

Molecular Spectrum of K-Ras Mutations in Colorectal Cancer Patients in Peshawar, Pakistan

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ABSTRACT

Background: The global incidence of colorectal cancer (CRC) has surged, presenting a significant health challenge with an annual occurrence of 1.7 million cases. The American Cancer Society reports that KRAS and BRAF mutations contribute to 20-50% of CRC cases. This study addresses the gap in data on KRAS mutations in CRC patients in Peshawar, Pakistan.

Objective: To determine the prevalence and types of KRAS mutations in colorectal cancer patients in Peshawar, Pakistan.

Methods: This retrospective, cross-sectional study was conducted at the Nuclear Medicine, Oncology, and Radiotherapy Institute (NORI) Hospital in Islamabad from April to November 2022. DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks of 58 CRC patients, regardless of age and gender, following informed verbal consent. KRAS mutation testing was performed using the Cobas Z480 Real-time thermal cycler.

Results: Among the 58 CRC patients, 34 (58.6%) were females, and 24 (41.4%) were males. The highest CRC incidence (36.2%) was observed in the 11-25 years age group. KRAS mutations at codons 12 and 13 were found in 18% of cases, with all isolates carrying the G12D mutation. Kaplan-Meier analysis revealed a lower survival probability for patients with codon 12 mutations compared to those with codon 13 mutations.

Conclusion: The study highlights the need for broader research on KRAS mutations across diverse ethnicities and geographical regions in Pakistan to better understand CRC prevalence and improve patient outcomes.

Keywords: BRAF mutation, colorectal cancer, CRC prevalence, G12D mutation, KRAS mutation.

INTRODUCTION

Colorectal cancer (CRC) is a collective term for malignancies affecting the colon and rectum, and it represents one of the most prevalent forms of cancer worldwide. Historically, the incidence of CRC has increased with the advancement of civilization, positioning it as a significant cause of morbidity and mortality globally. It is the second most common cancer among women, accounting for approximately 9.2% of all cancer cases, and the third most common among men, making up 10% of cases (1). Despite the higher incidence rates observed in developed nations, where 55% of CRC cases occur, mortality rates are disproportionately higher in developing countries, accounting for 52% of deaths compared to 48% in developed regions (2).

In Pakistan, particularly in the region of Khyber Pakhtunkhwa, the prevalence of CRC has shown a steady increase since the country's independence in 1947. A report from Shaukat Khanum Hospital in 2012 highlighted that CRC accounted for 5% of the total cancer diagnoses, being the sixth most common cancer among women and the second among men in a pool of 4,851 cancer patients (3). The development of CRC involves a complex multi-step process characterized by the accumulation of genetic and epigenetic alterations. These changes include mutations in critical oncogenes and tumor suppressor genes that disrupt normal cellular processes such as cell growth and apoptosis.

Mutations in the RAS and RAF gene families are particularly significant in metastatic CRC, with the Kirsten rat sarcoma viral oncogene homolog (KRAS) being one of the most frequently affected. KRAS mutations, primarily occurring in codons 12 and 13 of exon 2, are

implicated in about 85% of all RAS gene mutations in human cancers. These mutations lead to the continuous activation of signaling pathways such as PI3K/AKT and RAF/MEK/ERK, which are crucial for cell proliferation, migration, and survival (4, 5). Consequently, patients with CRC displaying these mutations often do not benefit from anti-epidermal growth factor receptor (EGFR) therapies, such as panitumumab and cetuximab, which are effective only in tumors expressing the wild-type KRAS gene (7).

Given the significant impact of KRAS mutations on CRC prognosis and treatment outcomes, the current study aims to delineate the mutation patterns of the KRAS gene, specifically within codons 12 and 13, among colorectal cancer patients in Peshawar, Pakistan. Understanding these mutation patterns will provide insights into the molecular mechanisms underlying CRC in this population and help refine therapeutic strategies for improved patient outcomes.

METHODS

The research was sanctioned by the Board of Study and the Advanced Study Research Board at the Department of Medical Laboratory Technology, University of Haripur, and conducted at the Nuclear Medicine, Oncology, and Radiotherapy Institute (NORI) Hospital, Islamabad. Ethical approval was in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

For this study, 58 colorectal cancer samples were collected from diagnosed patients at Hayatabad Medical Complex, Peshawar, using a non-probability convenient sampling method. The age and gender of the patients were not considered for selection. Samples, received from the Operation Theater, were preserved in 10% formalin within plastic containers and subsequently transported at temperatures ranging from 15-30 °C for further processing.

The formalin-fixed paraffin-embedded tissue (FFPET) samples, sectioned to 5µm thickness containing at least 50% tumor cells, were prepared for molecular analysis. DNA was isolated from FFPET-mounted slides following the protocol established by Patel et al. (9). The extracted DNA was then stored at -20 °C until further use.

For the detection of KRAS mutations, a quantitative real-time polymerase chain reaction (qPCR) was conducted using the Cobas z480 analyzer (Roche Molecular Diagnostics, Pleasanton, CA, USA) and the AmoyDx kit (Amoy Diagnostics, China). This kit facilitates the qualitative detection of mutations in codons 12, 13, 59, 61, 117, and 146 of the KRAS gene. Additionally, to confirm the qPCR results, polymerase chain reaction (PCR) was performed on all samples. The resulting DNA fragments were visualized via 2% agarose gel electrophoresis, validating the presence of mutations in codons 12 and 13 as identified by qPCR.

Table 1: PCR primers (10)

Gene	PCR primers	Product size
KRAS	GCTGAAAATGACTG (Forward)	113 bp
	TTGTTGGATCATATTCGTCCAC (Reverse)	

RESULTS

In this study, a detailed analysis was conducted on the prevalence and patterns of KRAS mutations among colorectal cancer (CRC) patients residing in the Peshawar area of Khyber Pakhtunkhwa, Pakistan—a region from which, to our knowledge, no previous CRC molecular data have been reported. The investigation into gene-environment interactions was particularly pertinent, given the diverse ethnic backgrounds and environmental exposures of the population studied. It was found that KRAS mutations in metastatic CRC patients varied significantly with tumor sidedness, impacting both the survival rates and progression of the disorder. Moreover, the type of RAS mutation has been demonstrated to predict the response to anti-epidermal growth factor receptor therapy (EGFR). The study encompassed a sample population of 58 CRC patients, among which 24 (41.4%) were males and 34 (58.6%) were females, with females being predominantly affected. This gender distribution aligns with another study which reported that 32 (62%) of CRC patients were male and 132 (68%) were female, suggesting that variations may stem from socio-demographic and clinical characteristics of the patients (11). Another relevant demographic feature observed was the age range of the patients, which spanned from 21 to 87 years, with a mean age of 59.08±15.55 years.

Mutation detection rates indicated that among the 58 samples analyzed, mutations in codons 12 and 13 of the KRAS gene were identified in 11 (18.9%) patients. The highest mutation rates were observed in the younger age group (11 to 25 years), where 21 samples were collected, and 5 (8.6%) exhibited mutations. Conversely, the lowest mutation rates were found in the age group of 41 to 55 years, with only 1 (1.2%) out of 13 patients showing mutations.

Analysis of the specific types of KRAS mutations revealed that Glycine to Aspartate substitution (G12D) was detected in 2 patients (18.18%), Glycine to Valine (G12V) in 6 patients (54.54%), and Glycine to Aspartate (G12D) again in 3 patients. According to Awidi et

al., KRAS mutations in exon 2 were prevalent in 76 out of 92 cases (83.6%), with G12D being the most common mutation observed (8).

The distribution of CRC across different parts of the large intestine showed varied involvement, with the rectum being the most affected area in 6 females and 2 males, followed by the colon in 2 males, and the sigmoid colon in 1 male. Comparative studies have highlighted that the prevalence of tumors in the recto-sigmoid region is significantly higher (54.7%) compared to other regions of the colon.

Furthermore, survival analysis using the Kaplan-Meier method indicated a statistically significant decrease in survival probability for patients with mutations in KRAS, particularly those with mutations in codon 12 (P=0.002). However, survival probability differences related to codon 13 mutations were not statistically significant (P=0.50), potentially reflecting the lower prevalence and different functional impacts of these mutations.

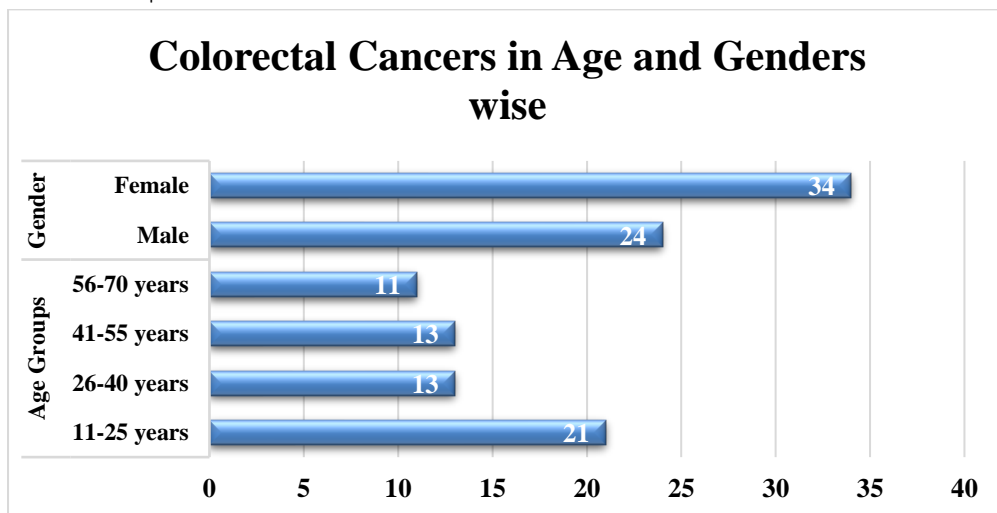


Figure 1 Age and gender wise frequency of colorectal cancer

Table 2: Group wise distribution of CRC patient

		Mutations in KRAS gene		Total
		Detected	Not detected (wild type)	
Age Groups	11 to 25 years	5 (8.6%)	16 (27.5%)	21 (36.2%)
	26 to 40 years	3 (5.1%)	10 (17.2%)	13 (22.4%)
	41 to 55 years	1 (1.2%)	12 (20.6%)	13 (22.4%)
	56 to 70 years	2 (3.4%)	9 (15.5%)	11 (18.9%)
Total		11 (18.9%)	47 (81.03%)	58 (100%)

Table 3: Gender wise distribution of KRAS mutation at codon 12 and 13

Codon	Nucleotide Substitution	Amino Acid Substitution	N	%
12	GGT to GTT	Glycine to Aspartate	02	18.18
12	GGT to GAT	Glycine to Valine	06	54.54
13	GGC to GAC	Glycine to Aspartate	03	27.27
	Total		11	100%

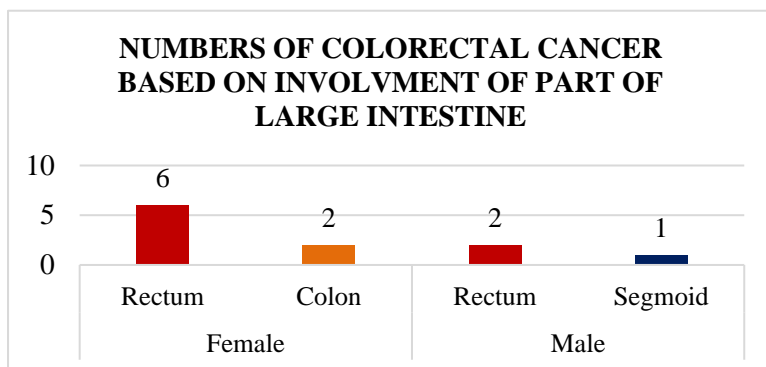


Figure 2 Numbers of colorectal cancer based on involvement of part of large intestine

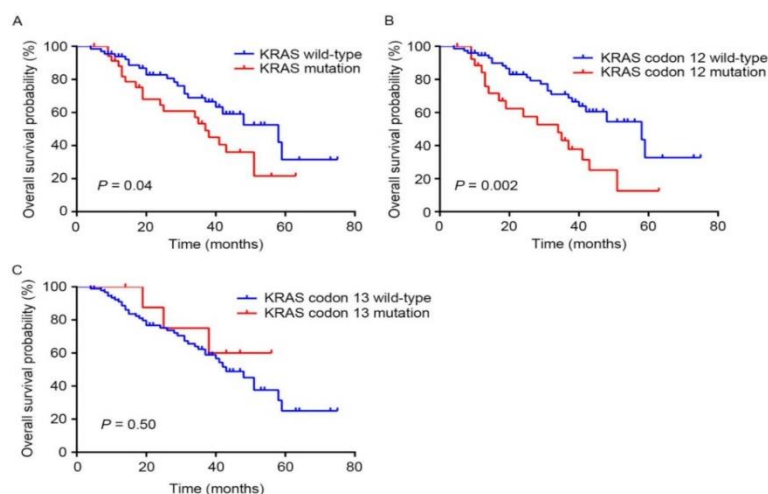


Figure 3 Kaplan-Meier curves of overall survival according to (A) KRAS mutation status, (B) KRAS codon 12 mutation status and (C) KRAS codon 13 mutation statuses in 58 patients.

(41.4%), which is somewhat in contrast to the findings of a similar study that reported a higher incidence in males (62%) compared to females (68%). This discrepancy may be attributed to socio-demographic and clinical characteristics specific to the population in Peshawar (17).

Age-wise, the highest mutation rates were observed in the younger age group (11-25 years), with a mutation rate of 8.6%. This finding diverges from many studies that typically report higher mutation rates in older age groups. A study reported that the average age of CRC patients with KRAS mutations was higher, emphasizing the need for further research to understand the age-related dynamics of KRAS mutations in this region (18).

Anatomical distribution of CRC in our study showed a higher prevalence of tumors in the rectum, particularly among females. This is in agreement with studies that highlight the recto-sigmoid region as the most affected area in CRC patients. For instance, a study by Arnold et al. (2017) reported that 54.7% of CRC cases involved the recto-sigmoid region, similar to our findings where the rectum was the most affected site (19).

Our study underscores the significant presence of KRAS mutations in CRC patients in Peshawar and their implications for disease prognosis and treatment strategies. The comparative analysis with previous studies highlights regional variations in mutation prevalence and demographic characteristics, pointing to the necessity of region-specific molecular studies to better understand and manage CRC (20).

CONCLUSION

This study has illuminated the molecular landscape of KRAS mutations among colorectal cancer patients in Pakistan, revealing a notably lower mutation rate compared to regional counterparts. Notably, the data indicates a higher prevalence of CRC in women than in men, which deviates from some global patterns where age, gender, and tumor type typically influence KRAS mutation rates.

DISCUSSION

This study provides a detailed analysis of KRAS mutations among colorectal cancer (CRC) patients in Peshawar, Pakistan, filling a significant gap in the molecular data from this region. Our findings reveal a noteworthy prevalence and pattern of KRAS mutations, particularly in codons 12 and 13, and highlight their implications for disease progression and treatment response (14).

The overall mutation detection rate in our study was 18.9%, with mutations predominantly found in codons 12 and 13 of the KRAS gene. This is somewhat lower compared to the findings of Awidi et al. (2020), who reported a prevalence of 83.6% KRAS mutations in exon 2 among CRC patients, with G12D being the most common mutation (Awidi et al., 2020). Specifically, in our study, the Glycine to Valine (G12V) substitution was the most frequent, occurring in 54.54% of cases with detected mutations, followed by Glycine to Aspartate (G12D) mutations (15).

Our results align with previous studies indicating that KRAS mutations are significant predictors of response to anti-EGFR therapy. The study in 2016 demonstrated that mutations in KRAS codon 12, but not codon 13, significantly impacted the effectiveness of anti-EGFR therapy, which is consistent with our findings where codon 12 mutations were associated with a statistically significant decrease in survival probability ($P=0.002$) (16). Gender distribution in our study showed a higher prevalence of CRC in females (58.6%) compared to males

Interestingly, young adults in Pakistan appear particularly susceptible to CRC, underscoring the critical need for early detection and screening in this group. Additionally, the findings suggest that mutations in KRAS codon 12 are associated with poorer survival outcomes than mutations in codon 13, highlighting potential implications for targeted therapies and patient management strategies.

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