	
≥ 61	2 (5.6%)	34 (94.4%)	36	
Gender				1.000
Male	3 (3.8%)	75 (96.2%)	78	
Female	2 (3.9%)	49 (96.1%)	51	

Clinicopathological Characteristic	Mutant NRAS	Wild-type NRAS	Total	p-value
Primary Tumor Location				1.000
Left	5 (4.6%)	104 (95.4%)	109	
Right	0 (0%)	20 (100%)	20	
Metastatic Site				
Liver	3 (4.8%)	59 (95.2%)	62	0.614
Lung	1 (4.3%)	22 (95.7%)	23	
≥ 2 Organs Involved	0 (0%)	19 (100%)	19	
Peritoneal	0 (0%)	16 (100%)	16	
Other	1 (11.1%)	8 (88.9%)	9	

BRAF

There was a significant association between BRAF mutation and primary tumor location ($p = 0.026$). However, no significant association was found between BRAF mutation and the other clinicopathological characteristics studied (Table 5).

Table 5: Fisher's exact test between BRAF mutation and the patient's clinicopathological characteristics.

Clinicopathological Characteristic	Mutant BRAF	Wild-type BRAF	Total	p-value
Age				0.688

Clinicopathological Characteristic	Mutant BRAF	Wild-type BRAF	Total	p-value
< 40	0 (0%)	19 (100%)	19	
40-60	3 (4.1%)	71 (95.9%)	74	
≥ 61	2 (5.6%)	34 (94.4%)	36	
Gender				0.383
Male	2 (2.6%)	76 (97.4%)	78	
Female	3 (5.9%)	48 (94.1%)	51	
Primary Tumor Location				0.026
Left	2 (1.8%)	107 (98.2%)	109	
Right	3 (15%)	17 (85%)	20	
Metastatic Site				
Liver	4 (6.5%)	58 (93.5%)	62	0.647
Lung	0 (0%)	23 (100%)	23	
≥ 2 Organs Involved	0 (0%)	19 (100%)	19	
Peritoneal	1 (6.2%)	15 (93.8%)	16	
Other	0 (0%)	9 (100%)	9	

DISCUSSION

Colon cancer is a multifaceted and diverse ailment influenced by genetic and environmental factors in its pathogenesis [8]. Of the numerous genetic modifications linked to colon cancer, mutations in the KRAS, NRAS, and BRAF genes stand out as the most prevalent. The frequency of these mutations within the KRAS, NRAS, and BRAF genes in CRC exhibits variability among diverse populations and geographical regions. We identified commonalities and distinctions when juxtaposing our study findings with those from other research endeavors.

Like our investigation, Elbjeirami et al. conducted a study in Jordan and reported a KRAS mutation rate of 44% in individuals with CRC [9]. Our study observed a prevalence of *KRAS* mutations at 40.3%. These findings suggest that the incidence of *KRAS* mutations in Jordan surpasses that reported in certain other Arabic nations, such as Lebanon (38.5%), Saudi Arabia (34.7%), and Egypt (27.4%) [10, 11].

Furthermore, the prevalence of KRAS mutations in Jordan, as evidenced by our study and that of Elbjeirami et al., closely mirrors findings from a study that aggregated data from 11 centers across seven European countries, reporting a similar prevalence of 40% [12].

Our study found that 3.9% of mCRC patients exhibited NRAS mutations, while an equal percentage had BRAF mutations. To provide context for these findings, we conducted a comparative analysis with a comprehensive meta-analysis comprising 275 studies and over 77,000 mCRC patients. This meta-analysis documented prevalence rates of 7.1% for BRAF mutations and 4.1% for NRAS mutations.

Remarkably, our study unveiled a slightly lower prevalence of NRAS mutations at 3.9%, compared to the meta-analysis rate of 4.1%. Conversely, we noted a more pronounced dissimilarity in the prevalence of BRAF mutations, with our study reporting a rate of 3.9%, while the meta-analysis indicated a prevalence of 7.1%. It is crucial to acknowledge that this disparity may stem from our study's relatively smaller sample size, which could account for the variability in prevalence estimates [13].

In our examination of the connection between KRAS, NRAS, and BRAF mutations and the clinicopathological characteristics of patients, we identified a noteworthy association between KRAS mutations and the primary tumor's location. Specifically, KRAS mutations were more frequently observed in tumors on the colon's right side. This observation aligns with prior research, which has consistently reported a heightened prevalence of KRAS mutations in right-sided colon tumors [14].

Furthermore, our study revealed a significant association between BRAF mutations and female patients. This discovery aligns with previous research that has consistently reported a higher incidence of BRAF mutations among female patients [15].

We did not detect any significant association between NRAS mutations and the clinicopathological characteristics of the patients.

ONCLUSIONS

Our study provides insights into the prevalence of KRAS, NRAS, and BRAF mutations among CRC patients in Jordan. We observed that the prevalence of KRAS mutations in Jordan exceeded that in other Middle Eastern nations, including Saudi Arabia and Lebanon, as well as several North African countries, such as Egypt and Algeria. Interestingly, it mirrored the prevalence seen in certain European countries. These findings underscore the significance of regional disparities in KRAS mutation prevalence in mCRC and emphasize the necessity for ongoing research to understand the underlying factors contributing to these variations.

CONFLICT OF INTEREST

None.

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None.

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